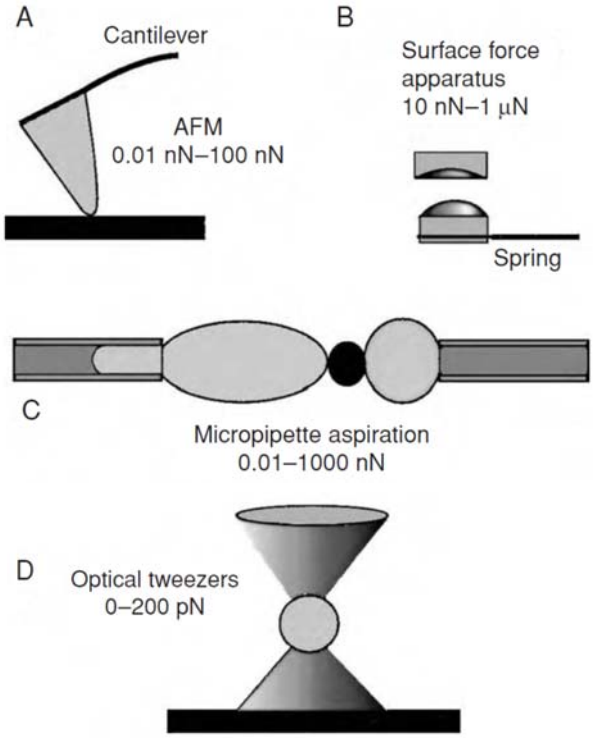
Typical magnitudes of quantities measured on the cellular scale (last column)

Quantity	SI units	"Micro SI" units (suitable for cells)
Distance	m	μm
Force	N	pN (= 10^{-12} N) to nN (= 10^{-9} N)[a]
Pressure, stress	Pa (= N/m^2)	$pN/\mu m^2$ (= 1 Pa) to $nN/\mu m^2$ (= 1 kPa)

[a] Forces of molecular bonds and those exerted by "soft" cells are in the picoNewton range. "Stiff" cells can exert forces in the nanoNewton range.

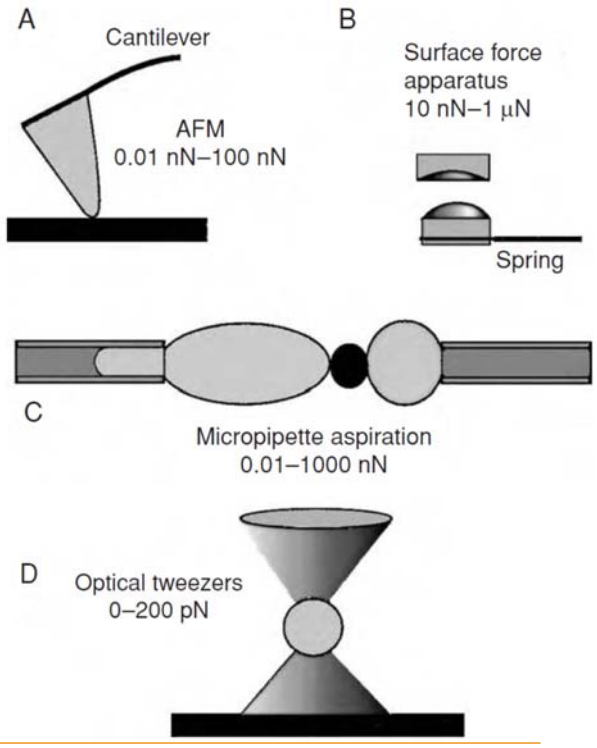
Methods to measure the mechanical properties of cells and biomolecules

- Force probes used to measure the mechanical properties of single cells and protein interaction forces.
- (A) An atomic force microscope (AFM) shows the probe tip, attached to the cantilever force transducer, coming in contact with a substrate.
- (B) A surface force apparatus, showing the crossed cylinders of the apparatus and the force-transducing spring.



Methods to measure the mechanical properties of cells and biomolecules

- (C) The bioforce probe, consisting of a cell partially aspirated into a micropipette. A bead (center black sphere) is attached to one cell (left), and a force between the bead and a second cell or glass bead (right) is exerted by aspirating the (left) cell into the pipette.
- (D) Optical tweezers. A bead is held in the optical trap, such that radiation pressure exerted on the bead applies force to adhesive contacts between materials on the bead surface and the substrate.



Van der Waals Forces

- In physical chemistry, the van der Waals force (or van der Waals interaction), named after Dutch scientist Johannes Diderik van der Waals, is the sum of the attractive or repulsive forces between molecules (or between parts of the same molecule) other than those due to covalent bonds, the hydrogen bonds, or the electrostatic interaction of ions with one another or with neutral molecules or charged molecules.

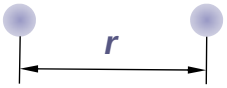
Gecko climbing glass (Wiki)

Van der Waals Forces

- The generalized interaction between molecules is given by the Mie pair potential

$$E(r) = -\frac{A}{r^n} + \frac{B}{r^m}$$

attractive repulsive



- A specific case of the Mie potential is the Lennard-Jones potential

$$E(r) = -\frac{A}{r^6} + \frac{B}{r^{12}}$$

where A and B are constants, e.g., for solid argon, $A = 8.0 \times 10^{-77}$ Jm^6 and $B = 1.12 \times 10^{-133}$ Jm^{12}.

Van der Waals Forces

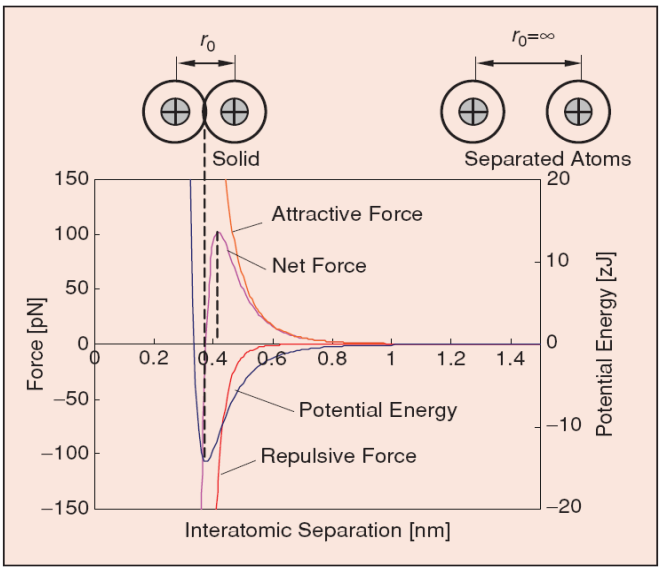
- The net van der Waals force is given by

$$F_{\mathrm{vdW}} = -\frac{dE}{dr}$$

- If r is scaled as $\sim L$, the attractive force scales as $\sim L^{-7}$, and thus its importance dramatically increases at the nanoscale. The repulsive force scales as $\sim L^{-13}$, which is important only at subnanometer scales.

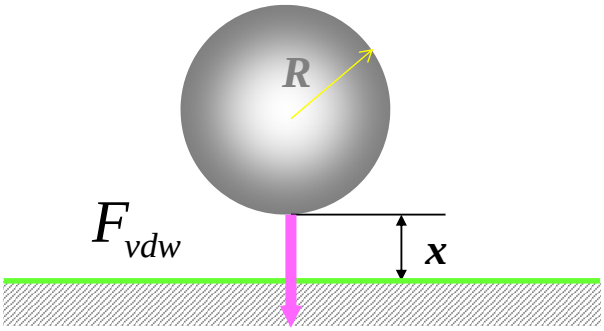
Intermolecular and Interatomic Forces

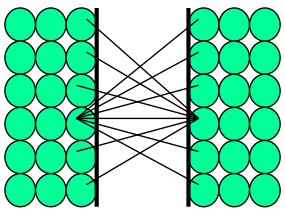
- The bond energy of van der Waals induced dipoles (such as argon solid shown here) is much smaller than electrostatic interaction based intramolecular ionic bonds (e.g., 3.2 eV for NaCl rock salt), metallic bonds (e.g., 3.1 eV for metal Cu) or covalent bonds (e.g., 4 eV for Si and 7.4 eV for C (diamond)), which scale as $\sim L^{-2}$.



The maximum value of F_{vdW} is obtained when $d^2E/dr^2 = 0$, or $r = (26B/7A)^{1/6} = 0.416$ nm, as $F_{vdW,\,max} = 102$ pN. Potential energy: 0.09 eV.

Van der Waals Forces





Volume Integration

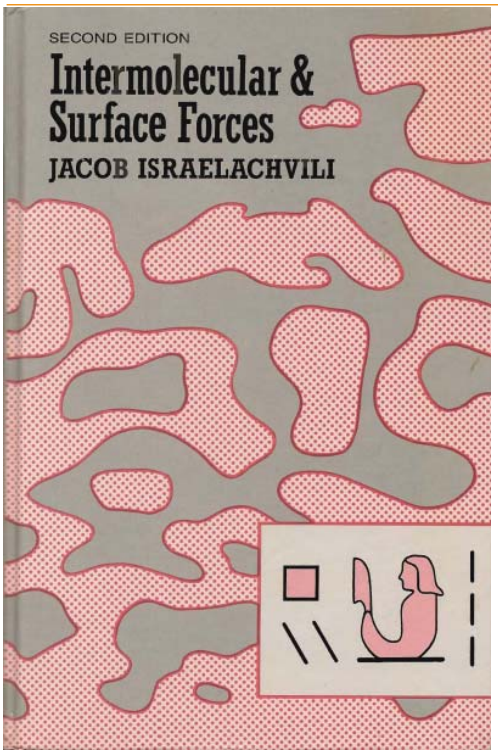
$$F_{vdw} = \frac{HR}{12x^2}$$

$F_{vdW} \sim x^{-2}$

$F_{vdW} \sim R^1$

H: Hamaker Constant x: Distance R: Radius of Bead

Van der Waals Forces



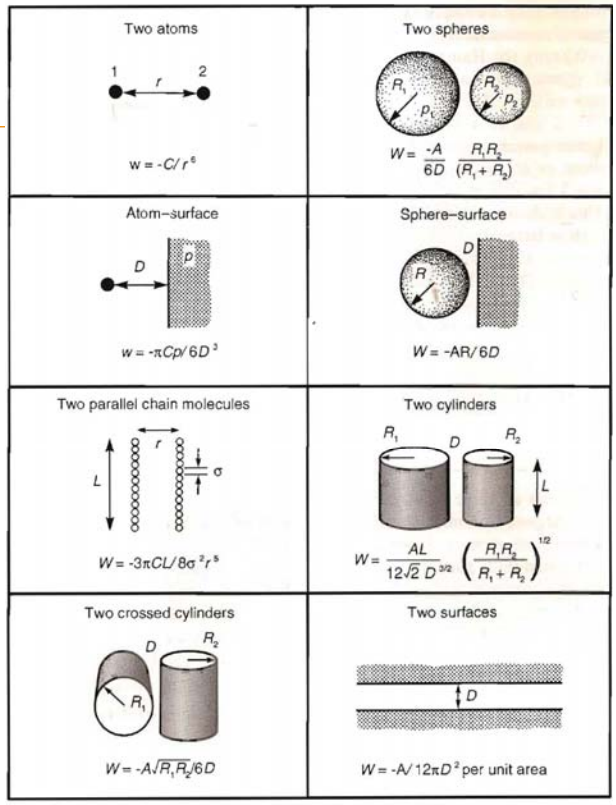


Fig. 11.1. Non-retarded van der Waals interaction free energies between bodies of different geometries calculated on the basis of pairwise additivity (Hamaker summation method). The *Hamaker constant* A is defined as $A = \pi^2 C \rho_1 \rho_2$ where ρ_1 and ρ_2 are the number of atoms per unit volume in the two bodies and C is the coefficient in the atom–atom pair potential (top left). A more rigorous method of calculating the Hamaker constant in terms of the macroscopic properties of the media is given in Section 11.3. The forces are obtained by differentiating the energies with respect to distance.

Van der Waals Forces

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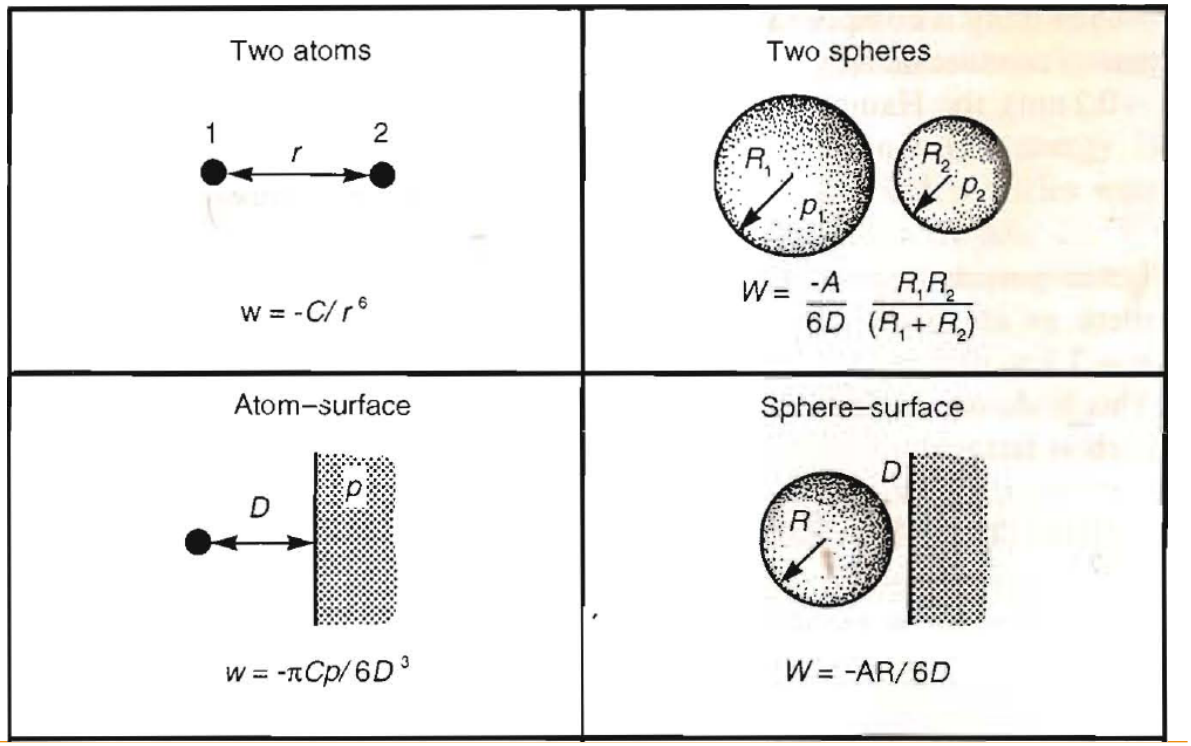
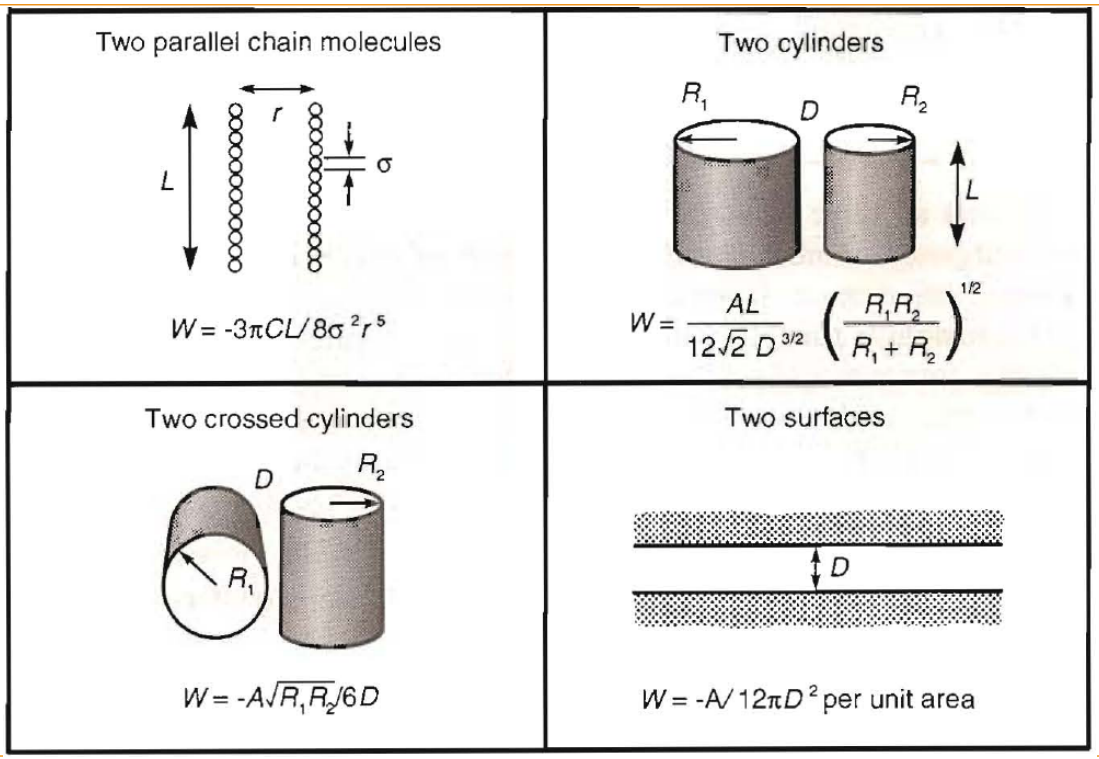


