Surfaces of Biomaterials

<u>Three lectures:</u>

1.23.05 – Surface Properties of Biomaterials

1.25.05 – Surface Characterization

1.27.05 – Surface and Protein Interactions

<u>Three points:</u>

1 – Surfaces have unique properties

2 – We can (and do) measure these properties

3 – Because they affect biocompatibility

Review

Bulk Materials are described by:

- Chemical / Molecular composition
- Atomic / Molecular structure (crystallinity, etc)
- Mechanics (elasticity, etc)
- Shape

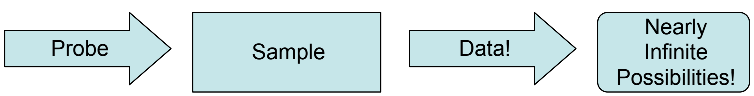
Surfaces of materials have unique descriptive properties:

- Excess surface free energy
- Atomic / Molecular composition different than bulk
- Chemical composition (reactivity) different than bulk
- Topography (vs. shape)

Surface characterization provides surface specific information about these properties.

Characterization

It's simple:



Characterization is the method by which one develops a data set that describes properties of the sample. Because of limited possibilities this process is:

- Discrete / Reliant on methodology
- Application specific
- Often material specific
- Resource limited

Surface sensitivity

The further your technique returns info from the sample, the less sensitive it is to the surface. This is usually related to the penetration depth of the probe.

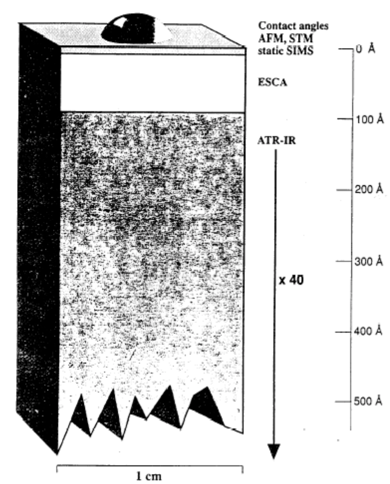
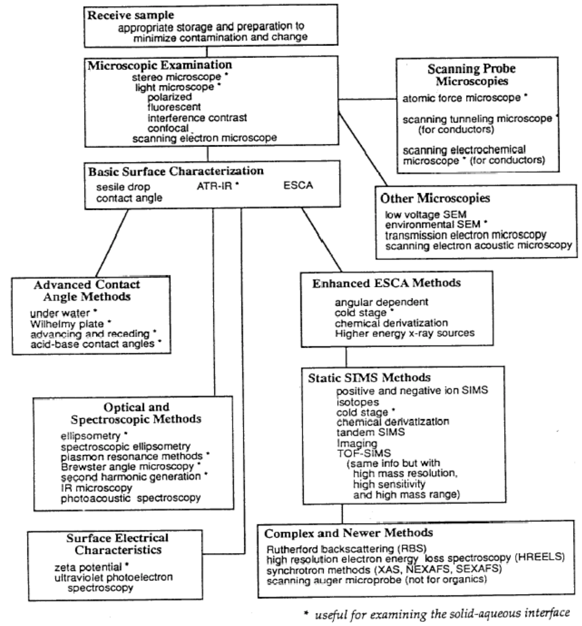


Figure 2. The major surface analysis methods probe to different depths. Contact angles, scanning tunneling microscopy, atomic force microscopy, and static secondary ion mass spectrometry are extremely surface localized.

Surface Analysis Techniques for Biomaterials

- Contact Angle Measurements, Dynamic Contact Angle (DCA)
- Electron Spectroscopy for Chemical Analysis (ESCA / XPS)
- Auger Electron Spectroscopy (AES)
- Near Edge X-ray Absorption Fine Structure (NEXAFS)
- Secondary Ion Mass Spectroscopy (SIMS)
- Scanning Probe Microscopy (AFM)
- Sum Frequency Generation (SFG)
- Surface Plasmon Resonance (SPR)
- Optical Imaging and Spectroscopy (microscopy, TIRF)
- Ellipsometry
- Scanning Electron Microscopy (SEM)
- Infrared Spectroscopy (FTIR)
- Many more techniques and more acronyms...

