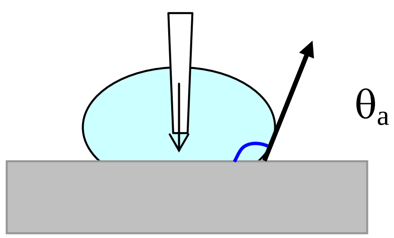
Lecture 12:
Biomaterials Characterization in Aqueous Environments

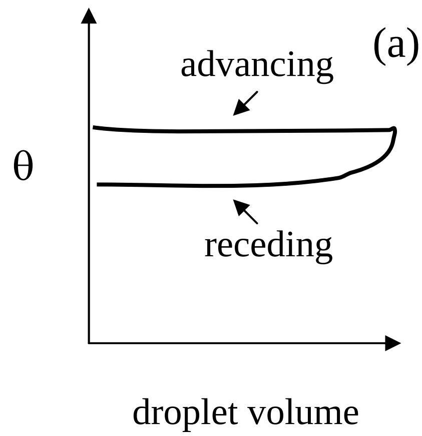
High vacuum techniques are important tools for characterizing surface composition, but do not yield information on surface structure or chemistry in a water-based environment.

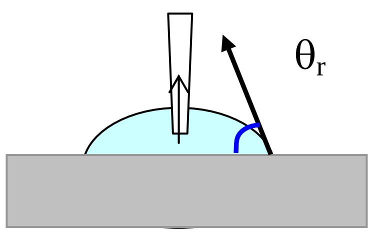
Aqueous-based methods for surface characterization are limited. Here we will consider three common techniques:

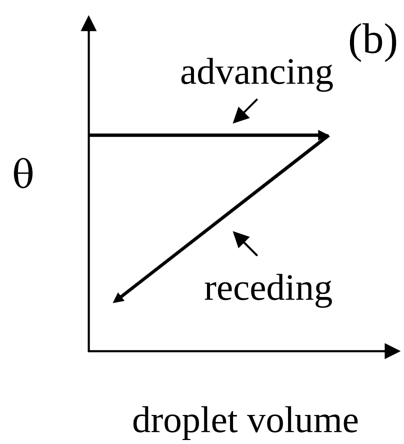
1. water contact angle studies

- surface reconstruction (a)
- water absorption (b)
- surface chemistry analysis









$$\cos\theta = f_1 \cos\theta_1 + f_2 \cos\theta_2$$

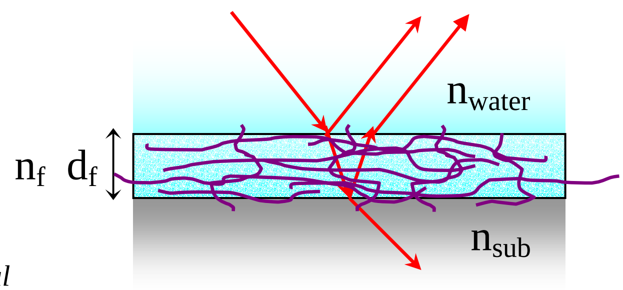
Cassie's eqn: use to determine fraction of surface area of components 1 & 2 ($f_1 + f_2 = 1$)