Van der Waals Forces



Van der Waals Forces

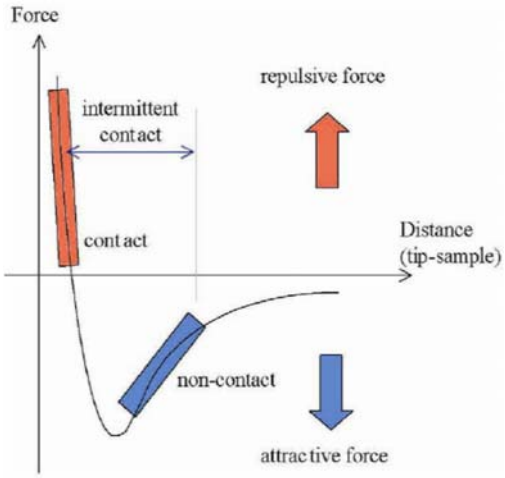
Fig. 11.1. Non-retarded van der Waals interaction free energies between bodies of different geometries calculated on the basis of pairwise additivity (Hamaker summation method). The *Hamaker constant* A is defined as $A = \pi^2 C\rho_1\rho_2$ where ρ_1 and ρ_2 are the number of atoms per unit volume in the two bodies and C is the coefficient in the atom–atom pair potential (top left). A more rigorous method of calculating the Hamaker constant in terms of the macroscopic properties of the media is given in Section 11.3. The forces are obtained by differentiating the energies with respect to distance.

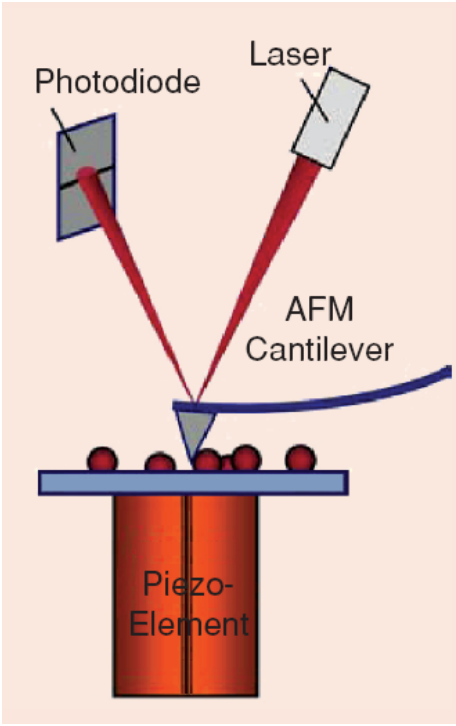
TABLE 11.1 Hamaker constants determined from pairwise additivity, Eq. (11.1).

Medium	C (10^{-79} J m^6)	ρ (10^{28} m^{-3})	A (10^{-19} J)
Hydrocarbon	50	3.3	0.5
CCl_4	1500	0.6	0.5
H_2O	140	3.3	1.5

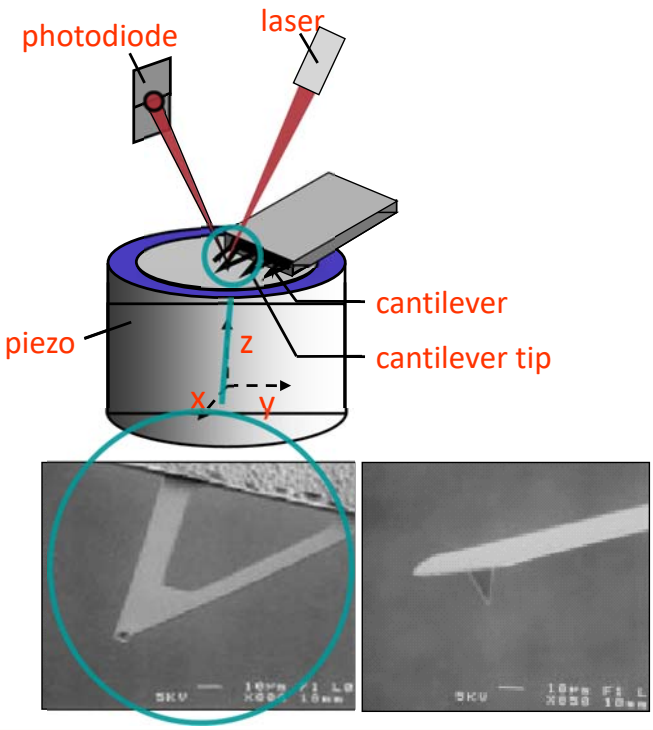
Atomic Force Microscopy (AFM)

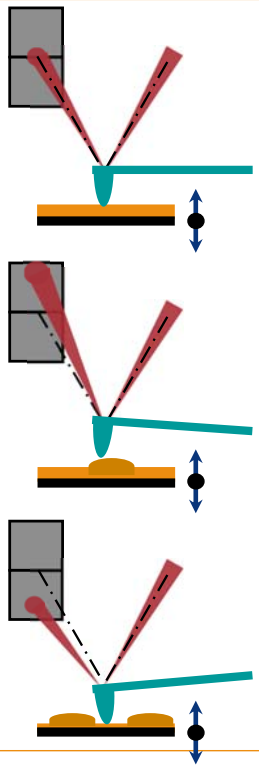
- Contact regime (a few hundreds of picometers)
  - Repulsive force (coulomb interaction)
- Non-contact regime (1-10 nanometers)
  - Attractive force (Van der Waals force)





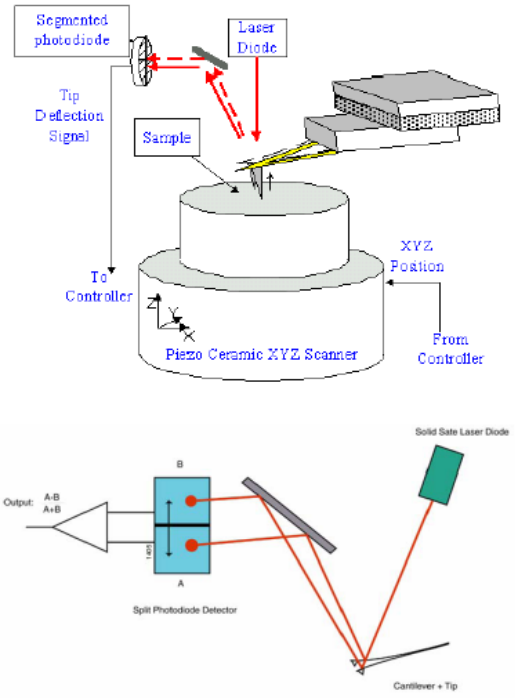
Atomic Force Microscopy



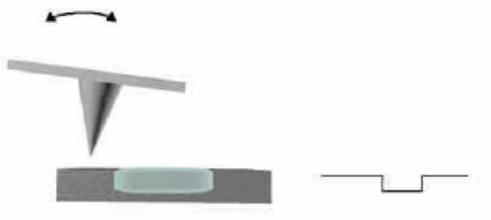


Atomic Force Microscopy

- Light lever used for contact mode, non-contact mode, and tapping mode.
- Long beam path (several cm) amplifies changes in beam angle.
- Laser light from a solid-state diode is reflected off the back of the cantilever and collected by a position sensitive detector (PSD).
- Angular displacement of cantilever results in one photodiode collecting more light than the other photodiode, producing an output signal which is proportional to the deflection of the cantilever.
- Detects cantilever deflections < 1Å (thermal noise limited).



Lateral Force Microscopy (Friction)



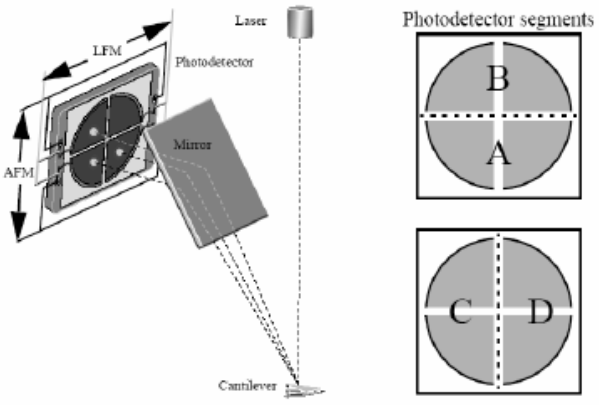
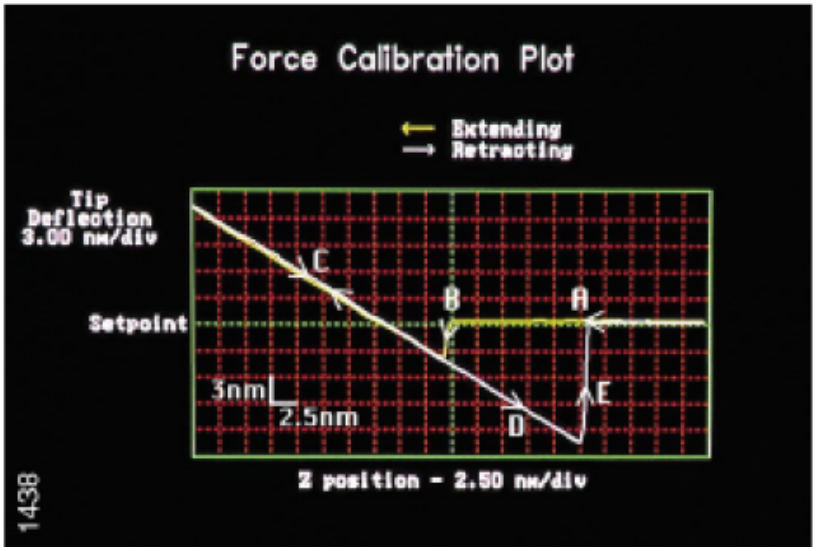


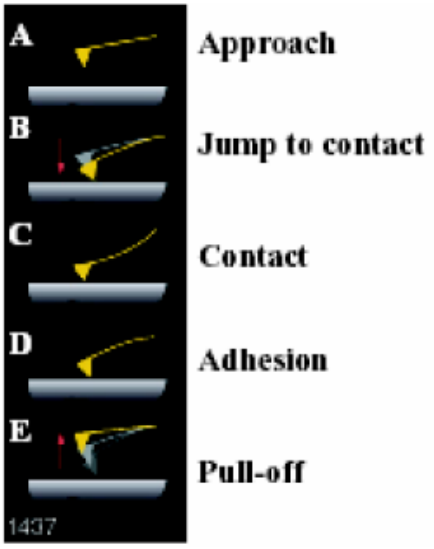
Figure 2.4. Quad photodetector arrangement. Different segments of the photodetector are used for generating AFM and LFM signals.

- The amount of torsion of the cantilever is controlled by changes in topography as well as changes in surface chemical properties.
- Possible to detect / image changes in material properties.

Force Curves

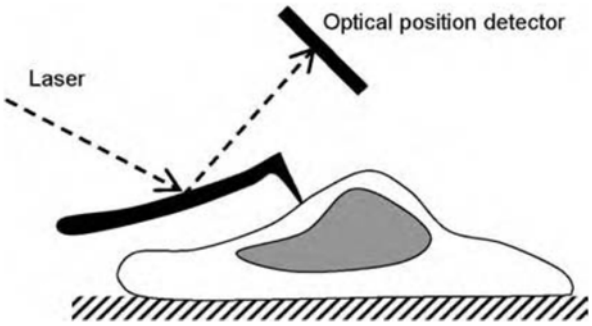
- Force curves are commonly used to set the imaging force in contact mode and to study attractive, repulsive, and adhesive interactions between the tip and the sample.





An atomic force microscope (AFM)

- Deflection of the laser beam is sensed and related to tip deflection by the known geometry of the optical pathway. Tip deflection is then related to applied force by knowledge of the mechanical characteristics of the cantilever arm supporting the tip.



Schematic description of the tip of the atomic force microscope contacting a cell, showing the path of the laser beam.

Force measurement using an AFM

- To understand the operation of the AFM it is important to delve into the operation of the cantilever arm in more detail. If the arm undergoes only small deflection in the vertical direction, then it can be treated as a linear spring with stiffness k_c. In this case, the force F needed to create a vertical deflection z can be written as

$$F = k_c z$$

- Typical values for k_c are about 0.02 to 5 Newton per meter (N/m).