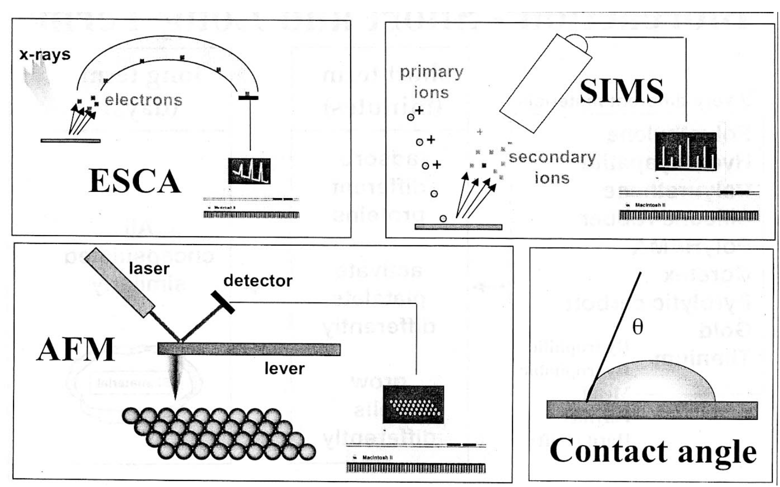


Biomaterials surface analysis flow chart

Surface Information

Property	Techniques
Composition	ESCA, Auger, SIMS, NEXAFS
Structure	SIMS, ESCA, NEXAFS, FTIR, SFG
Orientation	NEXAFS, FTIR, SFG
Spatial Distribution	Imaging SIMS, AFM, microscopy
Topography	AFM, profilometry
Thickness	ellipsometry, ESCA, AFM, SPR
Energetics	Contact angle

The Basic Repertoire



Contact angle methods

Modes:	Sessile drop, captive bubble, Wilhelmy plate, dynamic
Probe:	Small Drop of Liquid or Bubble
Data:	Contact Angle (θ)
Sample:	Any material interface that can support the probe
Principle: solid	Interfacial tension can be used to estimate surface energetics
Information:	Work of adhesion, Surface energetics
Depth:	Å's
Spatial Resolution:	mm^2
Sensitivity:	Depends on chemistry
Relative Cost:	Inexpensive
Other:	Similar techniques can be used for liquids
	Can be used to estimate solid surface energies

Instrument

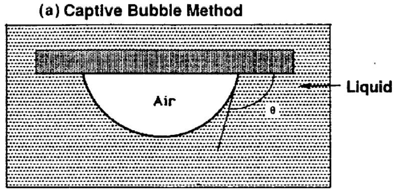
Force balance (equilibrium) method

Easy – Place a drop or bubble on a rigid surface and measure the geometry. The "contact angle" θ can be related to surface tension with the Young-Dupré equation:

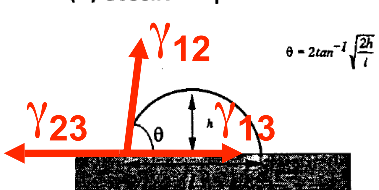
$$\gamma_{23} = \gamma_{12} \cdot \cos\theta_1 + \gamma_{13}$$

Slightly more complicated if angle is not measured directly.

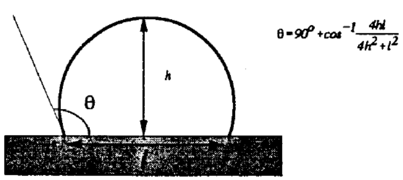
(a) Captive Bubble Method



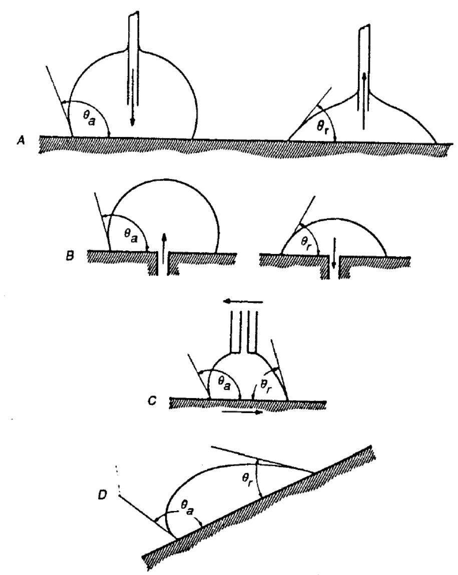
(b) Sessile Drop for θ < 90°



(c) Sessile Drop for θ > 90°



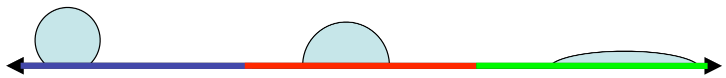
Dynamic method



If one observes "hysteresis" or a difference in θ_a vs. θ_r then it is likely that the surface is dynamically rearranging in response to interaction with the probe