2. *in situ* ellipsometry

- degree of hydration of a film

Ellipsometric angles Ψ and Δ

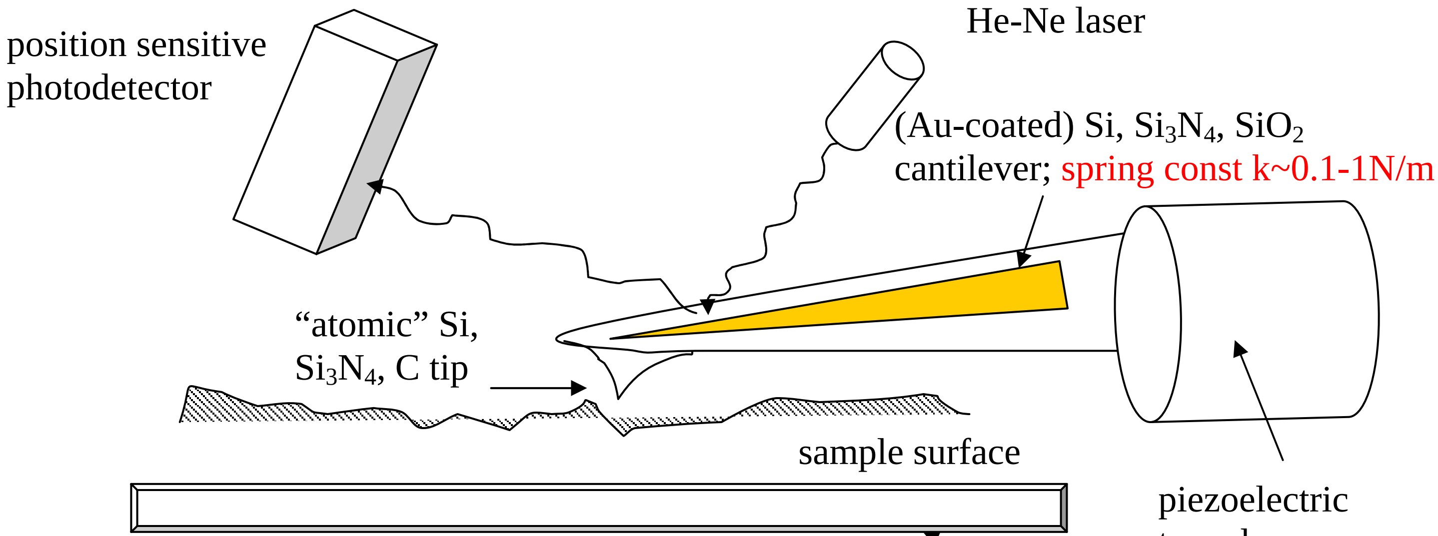
⇒ thickness (d_f) & refractive index (n_f) (3-layer model)



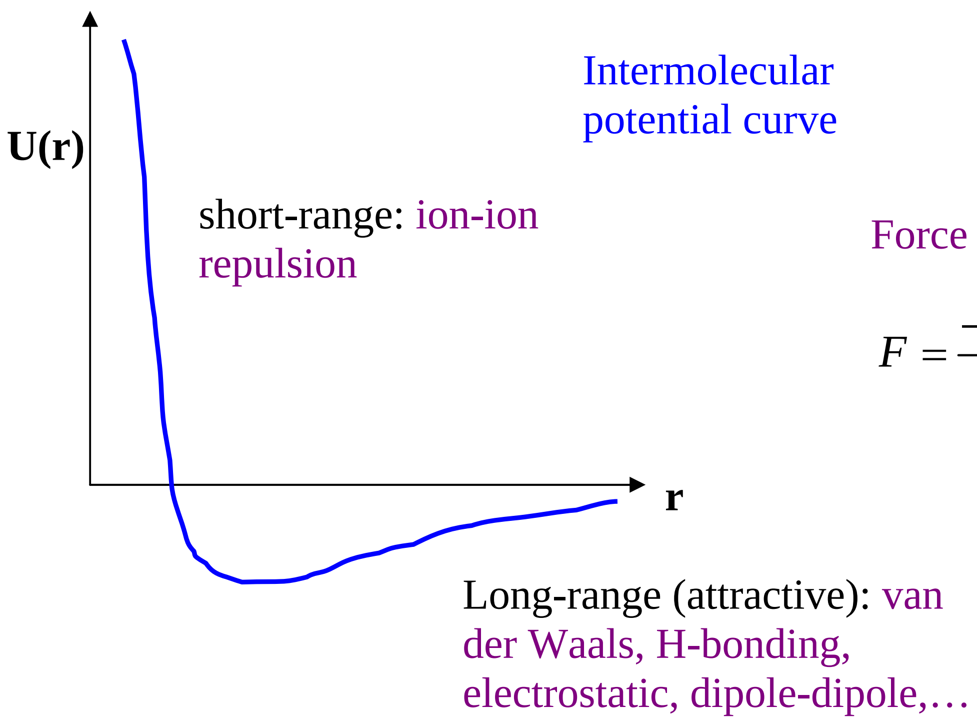
$$n_f = f_{water} n_{water} + f_{material} n_{material}$$

where f_{water} and $f_{material}$ are volume fractions.