Noise of force measurement

- Remember that the probe tip will typically be in an aqueous environment and will, therefore, be subject to random collisions from water molecules undergoing thermal motion.
- The average energy of a molecule at absolute temperature T is given by $1/2k_BT$, where k_B is Boltzmann's constant, 1.3807×10^{-23} J/K.
- The energy stored in a spring of stiffness k_c as it deflects by an amount z is $1/2k_cz^2$. Equating these two energies allows us to solve for the fluctuations, z, that will occur as a result of random collisions with water molecules as:

$$z = \sqrt{\frac{k_B T}{k_c}}$$

Noise of force measurement

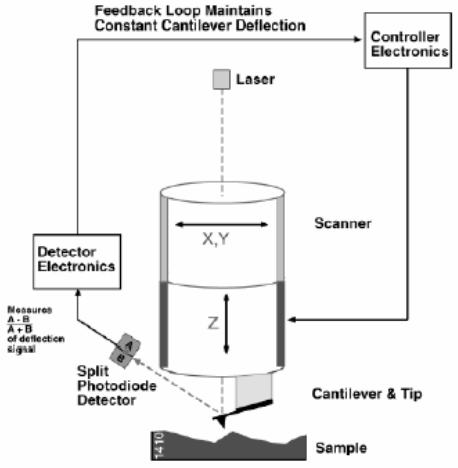
- This corresponds to a force

$$F = \sqrt{k_c k_B T}$$

- which represents the thermal “noise” that will be continually measured by the AFM tip.
- Forces appreciably smaller than this are not resolvable. For a sample at 37 °C (310 K), and an arm stiffness of 0.05 N/m, this corresponds to approximately 15 pN.

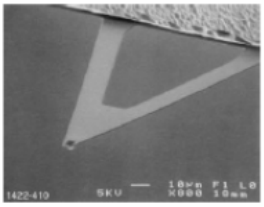
Contact Mode

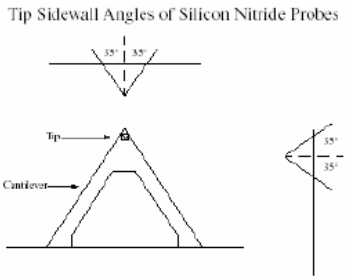
- Operates by scanning a tip across the sample surface while monitoring the change in cantilever deflection.
- A feedback loop maintains a constant deflection between the cantilever and the sample by vertically moving the scanner at each (x,y) data point.
- Force constants usually range from 0.01 to 1.0 N/m, resulting in forces ranging from nN to μN.
- The distance the scanner moves vertically at each (x,y) data point is stored to form the topographic image of the sample surface.
- Operation can take place in ambient and liquid environments.



Contact Mode Tips / Cantilevers

- Silicon nitride probes consist of a cantilever integrated with a sharp tip on the end.
- It is necessary to have a cantilever which is **soft** enough to be deflected by very small forces and has a **high enough resonant frequency** not to be susceptible to vibrational instabilities.
- This is accomplished by making the cantilever short to provide a high resonant frequency, and thin to provide a small force constant.



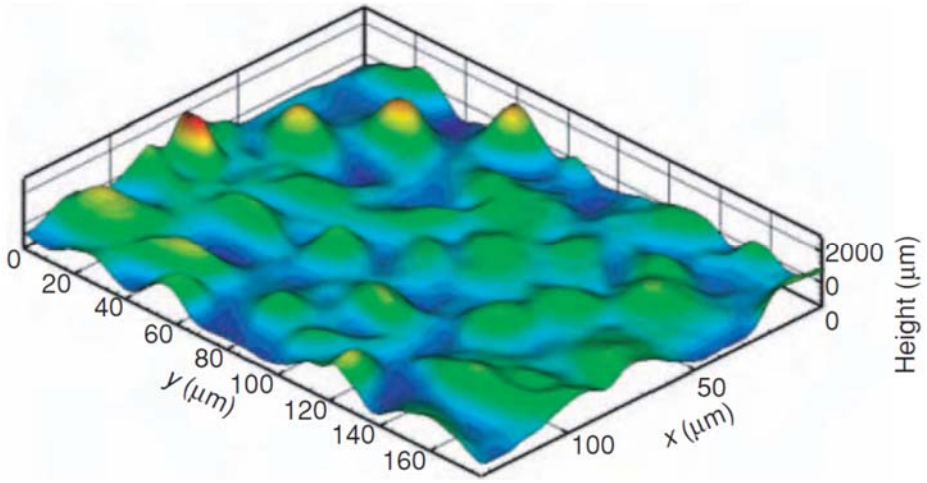


Silicon Nitride Probe Characteristics

Spring Constant (k)	0.58, 0.32, 0.12, 0.06 N/m [a]
Nominal Tip Radius of Curvature	20 - 60 nm
Cantilever Lengths	100 & 200 µm
Cantilever Configuration	V-shaped
Reflective Coating	Gold
Sidewall angles	35° on all 4 sides

a. Calculated spring constant values are based on the 0.6µm silicon nitride thickness; however, this value can actually vary from 0.4µm to 0.7µm. Thickness is cubed in the spring constant calculation, thus, actual values can vary substantially.

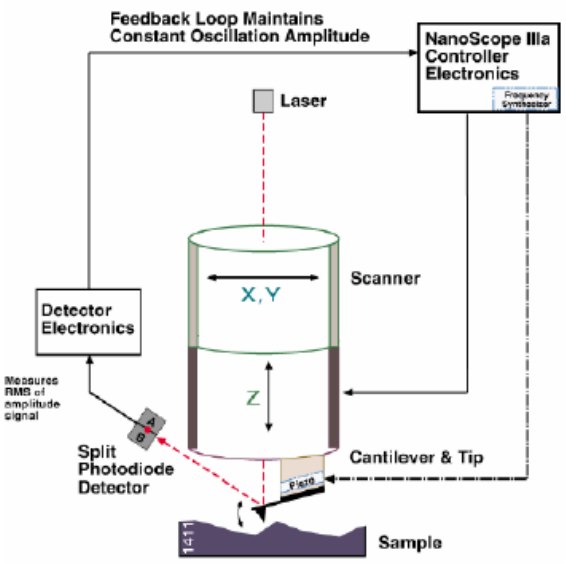
AFM map of cellular topography



- Atomic force microscopic map of cellular topography for confluent porcine aortic endothelial cells. The vertical scale has been exaggerated. Data courtesy of Dr. Shigeo Wada (Sendai University, Japan), and Mr. James Shaw and Dr. Chris Yip (University of Toronto).

Tapping Mode

- Tapping Mode AFM operates by scanning a tip on an oscillating cantilever across the sample surface.
- The cantilever is oscillated at or near its resonance frequency (amplitude typically from 20 to 100 nm).
- The tip lightly “taps” on the sample surface during scanning.
- The feedback loop maintains a constant oscillation amplitude by maintaining a constant RMS of the oscillation signal acquired by the split photodiode detector.
- Operation can take place in ambient and liquid environments.

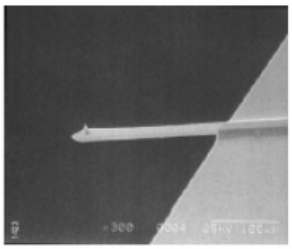


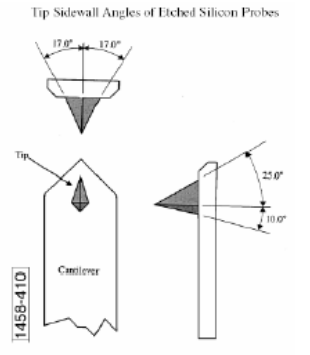
Tapping mode of AFM

- The cantilever arm is acoustically or magnetically driven so that it vibrates at or near its resonant frequency, and it is then moved vertically so that it approaches the cell surface.
- Far from the cell, the arm undergoes oscillations whose amplitude is determined by the magnitude of the driving signal.
- However, as the probe tip approaches the cell it begins to interact with the cell, which changes the magnitude of the arm’s vibrations.
- The position where this change in magnitude occurs is a measure of the topography of the cell at that lateral location. Because the probe tip just taps the specimen, rather than being dragged along it, this produces smaller lateral forces on the cell and less potential for damage.

Tapping Mode Tips / Cantilevers

- Silicon probes are used primarily for Tapping Mode applications.
- The tip and cantilever are an integrated assembly of single crystal silicon, produced by etching techniques.
- These probes can be **much stiffer than the silicon nitride probes**, resulting in larger force constants and resonant frequencies.





TappingMode Etched Silicon Probe (TESP) Characteristics

Spring Constant (k)	20 - 100 N/m
Resonant Frequency	200 - 400 kHz
Nominal Tip Radius of Curvature	5 - 10 nm
Cantilever Length	125 µm
Cantilever Configuration	Single Beam
Reflective Coating	Uncoated, Optional Al Coating

Force mapping mode of AFM

- Other interesting measurements can be made with the AFM on cells. For example, the probe tip can be vertically traversed towards the sample while the cantilever arm deflection is measured. This is known as “force mapping mode” and produces a curve of applied force versus surface deflection (a “force curve”). To understand this curve, we must remember that the probe tip is much harder than the relatively soft biological specimens, so that we effectively have a rigid cone penetrating into the cell, which we will treat as being locally planar and linearly elastic.

Force mapping mode of AFM

- The key result is that the deflection of the cell at the center of the probe tip, δ, due to an applied force F is

$$\delta^2 = \frac{\pi}{2}\frac{F(1-\nu^2)}{E\ \tan\alpha}$$

- where E and ν are the modulus and Poisson ratio for the cell and α is the known cone half-angle. A generalization of this solution that is useful for our purposes is:

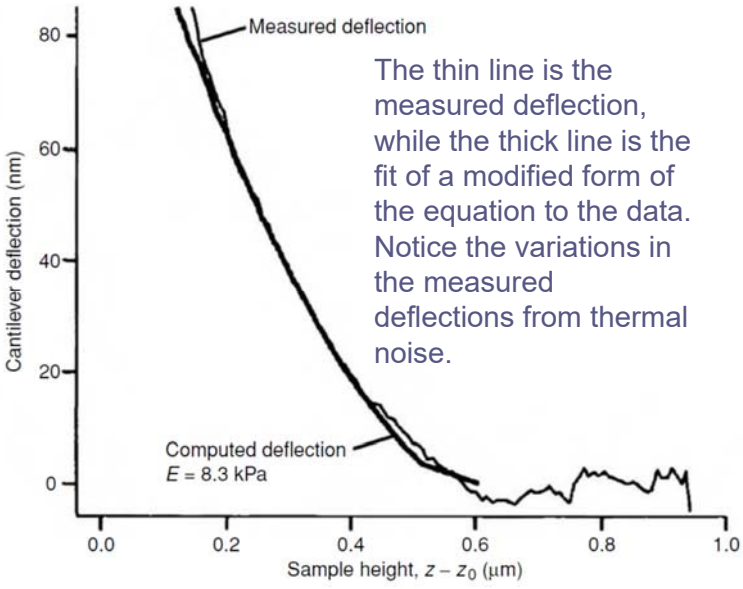
$$z - z_0 = \frac{F}{k_c} + \sqrt{\frac{\pi}{2}\frac{F(1-\nu^2)}{E\ \tan\alpha}}$$

- Here z_0 is the probe height at which the applied force becomes non-zero, and the first term on the right-hand side accounts for the deflection of cantilever arm.

A force curve

- The vertical axis is proportional to the force applied to the cell by the probe tip. The horizontal axis is the vertical offset of the base of the cantilever arm applied by the piezoelectric actuator, which we denoted by $z - z_0$ in equation

$$z - z_0 = \frac{F}{k_c} + \sqrt{\frac{\pi}{2}\frac{F(1-\nu^2)}{E\ \tan\alpha}}$$

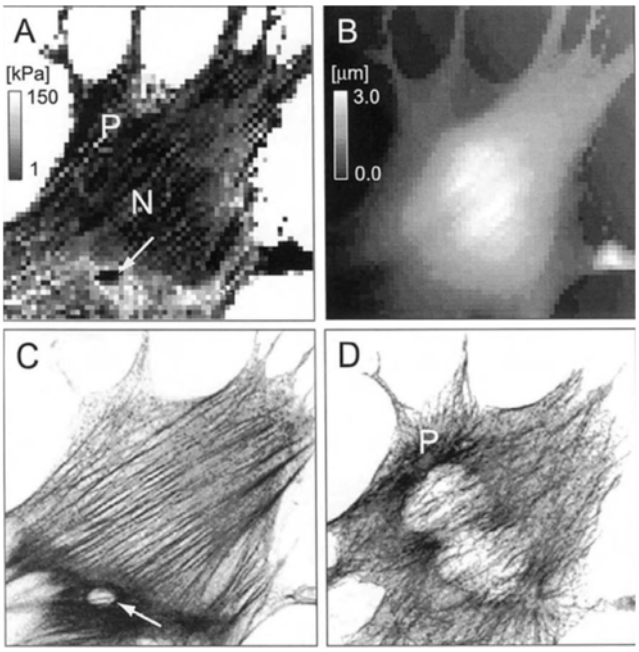


The thin line is the measured deflection, while the thick line is the fit of a modified form of the equation to the data. Notice the variations in the measured deflections from thermal noise.

Example force curve for measurement of cellular stiffness of an activated human platelet using the AFM.

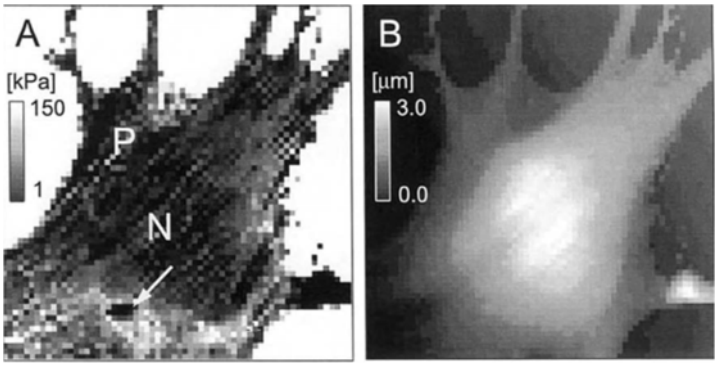
Elasticity and corresponding topography map

- By assuming a value for v (usually 0.5), the measured force–displacement data can be fit by Equation (2.5) to obtain z_0 and E. It will be appreciated that the value of E so obtained reflects a local Young's modulus and can be expected to vary over the surface of the cell. For example, the local stiffness has been related to the local cytoskeletal structure in fibroblasts.



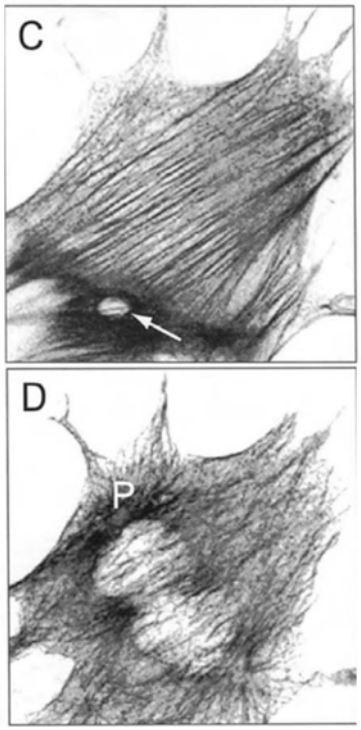
Elasticity and corresponding topography map

- Elasticity map (A) and corresponding topography map (B) of a living NIH3T3 fibroblast. The nuclear portion (N) is the softest, with a stiffness of approximately 4 kPa. A small softer "island" was observed in the perinuclear region (marked by arrow).