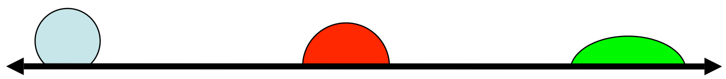
Θ indicates "wettability"

For water (γ_{LV} = 72.8 mN/m) wettability is inverse of hydrophobicity and it varies with material:



102º (Teflon, PTFE) | 72º (Mylar, PET) | ~5º (Glass)

One can also vary the probe, for example if Teflon is used:



102º (Water) | 71º (Methylene iodide) | 25º (Decane)

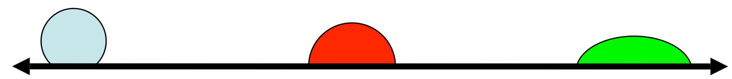
Surface energy of a solid

Remember that the surface tension of solids is not experimentally accessible.

One can use the multiple probe approach to estimate the surface energies of solids.

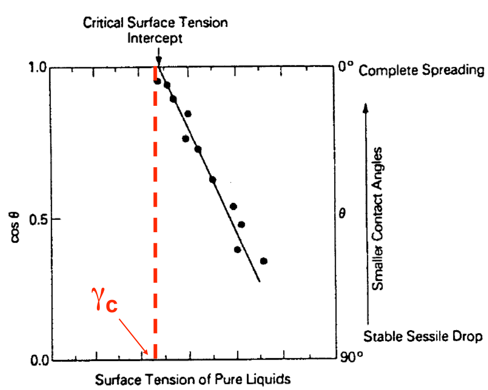
There are two prominent methods:

- Critical surface tension (γ_c) method (Zisman method)
- "Independent Surface Action" (Fowkes, Good et al. method)



Zisman's method

High surface energy liquids will not spread on low surface energy solids as this will not lower their excess surface free energy. Too bad, because a liquid that completely wets the surface could give you an estimate of the solid's surface tension. Fortunately, one can extrapolate.....:



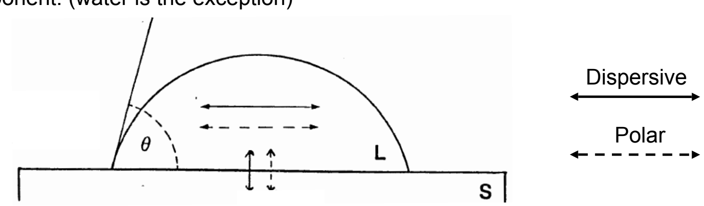
Possible if cos(θ) is a monotonic function of γ_L. The "critical surface tension" γ_c is a useful measure of the surface tension of the solid (maybe).

This only works well if the interaction between the probe liquids and the surface is dominantly *dispersive (non-polar)*.

Fowkes' method

Solid – liquid interaction can be considered to be due to addition of non-polar (dispersive or LW) and polar (Lewis acid-base, - and +) interactions between the probe and solid. These components of surface energy are known for a series of liquids – so use three probes, find θ's and solve the equation below for γ_S^{LW}, γ_S^{+} and γ_S^{-}.

A limitation of this technique is that most polar liquids do not have a strong γ_S^{+} component. (water is the exception)

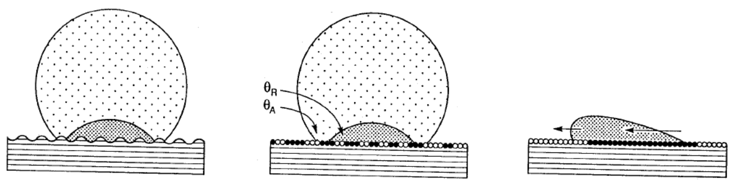


$$(1 + \cos\theta)\,\gamma_L^{TOT} = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{\oplus}\gamma_L^{\ominus}} + \sqrt{\gamma_S^{\ominus}\gamma_L^{\oplus}}\right)$$

Limitation of contact angles

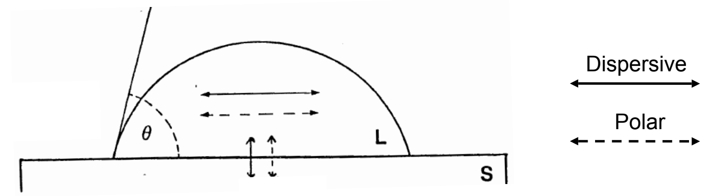
Measurement is highly operator dependant, affected by contamination, affected by surface roughness heterogeneity and rearrangements, and there are limited sample geometries.