3. Atomic Force Microscopy (or Surface Force Microscopy): imaging method that exploits intermolecular interactions between a small (~atomic) probe and molecules on surface



Scan area: 1×1 nm^2 to 250×250 μm^2
z-range: 8 μm
force range: 10^{-13}-10^{-6}N

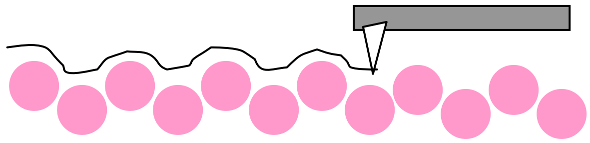


Force generated:

$$F = \frac{-\Delta U}{\Delta z}$$

Operation Modes

1. Contact mode (short-range)



- Tiny cantilever deflections detected by photodiode array

- Tip rastered over sample surface at fixed force (via photodetector-z-piezo feedback loop) generates topographical image
  ⇒ analogous to stylus on a record player

- Good for hard samples; *can drag soft materials!*

force applied: nN
x-y resolution: 1Å
z resolution: < 1Å

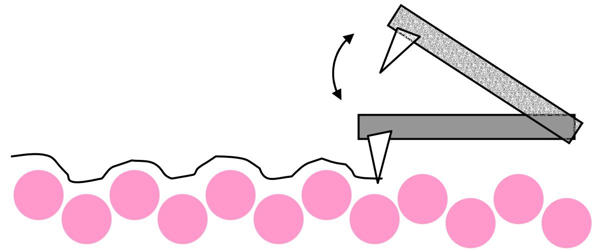
Photo removed for copyright reasons.

Contact mode images of TiO_2 (rutile) film surface

- No contrast at low resolution—flat surface

- High resolution—atoms of (001) plane are revealed

Figure 10 (a) and (b) from K.D. Jandt, *Surf. Sci.* **491** (2001) 303.



2. "Tapping" mode

- Tip oscillates in z-axis at high ω (~50-500 kHz in air, 10 kHz in fluids) with intermittent sample contact ⇒ eliminates shear forces

- Interactions between tip and sample cause amplitude attenuation (driven amplitude ~ 10 nm)

- Cantilever deflections used in feedback loop to maintain average applied force similar to contact mode

  oscillatory amplitude attenuation ⇒ "height" data

- Commonly used for soft samples, aqueous environments

x-y resolution: 1-2 nm

Tapping mode images in air (left) and water (below) of laminin (Ln-1) adsorbed onto mica.

- Cruciform molecular shape
- "Arms" can bend and fold

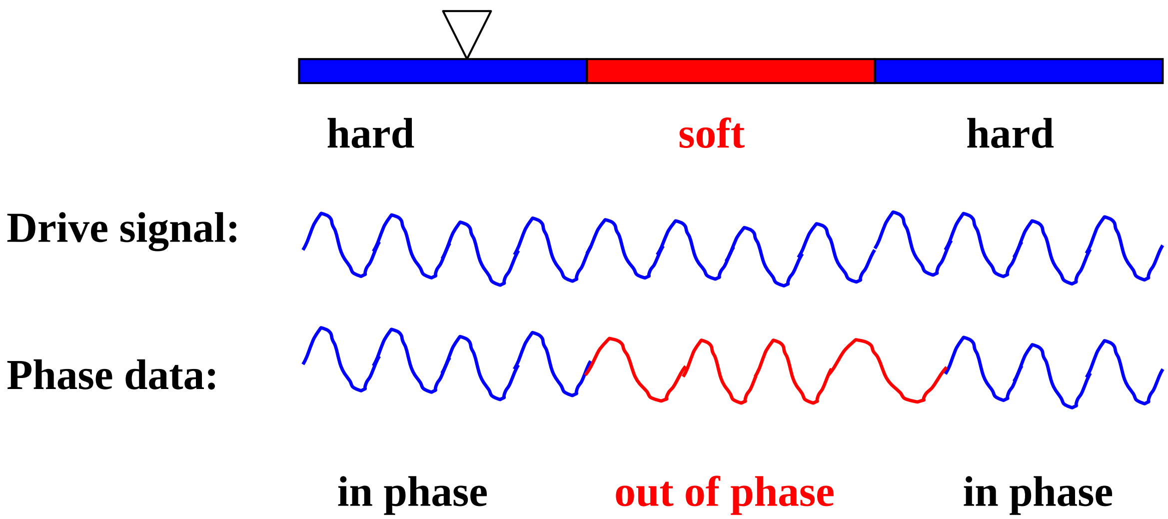
Photos removed for copyright reasons.