Elasticity and corresponding topography map

- (C,D) Immunofluorescence images of actin filaments (C) and microtubules (D) for the same cell shown in A and B. A bi-lobed nucleus is visible in the microtubule image. The small open area observed in the actin image (arrow in C) corresponds to the soft “island” observed in the elasticity map. Part of the cell with low actin density and high density of microtubules shows a low Young’s modulus (marked P in A and D). Each image size is 80 μm square. Reprinted from Haga *et al.* [35], with permission from Elsevier.



Maximum spatial resolution

- What sort of maximum spatial resolution can we expect with the AFM?
- A typical probe tip radius is 10–50 nm, but unfortunately this lateral resolution is not achievable when measuring cellular stiffness. That is because the probe sinks into the relatively soft cell and consequently measures over a larger area than just the tip. The magnitude of this effect depends on the applied force and the cell stiffness; typical values for lateral resolution are in the range of tens to several hundreds of nanometers. This is small enough to give reasonable resolution when mapping stiffness over a cell.

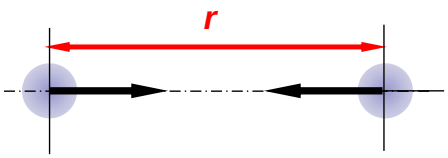
Interatomic van der Waals Forces

- The Lennard-Jones potential:
  - $E(r) = -\frac{A}{r^6} + \frac{B}{r^{12}}$

- van der Waals Forces between two atoms:
  - $F_{vdW} = -\frac{dE}{dr} = -\frac{6A}{r^7} + \frac{12B}{r^{13}}$
  - Attractive vdW Force: $F_{vdW,a} = -\frac{6A}{r^7}$
  - Repulsive vdW Force: $F_{vdW,r} = \frac{12B}{r^{13}}$
  - for solid argon, $A = 8.0 \times 10^{-77}$ Jm^6 and $B = 1.12 \times 10^{-133}$ Jm^{12}
  - If r is scaled as $\sim L$, the attractive force scales as $\sim L^{-7}$, and thus its importance dramatically increases at the nanoscale. The repulsive force scales as $\sim L^{-13}$, which is important only at subnanometer scales.

The minimum Lennard-Jones potential energy

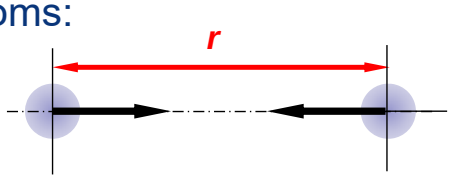
- The Lennard-Jones potential:
  - $E(r) = -\frac{A}{r^6} + \frac{B}{r^{12}}$



- The minimum value of $E(r)$
  - $\frac{dE}{dr} = \frac{6A}{r^7} - \frac{12B}{r^{13}} = 0$
  - $6Ar^6 - 12B = 0$
  - $r = \left(\frac{2B}{A}\right)^{\frac{1}{6}} \triangleq r_e$
  - For argon atoms:
    - $r_e = \left(\frac{2\times1.12\times10^{-133}}{8\times10^{-77}}\right)^{\frac{1}{6}} = 0.375[nm]$
    - $E_{vdW,min} = -\frac{A}{r_e^6} + \frac{2B}{r_e^{12}} = -\frac{8\times10^{-77}}{(0.375\times10^{-9})^6} + \frac{2\times1.12\times10^{-133}}{(0.375\times10^{-9})^{12}} = -2.877 \times 10^{-20}[J] + 2.896 \times 10^{-20}[J] = 1.974 \times 10^{-22}[J] = 0.0012[eV]$
    - Note 1J = 6.24150913 × 10^{18} eV

Interatomic van der Waals Forces

- van der Waals Forces between two atoms:
  - $F_{vdW} = -\frac{dE}{dr} = -\frac{6A}{r^7} + \frac{12B}{r^{13}}$

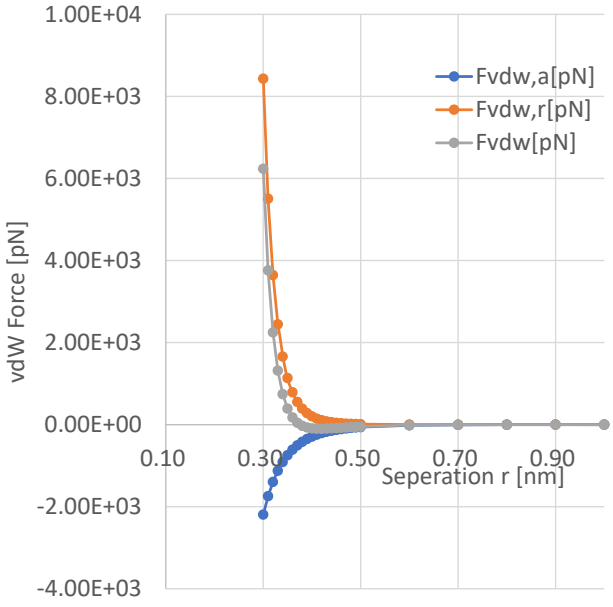


- The maximum value of F_{vdW}
  - $\frac{dF_{vdW}}{dr} = 0$
  - $\frac{7\times6A}{r^8} - \frac{13\times12B}{r^{14}} = 0$
  - $7 \times 6Ar^6 - 13 \times 12B = 0$
  - $r = \left(\frac{13\times12B}{7\times6A}\right)^{\frac{1}{6}} = \left(\frac{26B}{7A}\right)^{\frac{1}{6}} \triangleq r_0$
  - For argon atoms:
    - $r_0 = \left(\frac{26\times1.12\times10^{-133}}{7\times8\times10^{-77}}\right)^{\frac{1}{6}} = 0.416[nm]$
    - $F_{vdW,max} = -\frac{6A}{{r_0}^7} + \frac{12B}{{r_0}^{13}} = -\frac{6\times8\times10^{-77}}{(0.416\times10^{-9})^7} + \frac{12\times1.12\times10^{-133}}{(0.416\times10^{-9})^{13}} = -222.63[pN] + 120.28[pN] = -102.35[pN]$ ("–" means attractive force)

Interatomic van der Waals Forces

r [nm]	Fvdw,a[pN]	Fvdw,r[pN]	Fvdw[pN]
10.00	-4.80E-08	1.34E-16	-4.80E-08
5.00	-6.14E-06	1.10E-12	-6.14E-06
1.00	-4.80E-01	1.34E-03	-4.79E-01
0.90	-1.00E+00	5.29E-03	-9.98E-01
0.80	-2.29E+00	2.44E-02	-2.26E+00
0.70	-5.83E+00	1.39E-01	-5.69E+00
0.60	-1.71E+01	1.03E+00	-1.61E+01
0.50	-6.14E+01	1.10E+01	-5.04E+01
0.40	-2.93E+02	2.00E+02	-9.27E+01
0.30	-2.19E+03	8.43E+03	6.24E+03
0.20	-3.75E+04	1.64E+06	1.60E+06
0.10	-4.80E+06	1.34E+10	1.34E+10
0.09	-1.00E+07	5.29E+10	5.29E+10
0.08	-2.29E+07	2.44E+11	2.44E+11
0.07	-5.83E+07	1.39E+12	1.39E+12
0.06	-1.71E+08	1.03E+13	1.03E+13
0.05	-6.14E+08	1.10E+14	1.10E+14
0.04	-2.93E+09	2.00E+15	2.00E+15
0.03	-2.19E+10	8.43E+16	8.43E+16
0.02	-3.75E+11	1.64E+19	1.64E+19
0.01	-4.80E+13	1.34E+23	1.34E+23

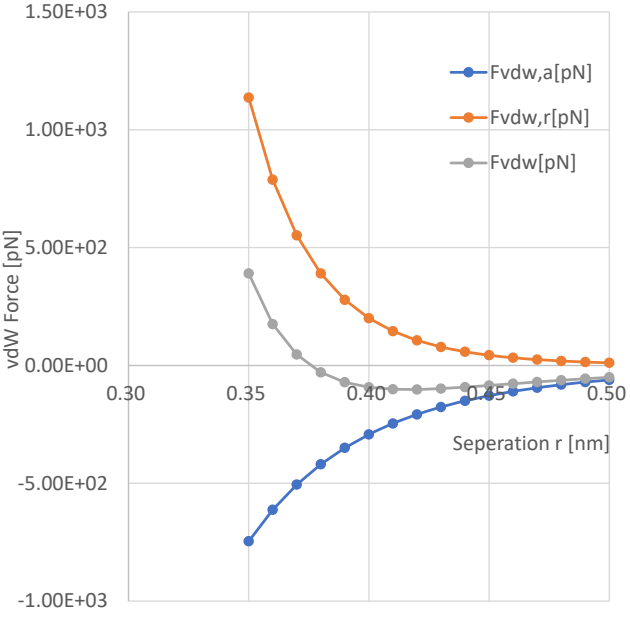
- for argon atoms, $A = 8.0 \times 10^{-77}$ Jm^6 and $B = 1.12 \times 10^{-133}$ Jm^{12}



Interatomic van der Waals Forces

r [nm]	Fvdw,a[pN]	Fvdw,r[pN]	Fvdw[pN]
0.50	-6.14E+01	1.10E+01	-5.04E+01
0.49	-7.08E+01	1.43E+01	-5.65E+01
0.48	-8.18E+01	1.87E+01	-6.30E+01
0.47	-9.47E+01	2.46E+01	-7.01E+01
0.46	-1.10E+02	3.25E+01	-7.76E+01
0.45	-1.28E+02	4.33E+01	-8.51E+01
0.44	-1.50E+02	5.80E+01	-9.23E+01
0.43	-1.77E+02	7.82E+01	-9.84E+01
0.42	-2.08E+02	1.06E+02	-1.02E+02
0.41	-2.46E+02	1.45E+02	-1.01E+02
0.40	-2.93E+02	2.00E+02	-9.27E+01
0.39	-3.50E+02	2.78E+02	-7.14E+01
0.38	-4.20E+02	3.90E+02	-2.94E+01
0.37	-5.06E+02	5.52E+02	4.62E+01
0.36	-6.13E+02	7.88E+02	1.75E+02
0.35	-7.46E+02	1.14E+03	3.90E+02
0.34	-9.14E+02	1.66E+03	7.43E+02
0.33	-1.13E+03	2.44E+03	1.32E+03
0.32	-1.40E+03	3.64E+03	2.25E+03
0.31	-1.74E+03	5.50E+03	3.76E+03
0.30	-2.19E+03	8.43E+03	6.24E+03

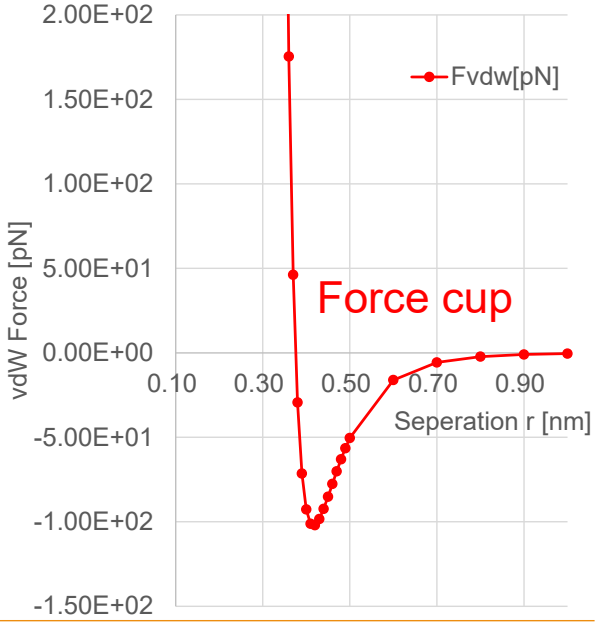
- for argon atoms, $A = 8.0 \times 10^{-77}$ Jm^6 and $B = 1.12 \times 10^{-133}$ Jm^{12}



Interatomic van der Waals Forces

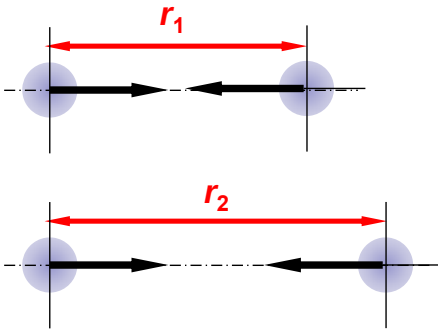
r [nm]	Fvdw,a[pN]	Fvdw,r[pN]	Fvdw[pN]
0.50	-6.14E+01	1.10E+01	-5.04E+01
0.49	-7.08E+01	1.43E+01	-5.65E+01
0.48	-8.18E+01	1.87E+01	-6.30E+01
0.47	-9.47E+01	2.46E+01	-7.01E+01
0.46	-1.10E+02	3.25E+01	-7.76E+01
0.45	-1.28E+02	4.33E+01	-8.51E+01
0.44	-1.50E+02	5.80E+01	-9.23E+01
0.43	-1.77E+02	7.82E+01	-9.84E+01
0.42	-2.08E+02	1.06E+02	-1.02E+02
0.41	-2.46E+02	1.45E+02	-1.01E+02
0.40	-2.93E+02	2.00E+02	-9.27E+01
0.39	-3.50E+02	2.78E+02	-7.14E+01
0.38	-4.20E+02	3.90E+02	-2.94E+01
0.37	-5.06E+02	5.52E+02	4.62E+01
0.36	-6.13E+02	7.88E+02	1.75E+02
0.35	-7.46E+02	1.14E+03	3.90E+02
0.34	-9.14E+02	1.66E+03	7.43E+02
0.33	-1.13E+03	2.44E+03	1.32E+03
0.32	-1.40E+03	3.64E+03	2.25E+03
0.31	-1.74E+03	5.50E+03	3.76E+03
0.30	-2.19E+03	8.43E+03	6.24E+03

- for argon atoms, $A = 8.0 \times 10^{-77}$ Jm^6 and $B = 1.12 \times 10^{-133}$ Jm^{12}



Scaling of Interatomic van der Waals Forces

- If we know the vdW force for a certain separation,
  - $F_{vdW,1} = F_{vdW,a1} - F_{vdW,r1} = -\frac{6A}{r_1^7} + \frac{12B}{r_1^{13}}$,
- how to calculate the vdW force for a different separation?
  - $r_2 = kr_1$ *or* $k = \frac{r_2}{r_1}$
  - $F_{vdW,2} = -\frac{6A}{r_2^7} + \frac{12B}{r_2^{13}} = -\frac{6A}{(kr_0)^7} + \frac{12B}{(kr_0)^{13}}$
  - $= -\frac{6A}{k^7(r_0)^7} + \frac{12B}{k^{13}(r_0)^{13}} = -k^{-7}\frac{6A}{(r_0)^7} + k^{-13}\frac{12B}{(r_0)^{13}}$
  - For argon atoms:
    - Take $r_0 = r_{max} = 0.416[nm]$
    - $F_{vdW,0} = F_{vdW,max} = -222.63[pN] + 120.28[pN] = -102.35[pN]$



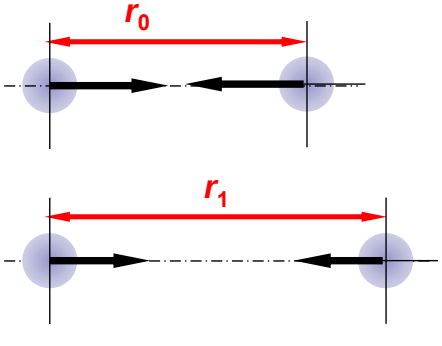
e.g.