Biggest problem – so inexpensive and easy it is often done poorly and misinterpreted.



Surface Roughness | Heterogeneity | Rearrangments

Intermission: CA lab



$$(1 + \cos\theta)\,\gamma_L^{TOT} = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{\oplus}\gamma_L^{\ominus}} + \sqrt{\gamma_S^{\ominus}\gamma_L^{\oplus}}\right)$$

= non-polar work + polar work of adhesion

$= W_{LW} + W_p$

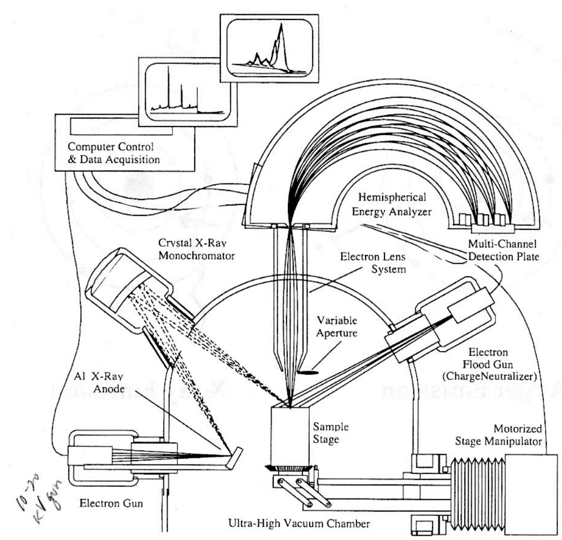
Plasma treatment of materials? What does it do to material's surface?

$W_{\text{(increased polarity)}} = W_{\text{(after plasma treatment)}} - W_{\text{(before plasma treatment)}}$

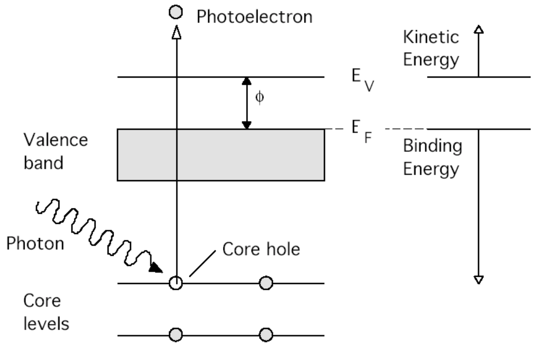
Photoelectric Techniques

Alias:	ESCA (Electron Spectroscopy for Chemical Analysis)
Modes:	XPS (X-ray photoelectron spectroscopy), Auger Spectroscopy, UPS (UV photoelectron spectroscopy)
Probe:	Photons (X-rays, UV)
Signal:	Electrons
Information:	Elemental composition and chem. bonding
Sample:	Any that can withstand ultra-high vacuum
Principle:	Photoelectric effect (think Einstein)
Depth:	100 Å (1000+ Å in destructive mode)
Spatial Resolution:	μm^2
Sensitivity:	few % error not unreasonable
Relative Cost:	Very expensive
Other:	Semi-quantitative to quantitative
	NEXAFS is an extension of this technique that gives orientation information
	Imaging modes

XPS instrument



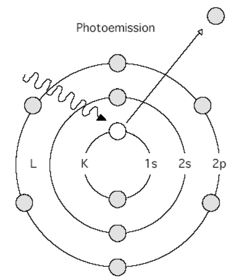
The photoemission process

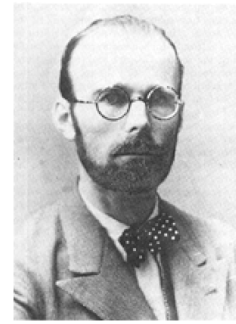


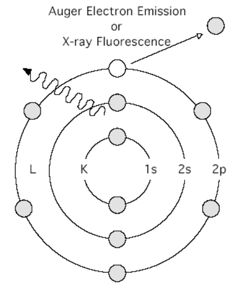
$$KE = h\nu - BE - \phi$$

Auger... Pierre Auger

What is fate of core hole?