Figures 1 and 4 from C.H. Chen, D.O. Clegg & H.G. Hansma, *Biochemistry* **37**, 1998, 8262.

Phase imaging (in conjunction with tapping mode)

- Tip oscillated in z-axis, making intermittent sample contact
- Simultaneous measurement of amplitude attenuation & phase lag of cantilever signal vs. signal sent by piezo-driver

oscillation amplitude attenuation ⇒ "height" data
oscillation phase-shift ⇒ "elasticity" map



AFM image of polystyrene-*b*-poly(lauryl methacrylate) block copolymer film.

Height data: variation in film thickness seen at polymer droplet edge

Figure removed for copyright reasons.

Phase data: microdomains of soft PLMA block (Tg~-35C) and hard PS block (Tg~100C) are distinguished

Figure 6 from M.J. Fasolka et al., *Macromolecules* **33**, 2000, 5702.

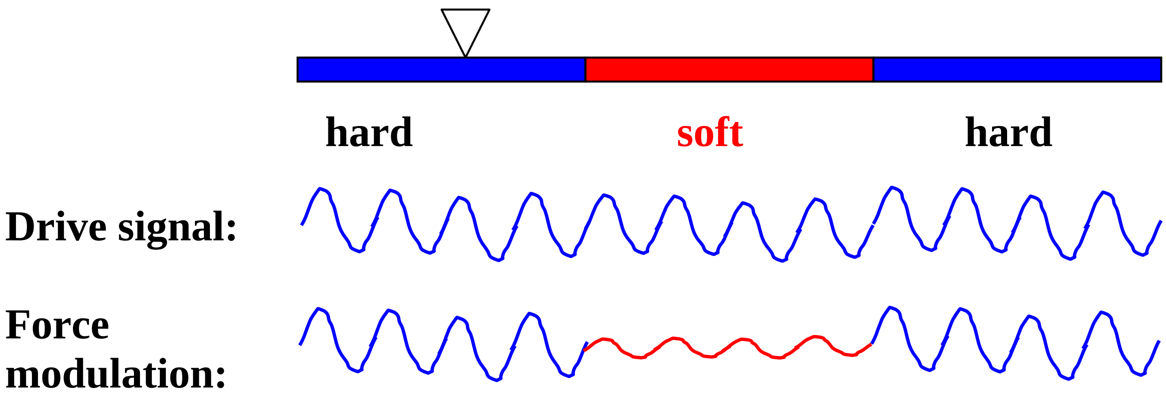
3. Force modulation mode

- Tip oscillates in z-axis at $\omega < \omega_o = (k/m)^{1/2}$ (cantilever resonance frequency), making intermittent sample contact; ω ~3-120kHz.

- Interactions between tip and sample cause amplitude attenuation

- Contact force applied to sample is modulated, giving elasticity information

  cantilever deflection amplitude ⇒ "elasticity" map



4. Non-contact AFM

- Oscillation near resonance frequency *without* tip-surface contact (long-range forces in U(r) curve; r > 0.6 nm, typical F <1 pN)

- Force gradients from surface interactions shift resonance frequency

$$\omega - \omega_o = \frac{-dF}{dz}\frac{1}{2k}$$

dF/dz >0 ⇒ attractive force
dF/dz <0 ⇒ repulsive force

- Force gradients used to map secondary interactions
  (difficult in fluids due to damping; good for soft samples)

resolution: dF/dz ~ 10 μN/m
(0.1 pN at a gap of 1 nm)

5. Force-Distance Profiles

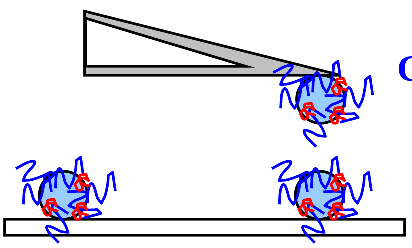
- As sample is brought towards probe tip, measured force: $\Delta F = k\Delta z_c$ (Δz_c = cantilever deflection)

D > 10 nm hydrophobic interactions, electrostatic interactions, steric repulsion of polymer "brush" layer

D < 10 nm van der Waals attraction

- Obtain F(z) of species w/ surface by coating tip with receptor, antibody, ligand, colloid, cell, etc.