$r_1 = 10r_0 = 4.16\text{nm}$

$F_{vdW,1} = -10^{-7}F_{vdW,a0} + 10^{-13}F_{vdW,r0} = -10^{-7}222.63[pN] + 10^{-13}120.28[pN] \approx -22.63[aN]$

Scaling of Interatomic van der Waals Forces

- If we know the vdW force for a certain separation,
  - $F_{vdW,1} = F_{vdW,a1} - F_{vdW,r1} = -\frac{6A}{r_1^7} + \frac{12B}{r_1^{13}}$,
- how to calculate the vdW force for a different separation?
  - $r_2 = kr_1$
  - $F_{vdW,2} = -\frac{6A}{r_2^7} + \frac{12B}{r_2^{13}} = -\frac{6A}{(kr_0)^7} + \frac{12B}{(kr_0)^{13}}$
  - $= -\frac{6A}{k^7(r_0)^7} + \frac{12B}{k^{13}(r_0)^{13}} = -k^{-7}\frac{6A}{(r_0)^7} + k^{-13}\frac{12B}{(r_0)^{13}}$
  - For argon atoms:
    - Take $r_0 = r_{max} = 0.416[nm]$
    - $F_{vdW,0} = F_{vdW,max} = -222.63[pN] + 120.28[pN] = -102.35[pN]$



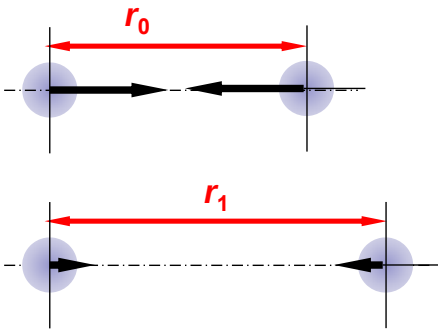
e.g.

$r_1 = 1.1r_0 = 0.4576\text{nm}$

$F_{vdW,1} = -1.1^{-7}F_{vdW,a0} + 1.1^{-13}F_{vdW,r0} = -0.513 \times 222.63[pN] + 0.290 \times 120.28[pN] = -79.33[pN]$

Scaling of Interatomic van der Waals Forces

- If we know the vdW force for a certain separation,
  - $F_{vdW,1} = F_{vdW,a1} - F_{vdW,r1} = -\frac{6A}{r_1^7} + \frac{12B}{r_1^{13}}$,
- how to calculate the vdW force for a different separation?
  - $r_2 = kr_1$
  - $F_{vdW,2} = -\frac{6A}{r_2^7} + \frac{12B}{r_2^{13}} = -\frac{6A}{(kr_0)^7} + \frac{12B}{(kr_0)^{13}}$
  - $= -\frac{6A}{k^7(r_0)^7} + \frac{12B}{k^{13}(r_0)^{13}} = -k^{-7}\frac{6A}{(r_0)^7} + k^{-13}\frac{12B}{(r_0)^{13}}$
  - For argon atoms:
    - Take $r_0 = r_{max} = 0.416[nm]$
    - $F_{vdW,0} = F_{vdW,max} = -222.63[pN] + 120.28[pN] = -102.35[pN]$



e.g.

$r_1 = 1.5r_0 = 0.4576\text{nm}$

$F_{vdW,1} = -1.5^{-7}F_{vdW,a0} + 1.5^{-13}F_{vdW,r0}$

$= -0.059 \times 222.63[pN] + 0.005 \times 120.28[pN]$

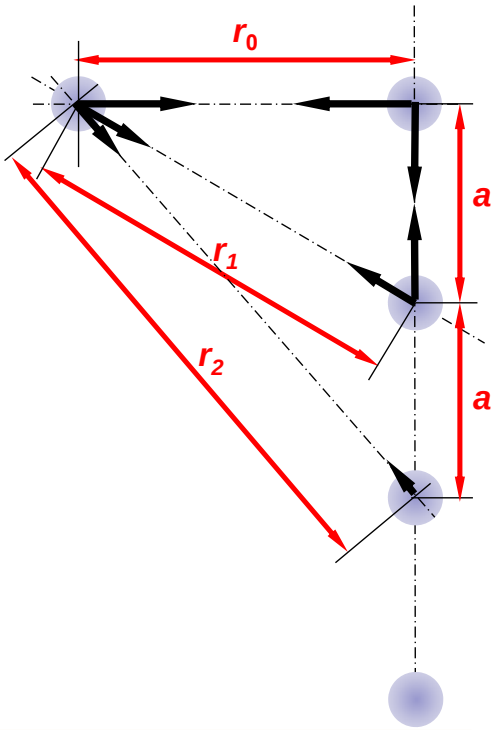
$= -12.53[pN]$

van der Waals Forces between an atom and a chain of atoms

- The net vdW force for atom A,
  - $\vec{F}_{vdW,net} = \vec{F}_{vdW,0} + \vec{F}_{vdW,1} + \vec{F}_{vdW,2} + \cdots$
  - If $a \approx r_0$,
  - $r_1 = \sqrt{2}r_0 = 1.414\ r_0$,
  - $r_2 = \sqrt{5}r_0 = 2.236\ r_0$, $\vec{F}_{vdW,2}$ *is ignorable*
  - $\vec{F}_{vdW,net} = \vec{F}_{vdW,0} + \vec{F}_{vdW,1} + \vec{F}_{vdW,2} + \cdots$

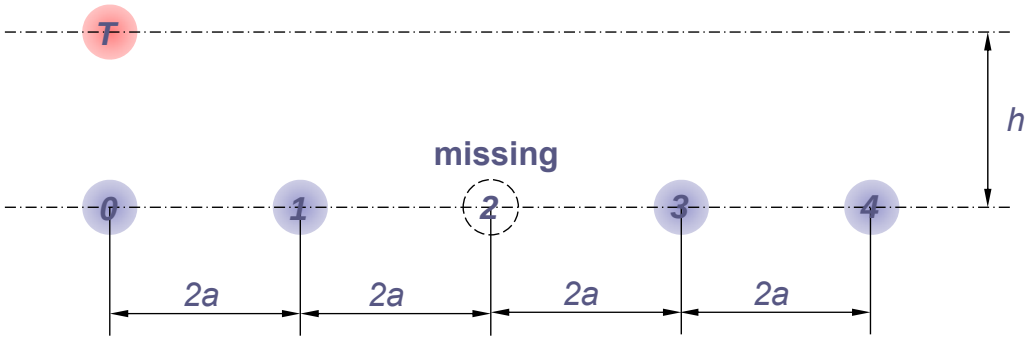
    $\approx \vec{F}_{vdW,0} + \vec{F}_{vdW,1}$ (error is acceptable)

    $\approx \vec{F}_{vdW,0}$ (error is acceptable)
  - Calculate the errors by yourself



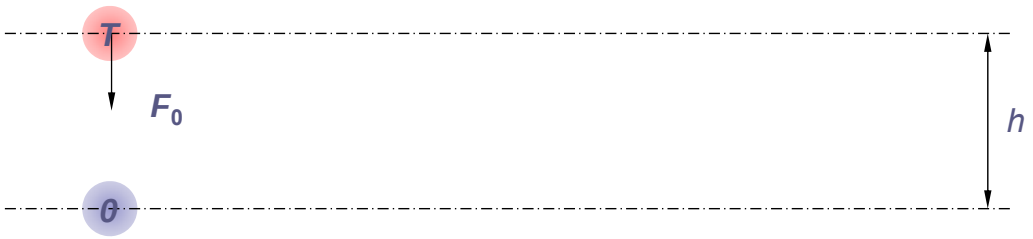
van der Waals Forces between a sliding atom and a chain of atoms

- Ignore the repulsive force, given $A = 10^{-77}$ Jm^6 and $h = 2a = 0.45$ nm, when an atom T slides over a chain of atoms (fixed), how the vdW force in the vertical direction on it will change?



van der Waals Forces between a sliding atom and a chain of atoms

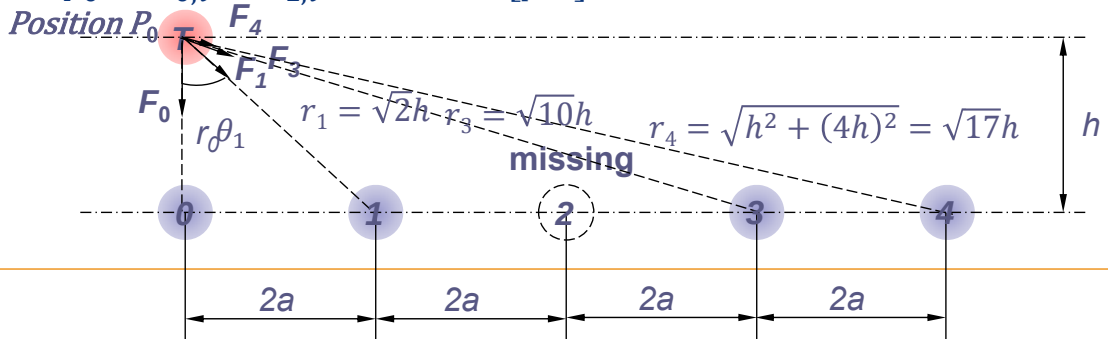
- Calculate the vdW force for a simple case like



$$F_0 = -\frac{6A}{h^7} = -\frac{6 \times 10^{-77}}{(0.45 \times 10^{-9})^7} = -16.06[pN]$$

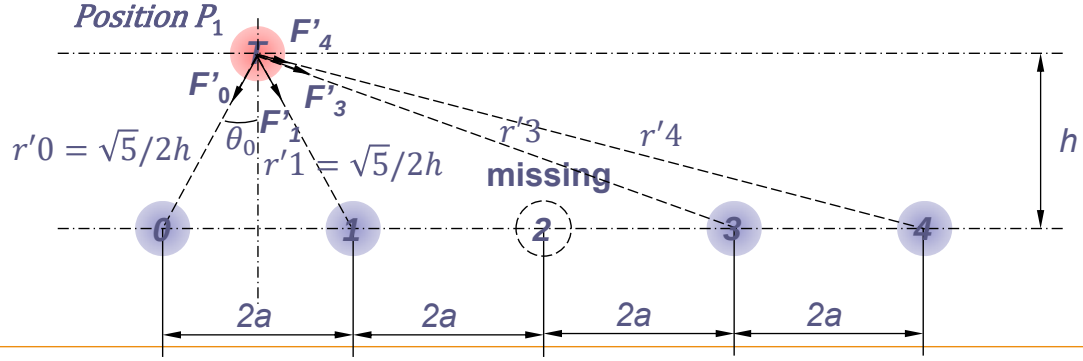
van der Waals Forces between a sliding atom and a chain of atoms

- $F_0 = -16.06[pN]$
- $F_1 = k^{-7} \times F_0 = (\frac{r_1}{r_0})^{-7} \times F_0 = (\sqrt{2})^{-7} \times F_0 = 0.088 \times (-16.06)[pN] = -1.42[pN]$
  - $F_{1,v} = F_1 cos\theta_1 = -1.42 cos45° = -1.00[pN]$ (ignore this will cause 6% error)
- $F_3 = (\sqrt{10})^{-7} \times F_0 = 0.00032F_0$ (negligible)
- $F_4 = (\sqrt{17})^{-7} \times F_0 = 0.000049F_0$ (negligible)
- $F_{P0} = F_{0,v} + F_{1,v} = -17.06[pN]$



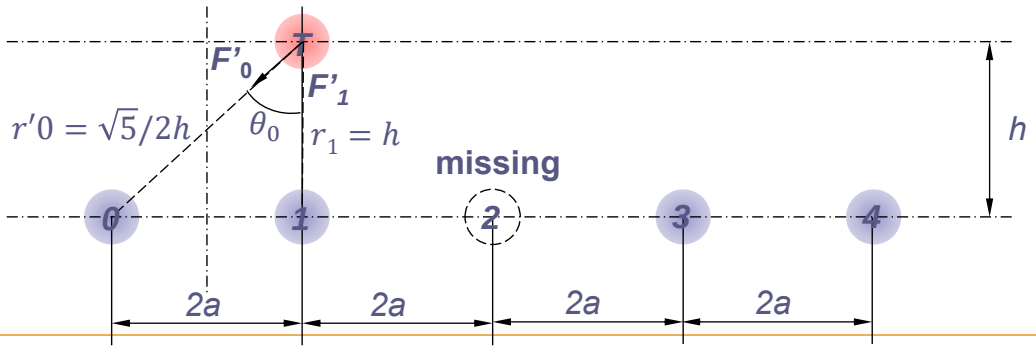
van der Waals Forces between a sliding atom and a chain of atoms

- $r'_0 = r'_1 = \sqrt{h^2 + (h/2)^2} = \sqrt{5}/2h$
- $F'_0 = F'_1 = (\frac{r'_0}{r_0})^{-7} \times F_0 = (\sqrt{5}/2)^{-7} \times F_0 = 0.4579 \times (-16.06)[pN] = -7.35[pN]$
  - $F'_{0,v} = F'_{1,v} = F_0 cos\theta_0 = -7.35 \times 2/\sqrt{5} = -6.58[pN]$
- F'_3, F'_4 (negligible)
- $F_{P1} = F'_{0,v} + F'_{1,v} = -13.16[pN]$



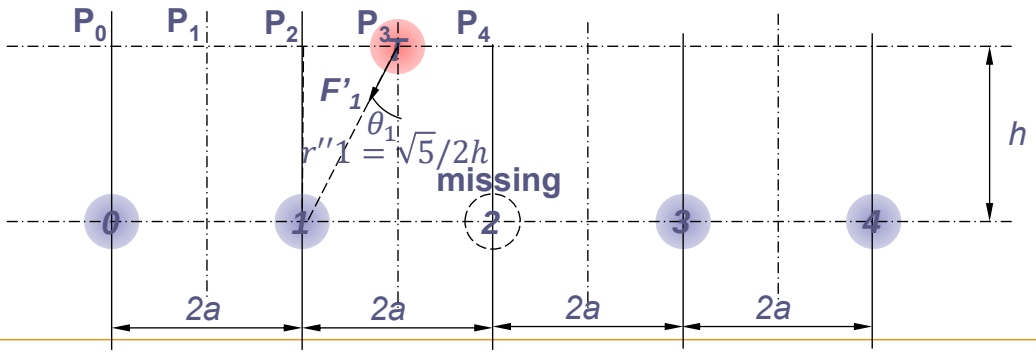
van der Waals Forces between a sliding atom and a chain of atoms

- Position P_2 –The same as P_1
- $F_{P2} = -17.06[pN]$



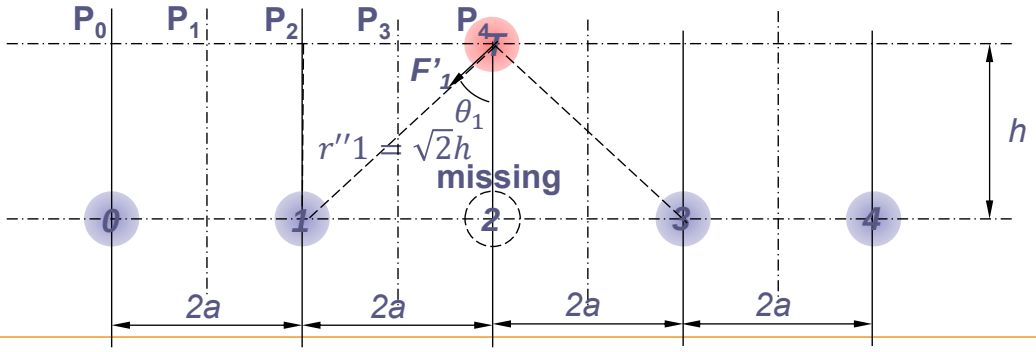
van der Waals Forces between a sliding atom and a chain of atoms