Colloidal Particle Force Spectroscopy



Grafted hydrophilic chains
$(EO)_{22}$ ⇒ "cloaking"

pure repulsion

NOTE: ↑ tip size = loss of x-y imaging resolution

Mixed grafted chains
$(EO)_{22}$ ⇒ "cloaking"
$C_{16}H_{37}$ ⇒ "binding"

repulsion-attraction-repulsion

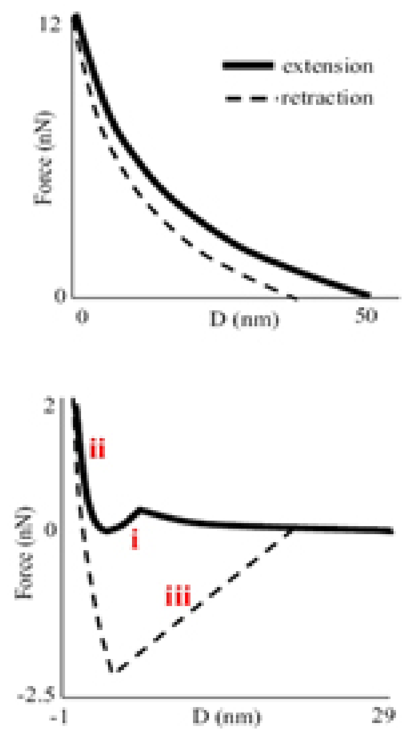


Figure by MIT OCW.

- jump to contact seen for $\frac{-U(D)}{D} > kD$ (i)

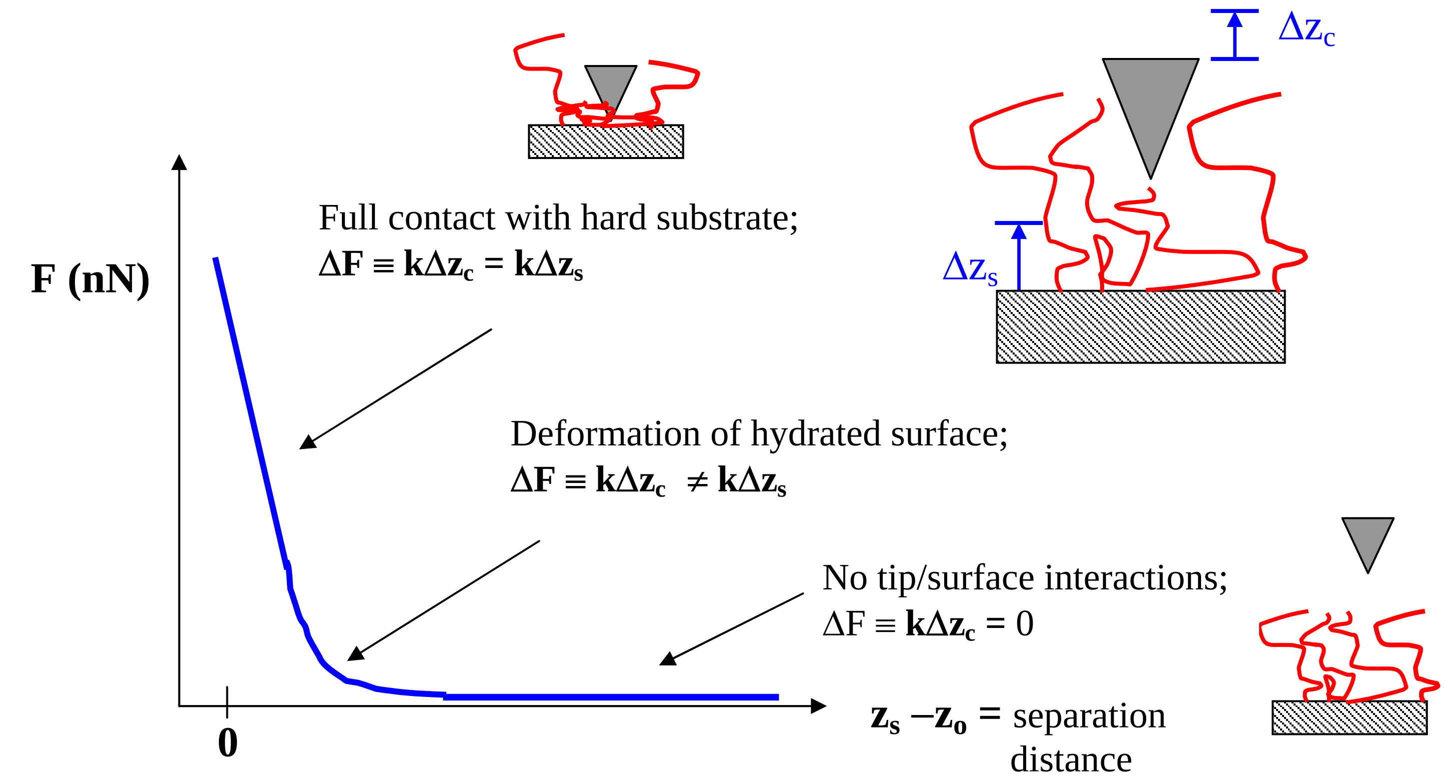
- further approach bends cantilever (ii)

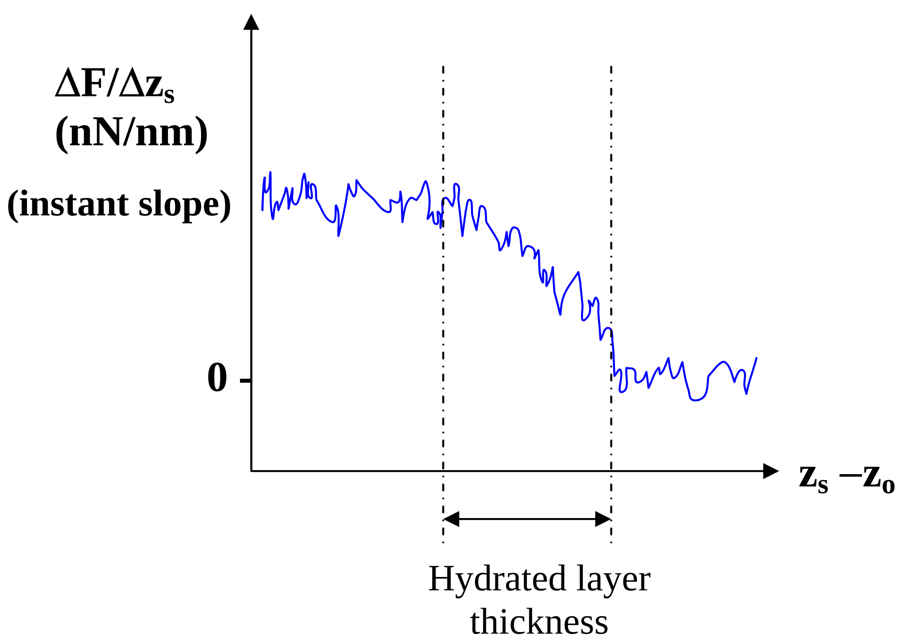
- on retraction, tip "sticks" from adhesion forces (iii)

after S.C. Olugebefola et al., *Langmuir* **18**, 2002, 1098.

- Measure height of hydrated surface layer via nonlinear regimes

Sample height interval: $\Delta z_s = z_{s,j} - z_{s,j-1}$
Force increment from cantilever deflection: $\Delta F \equiv k\Delta z_c$
Sample deformation: $\Delta z_s - \Delta z_c$





<u>Biomaterials-relevant SFM/AFM Studies</u>

- protein adsorption
- cell membrane integral proteins
- initiation of clot formation
- hydrated surface layers
- chemical mapping
- ligand-receptor interactions
- cell adhesion
- surface charge mapping
- surface topography
- surface elasticity
- protein structure (single chain expts)…

References

C.A. Siedlecki and R.E. Marchant, "AFM for characterization of the biomaterial interface", *Biomaterials* **19** (1998), 441-454.

K.D. Jandt, "Atomic force microscopy of biomaterials surfaces and interfaces", *Surface Science* **491** (2001), 303-332.

S. Kidoaki and T. Matsuda, "Mechanistic aspects of protein/material interactions probed by atomic force microscopy", *Colloids and Surfaces B: Biointerfaces* **23** (2002) 153-163.