- Position P_3 –Similar to P_1 but the value is a half of that
- $F_{P3} = -6.58[pN]$



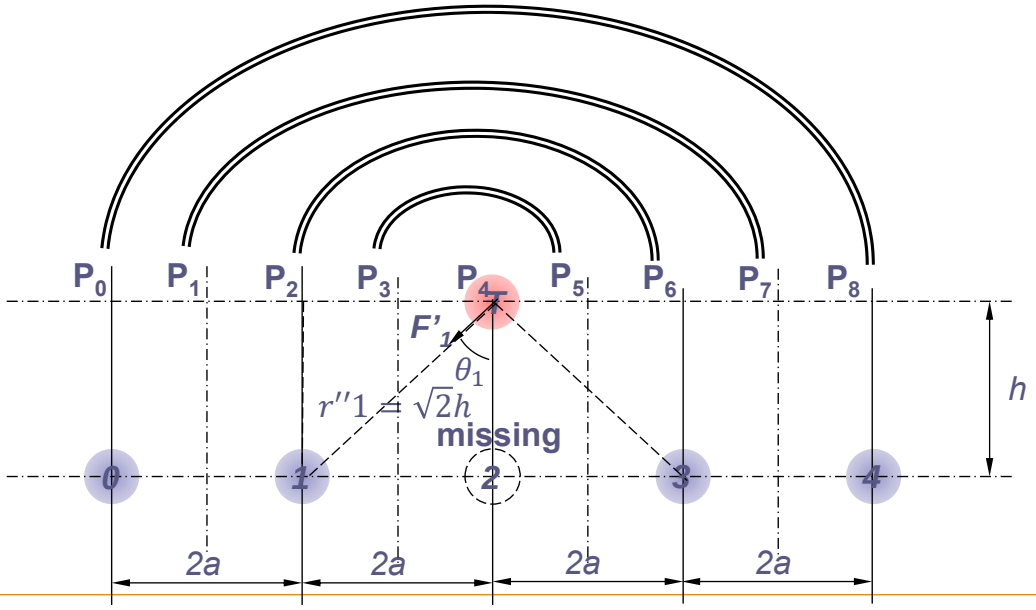
van der Waals Forces between a sliding atom and a chain of atoms

- Position P_4
- $F_{P4} = -2.00[pN]$

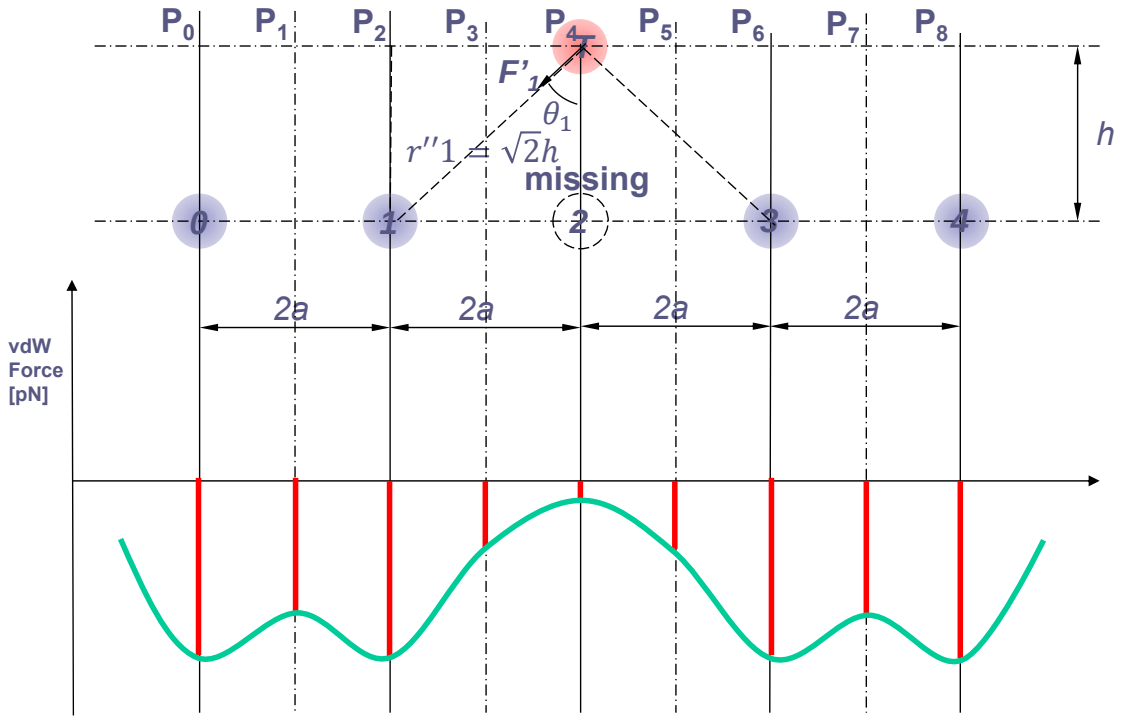


van der Waals Forces between a sliding atom and a chain of atoms

- Position P_4 –Similar to P_1 but the value is a half of that
- $F_{P3} = -2.00[pN]$

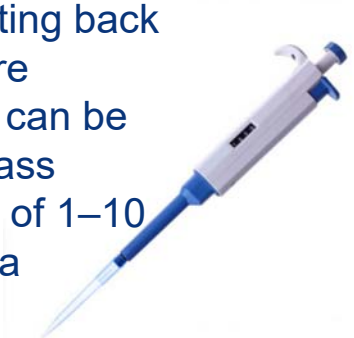


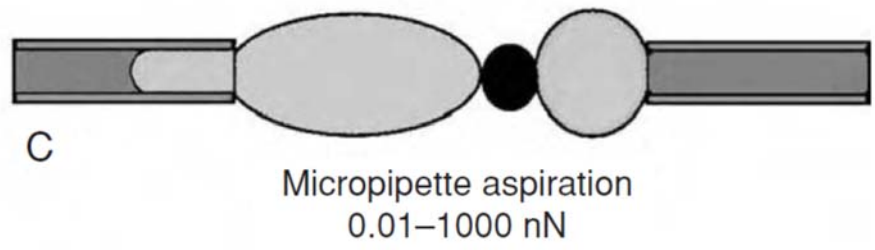
van der Waals Forces between a sliding atom and a chain of atoms



Micropipette aspiration

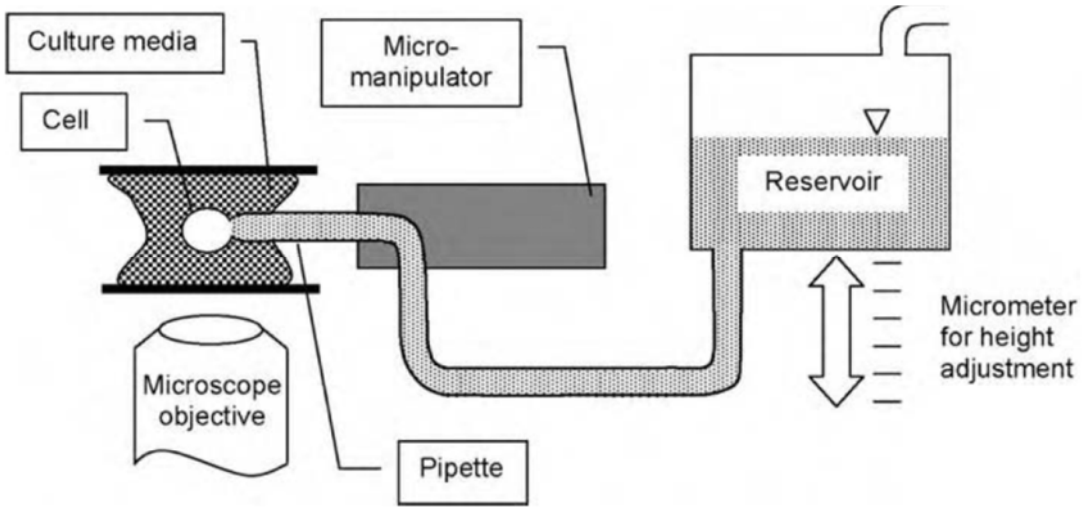
- The third methodology is micropipette aspiration. This is one of the oldest techniques for measuring cellular (and subcellular) biomechanical properties, dating back to the middle of the twentieth century. There are different ways in which micropipette aspiration can be used, but all approaches employ a very fine glass micropipette, typically having internal diameter of 1–10 μm and with a tip that can be moved about by a micromanipulator.





Micropipette aspiration
0.01–1000 nN

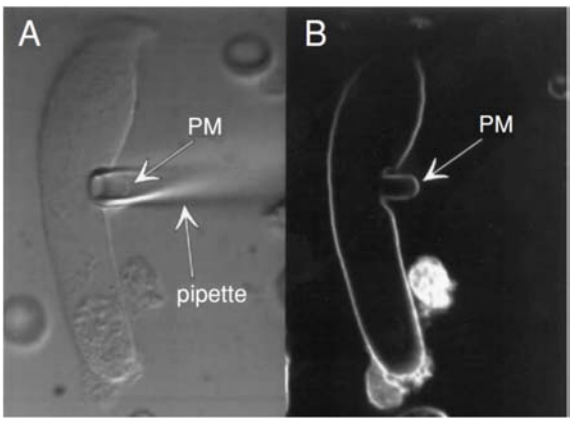
Micropipette aspiration



- Schematic overview of apparatus used for micropipette aspiration of a living cell (not to scale). Adapted from Shao and Hochmuth with kind permission of the authors and the Biophysical Society.

Micropipette aspiration

- In the simplest form of micropipette aspiration, the pipette tip is brought into contact with a cell and a small suction (aspiration) pressure is generated by the reservoir. Direct microscopic observation reveals the cell's deformation and motion in real-time as it is acted upon by the micropipette. From knowledge of the reservoir height and the pipette tip cross-sectional area, the force applied to the cell can be determined.

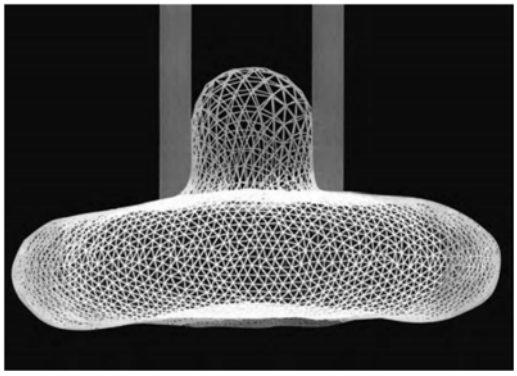


Micropipette aspiration

- This apparatus can generate forces ranging from 10 pN to about 10^4 nN, which is sufficient to cause appreciable cellular deformation. The smallest resolvable force in this technique depends on the precision with which the reservoir can be positioned; typical vertical positioning accuracy is several μm, which translates into a force of order 1–10 pN for a 10 μm diameter pipette.

Micropipette aspiration

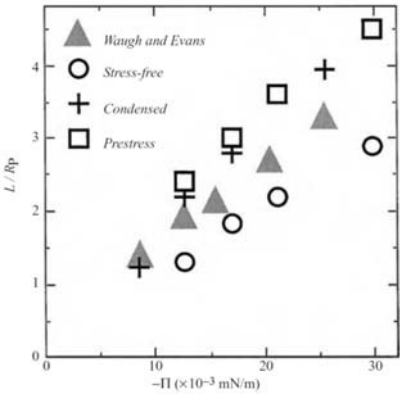
- When red cells are aspirated by a micropipette, a portion of the red cell is drawn inside the lumen of the pipette and is elongated.
- Simulation of a flaccid red cell being aspirated by a micropipette. The spectrin network within the cell is modeled as a network of non-Hookean springs, shown in this image as white segments. There are 6110 vertex nodes, each of which is connected to six neighbors by spectrin strands. From Discher *et al.* with kind permission of the authors and the Biophysical Society.



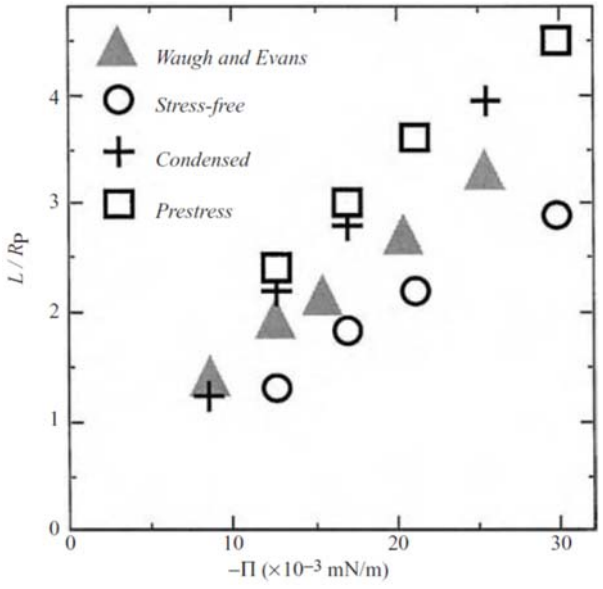
D. E. Discher, D. H. Boal and S. K. Boey. Simulations of the erythrocyte cytoskeleton at large deformation. II. Micropipette aspiration. *Biophysical Journal*, **75** (1998), 1584–1597.

Micropipette aspiration

- If the applied suction pressure is low enough to avoid red cell rupture, an equilibrium is established in which the applied suction pressure is balanced by mechanical stresses within the cortical cytoskeleton of the red cell. One measure of the cell's elastic properties is the distance that this aspirated segment extends into the pipette, *L*. Experimental measurements of this distance as a function of the applied pressure are shown in the figure, where we see that there is an approximately linear relationship between *L* and applied pressure, at least over the range of pressures plotted.



Micropipette aspiration



R. Waugh and E. A. Evans. Thermoelasticity of red blood cell membrane. *Biophysical Journal*, **26** (1979), 115–131.

- Length of the aspirated segment, *L*, as a function of aspiration pressure for micropipette aspiration of a flaccid human red cell. The aspirated segment length has been non-dimensionalized by inner pipette radius *R*p. The quantity Π is defined to be $2PR_p$, where *P* is the aspiration pressure (taken to be <0 for suction). This implies that Π values < 0 correspond to tension in the cortical cytoskeleton/membrane. The grey triangles are experimental data from Waugh and Evans.

Electrostatic Forces

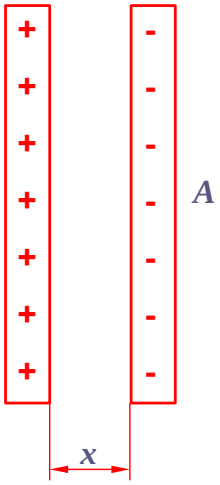
- Let us consider the electrostatic force between two parallel plates and examine how that force is affected by scaling. Let *A* denote the surface area of the plates, and let *x* be the separation distance. Assume *x* is small relative to the dimensions of the plates. The capacitance is given by

$$C = \varepsilon \frac{A}{x}$$

where *ε* is the permittivity of the dielectric material separating the plates. The capacitance relates a voltage *U* that is applied to the plates to the charge Q that is accumulated on each plate: Q = *CU*. The electrostatic co-energy stored in the capacitor can be expressed by

$$W = \frac{1}{2} C U^2$$

and the attractive force between the plates is computed as *F* = −*dW*/*dx*.



Electrostatic Forces

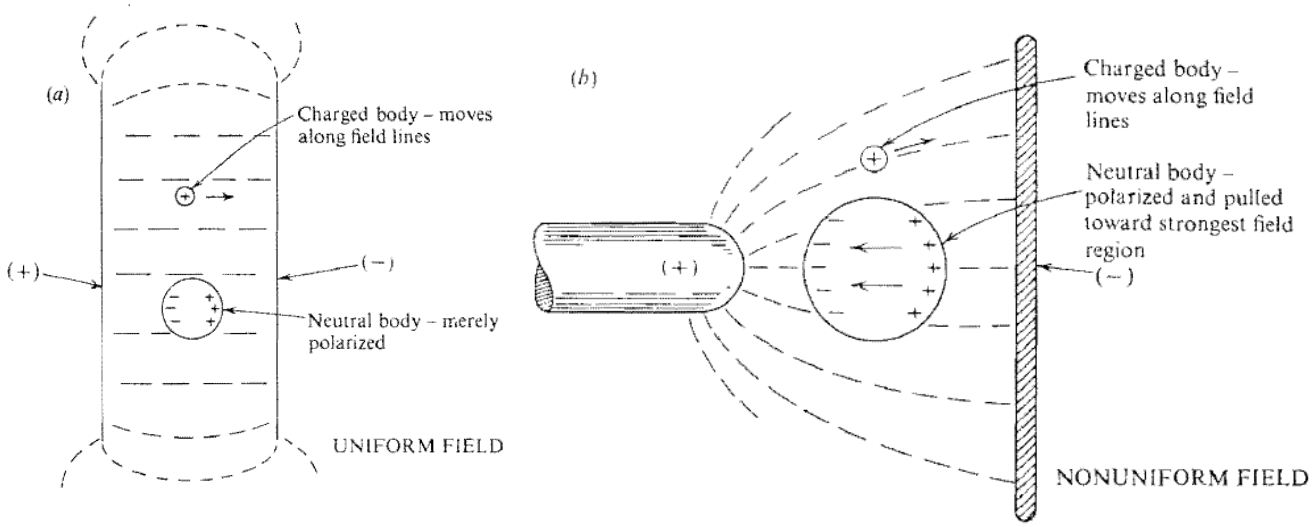
- In the case of constant voltage

$$W = \frac{\varepsilon A}{2x} U^2 \qquad F = -\frac{dW}{dx} \qquad F_U = \frac{\varepsilon A U^2}{2x^2}$$

- In the case of constant charge:

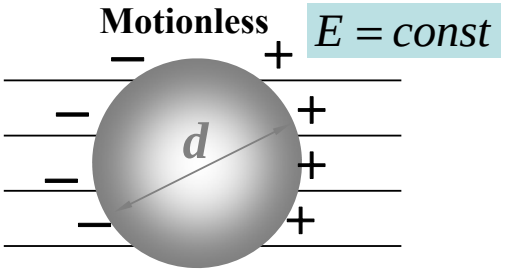
$$W = \frac{1}{2}\frac{Q^2}{C} = \frac{1}{2}\frac{Q^2 x}{\varepsilon A} \qquad F = -\frac{dW}{dx} \qquad F_Q = \frac{Q^2}{2\varepsilon A}$$

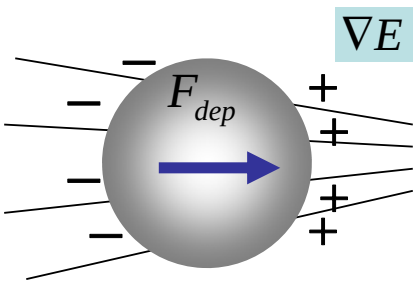
Dielectrophoretic (DEP) Forces



Dielectrophoretic (DEP) Forces

Forces subjected on neutral objects inside a nonuniform electric field





$$F_{dep} = \frac{1}{4}\pi d^3 \frac{\varepsilon_0(\varepsilon - \varepsilon_0)}{\varepsilon + 2\varepsilon_0}\nabla|\mathbf{E}|^2$$

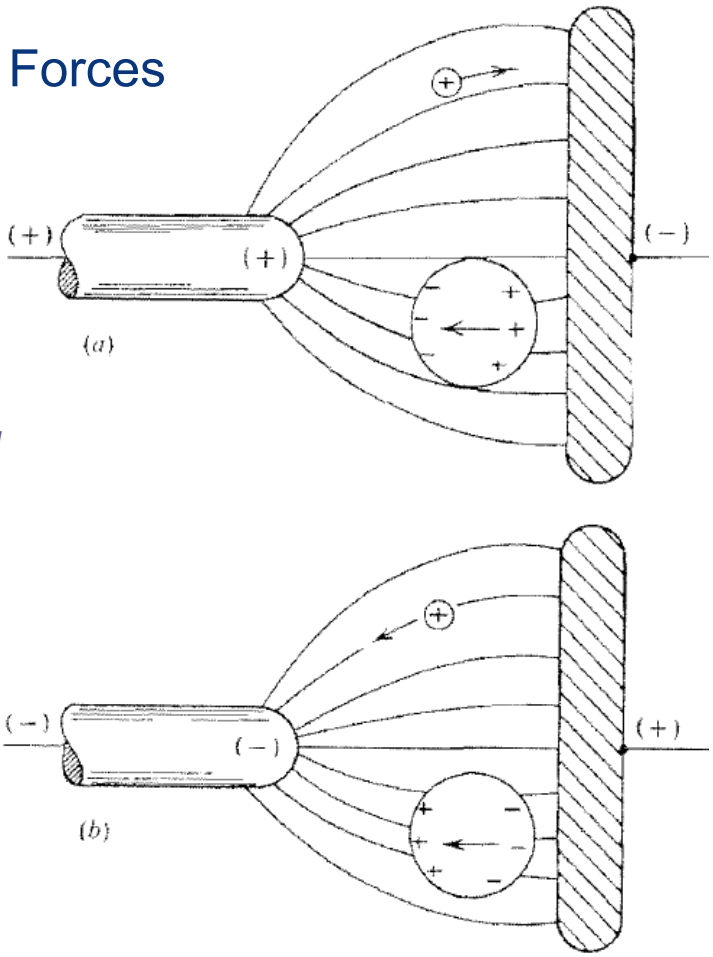
E: Electric field intensity

ε : Dielectric coefficient of object

ε_0 : Dielectric coefficient of medium

Dielectrophoretic (DEP) Forces

(a) Positively charged body moves towards negative electrode. Neutral body is polarized, then is attracted towards point where field is strongest. Since the two charge regions on the neutral body are equal in amount of charge, but the force is proportional to the local field, a net force towards the region of more intense field results.
(b) Positively charged body moves towards the negative electrode. Again, the neutral body is polarized, but does not reverse direction although the field is reversed. It still moves towards the region of **higbest field intensity.**



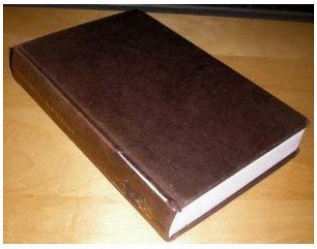
Dielectrophoresis (DEP)

Dielectrophoresis

The behavior of neutral matter in nonuniform electric fields

HERBERT A. POHL

Professor of Physics, Oklahoma State University, Stillwater, Oklahoma



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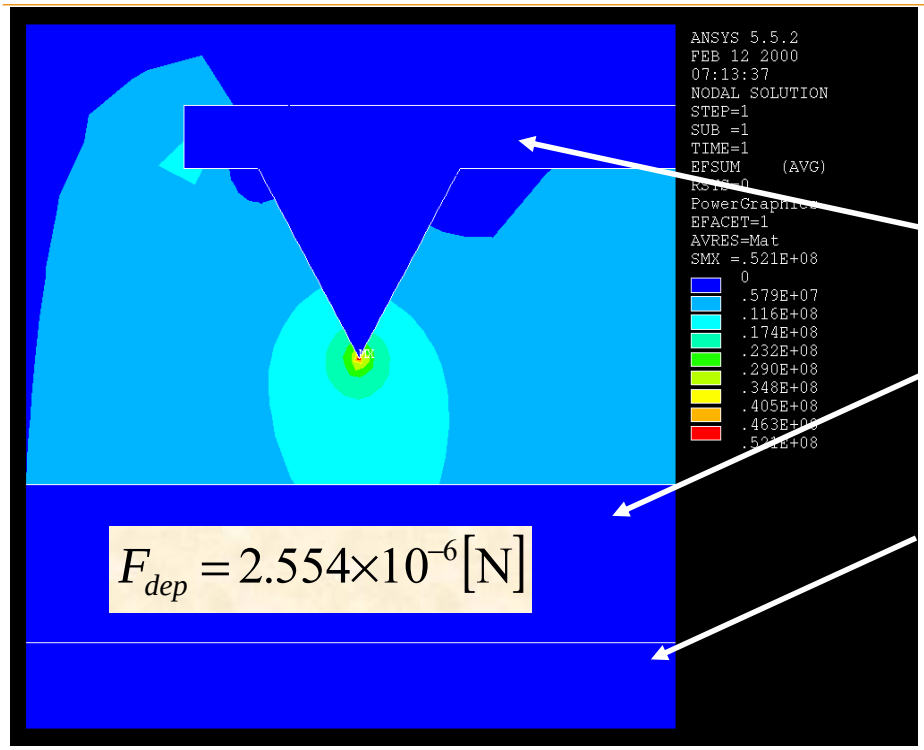
- The behavior of matter in electrical fields, especially nonuniform fields, is of interest to scientists of various branches: physics, chemistry, engineering, or life science.
- To chemists and physicists it is a science of many and varied phenomena.
- To engineers it is a source of new and useful techniques for separating materials or improving materials behavior.
- To ecologists it provides a major means of minimizing pollution.
- To life scientists it offers new ways to study and manipulate cells and their sub-particles, and to help unravel the nature of living systems. It constitutes a novel method of separating and distinguishing cells that differ in kind or physiological state.

Electric Field Analysis with FEM



Analytic Conditions

Vacuum
Permittivity:
8.85×10^{-12}[F/m]

Cantilever
Material: gold
Relative Permittivity:
∞

Insulate Film
Material:
Polyimide
Relative
Permittivity:2.2

Coating Film
Material: Al
Relative Permittivity: ∞

Voltage Applied
200 [V]

Magnetic Forces

- Consider the force between two identical magnets with magnetization *M and volume v,* aligned along their dipole axes, and separated by a distance *x. The field created by one magnet* along its axis is expressed using the point dipole model as

$$H(x) = \frac{M v}{2\pi x^3}$$

- The magnitude of the attractive/repulsive force on the other magnet is then given by

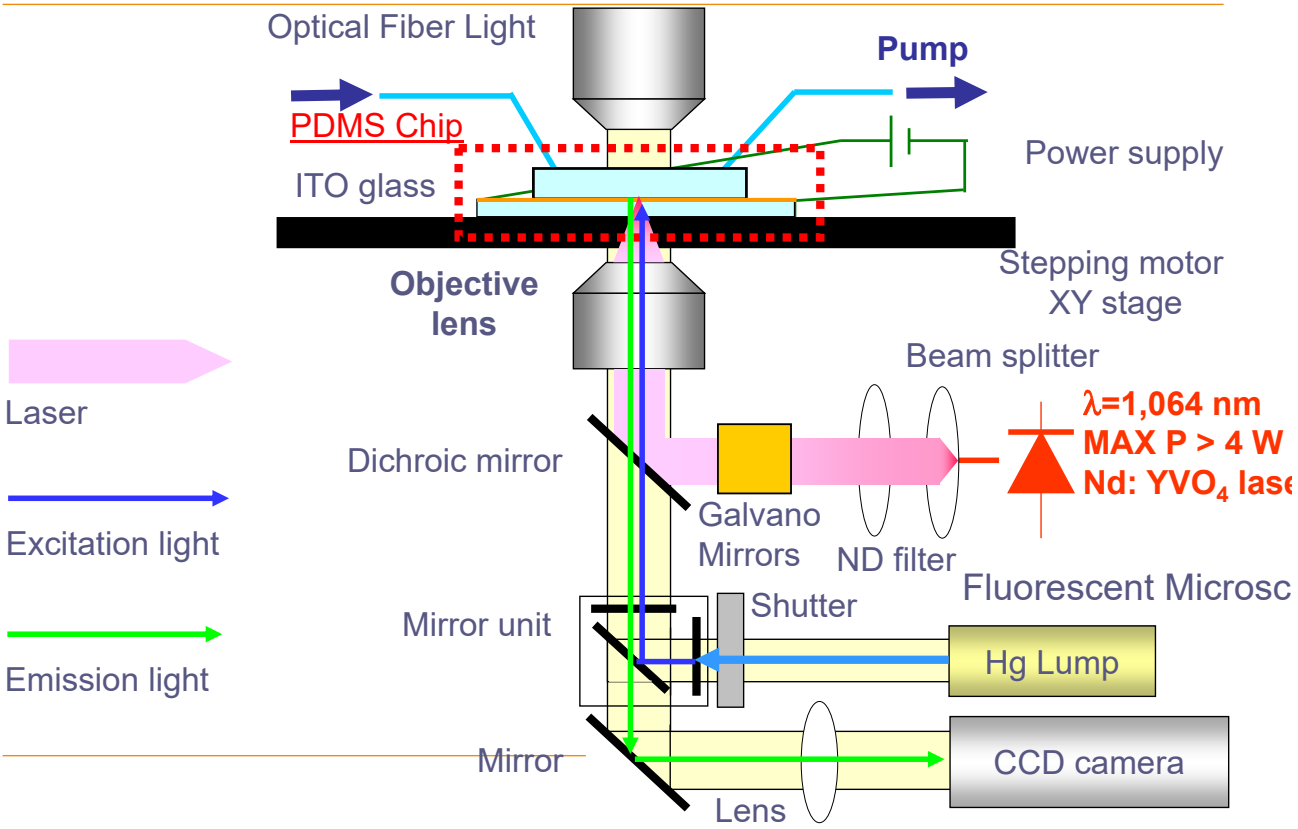
$$F_m = \mu_0 M v \left| \frac{\partial H}{\partial x} \right| = \frac{3\mu_0 M^2 v^2}{2\pi x^4}$$

where μ_0 is the permeability of free space. Note that the magnetization *M* remains constant for scaling as it is an intrinsic physical property of the magnets.

Optical trapping (“optical tweezers”)

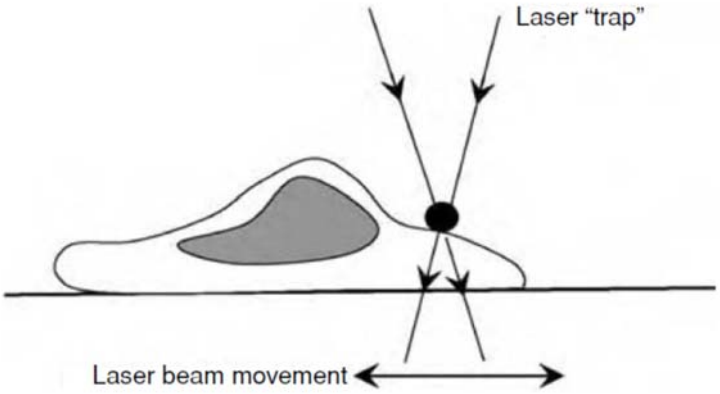
- Photons carry momentum; consequently when light shines on a surface there is an effective force exerted on that surface.
- Usually this effect is very small and can be neglected.
- However, for intense light shining on a small particle, forces in the range of 1 to 200 pN can be generated. An extension of this concept is to create a specially focused light beam that creates a potential “well” that traps a bead or small particle, typically 1–2 μm in diameter.

Configuration of System



Optical tweezers for biological measurements

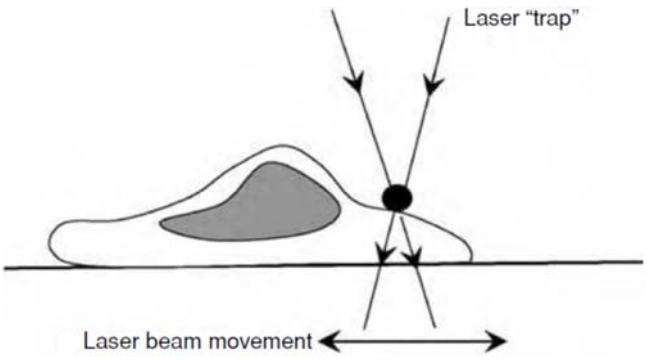
- This principle can be used for biological measurements by coating the bead with fibronectin (or some other molecule that will bind to receptors on the cell surface) so that the bead adheres to a given location on the cell. The beam is then moved laterally, and the motion of the bead is observed microscopically.



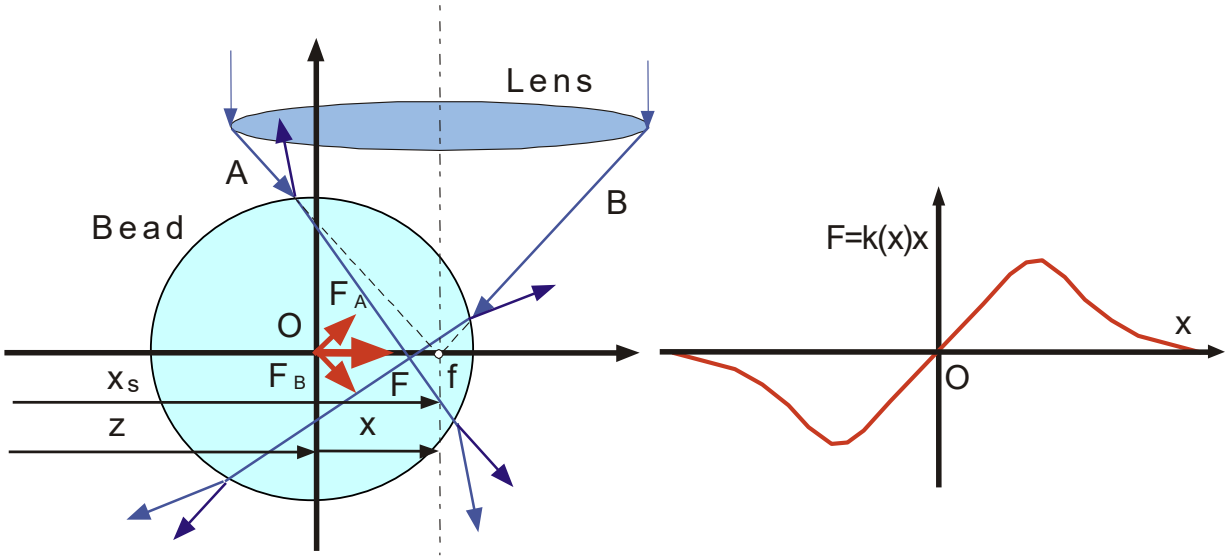
Optical tweezers for biological measurements

- From knowledge about the characteristics of the light trap, the force exerted on the bead by the moving light beam can be determined from the bead position relative to the center of the optical trap. Since the displacement of the bead is simultaneously monitored, this information can be used to determine the local stiffness of the cell. Essentially the same technique can be used to measure the force exerted by individual molecules.

Schematic description of the "optical tweezers" manipulating a bead that is attached to a cultured cell. Bead position is monitored by microscopy and so the relative position of the bead and the optical trap center are known.

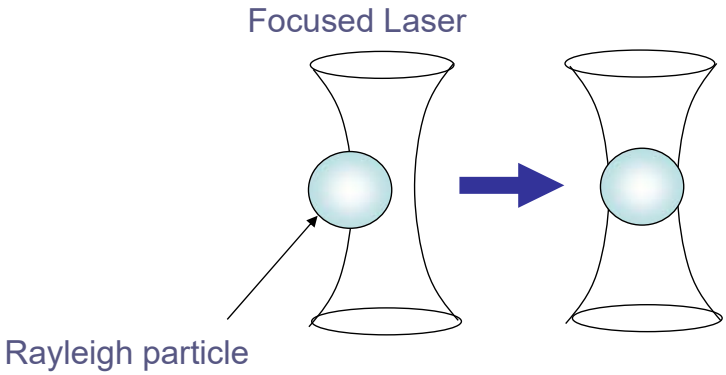


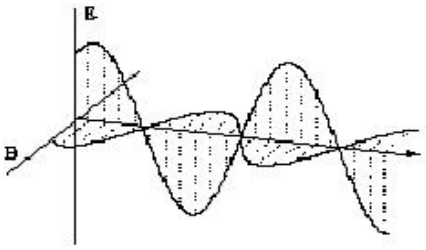
Laser Trap Model in Ray Optics Regime



Coordinate system **Optical trapping force image**

Laser Traps for High Dielectric Objects





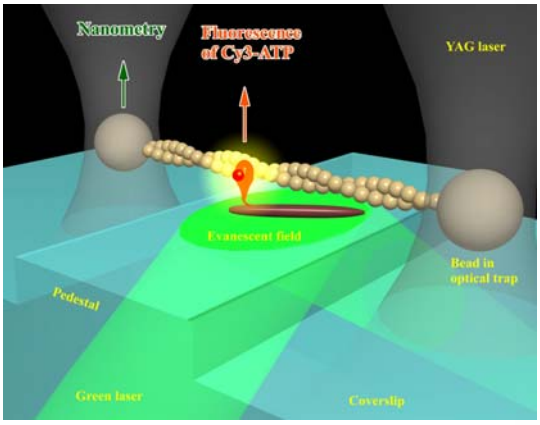
$$F = \alpha\left\{\frac{\nabla E^2}{2} + \frac{d(E\times B)}{dt}\right\}$$

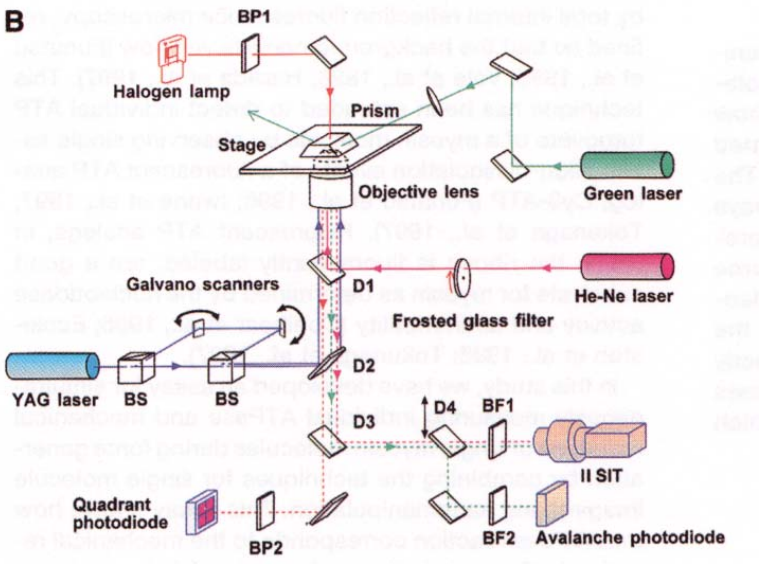
Gradient force **Scattering force**

Any object which has a higher dielectric constant than the surrounding material, which enters the optical trap, will become trapped.

Simultaneous Observation of Individual ATPase and Mechanical Events by a Single Myosin Molecule during Interaction with Actin

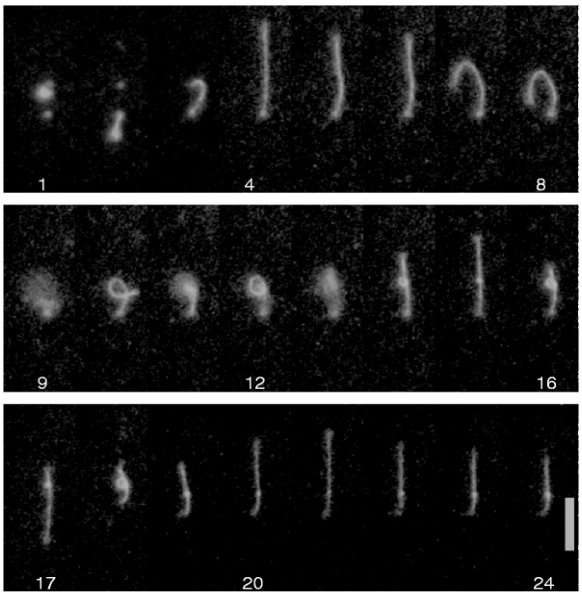
Akihiko Ishijima,*† Hiroaki Kojima,* Takashi Funatsu,* Makio Tokunaga,* Hideo Higuchi,* Hiroto Tanaka,‡ and Toshio Yanagida*‡§





A. Ishijima, Nagoya Univ.

Knotting a single DNA molecule

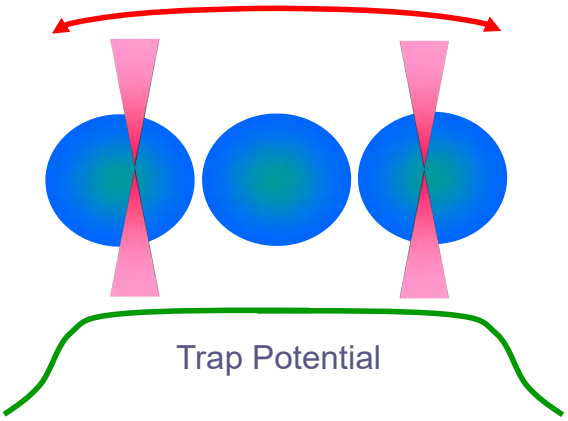


Knotting a single DNA molecule. Two beads were held with optical tweezers, and a DNA molecule stained with POPO-3 was attached to the lower bead by moving the microscope stage (images 1 and 2). The knot diameter in image 21 is estimated as <0.2 μm from its intensity. Scale bar, 10 μm

Nature 1999, Kinoshita et al.

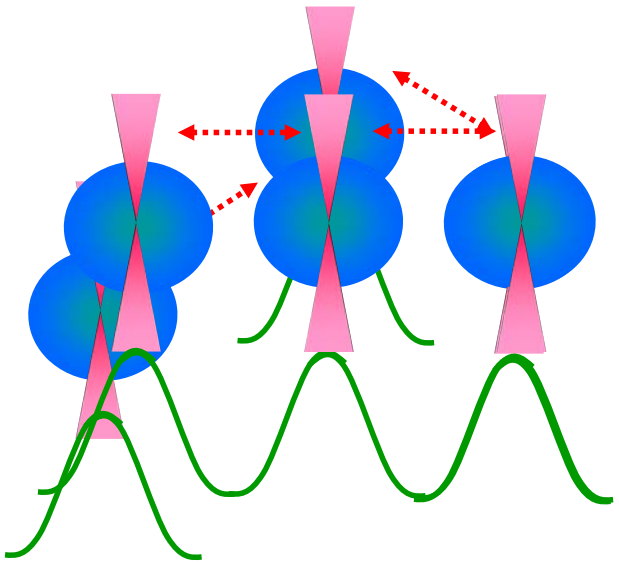
SLM for Trajectory Control of Multiple Objects

Laser Scanning Micromanipulation



Scanning Laser Micromanipulation
Masuhara et al., 1991

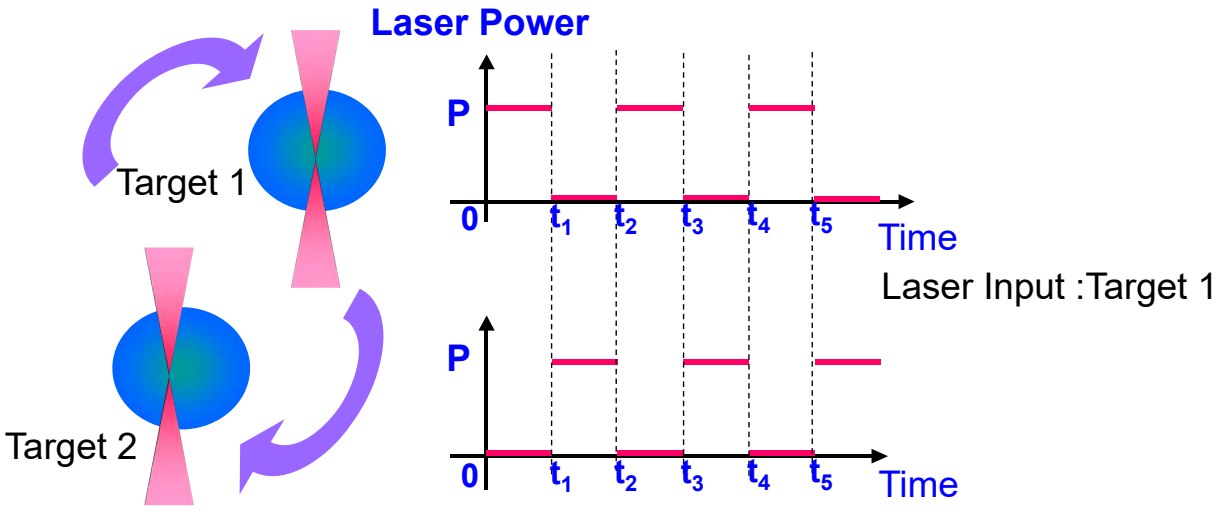
Synchronized Laser Micromanipulation



A single laser scanned in high speed

Synchronized Laser Micromanipulation: SLM

Trajectory control of multiple targets by a single laser



High Speed Scanning Laser

Discontinuous laser trap pattern

Dancing with Cells

- Robot hand
  - Laser
- Cells
  - 5µm

ICRA2003 Video Proceedings
6 Dancing Yeast Cells