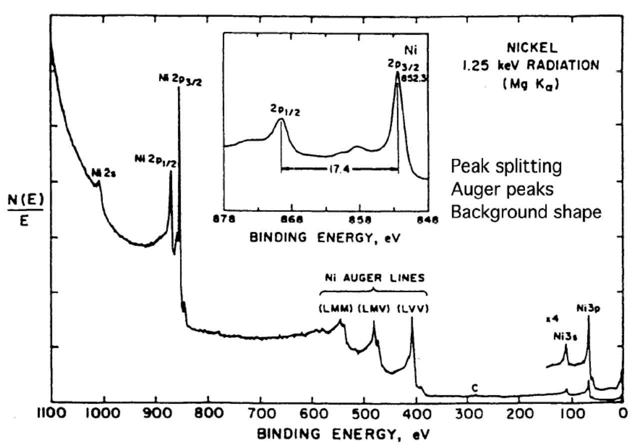
- Auger electron emission - basis of Auger electron spectroscopy (AES)
- X-ray fluorescence

Data from ESCA

Steps due to inelastic background (function of orbitals)

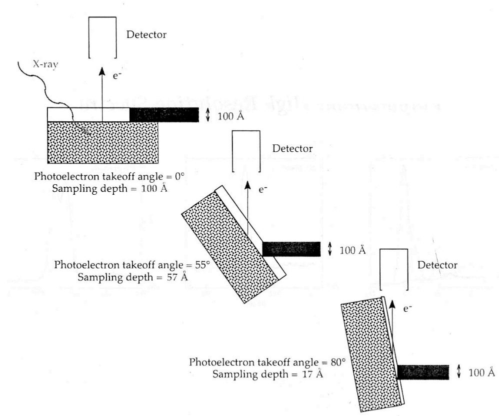
Auger lines always present

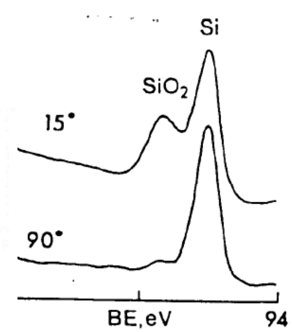
Need to compare to established binding energy curves



Depth Scanning

ESCA and related techniques can be made more surface sensitive by angling in the beam.



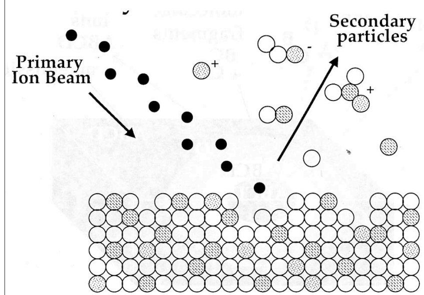


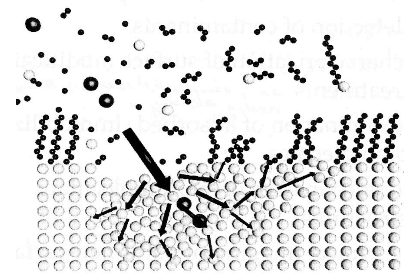
SIMS

Alias:	Secondary Ion Mass Spectroscopy, ToF-SIMS
Modes:	static and dynamic, secondary electron
Probe:	Ions (Ar^+, Ga^+, Cs^+, C_{60}^+, etc.), keV
Signal:	Secondary Ions (from sample)
Information:	Elemental and Molecular Composition
Sample:	Any that can withstand ultra-high vacuum
Principle:	Bombardment ions liberate secondary particles from the surface, secondary ions can be detected for mass
Depth:	10 Å (more in dynamic mode)
Spatial Resolution:	less than 1 μm^2
Sensitivity:	"very high"
Relative Cost:	Expensive
Other:	Semi-quantitative to quantitative
	Can resolve isotopes
	Imaging modes
	ToF detectors lead to exact mass detection

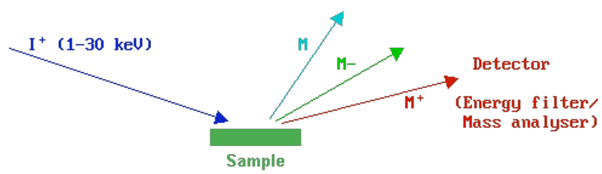
SIMS

This is a (by design) a destructive technique…

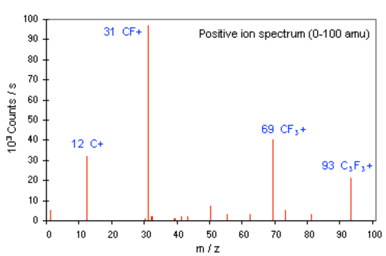


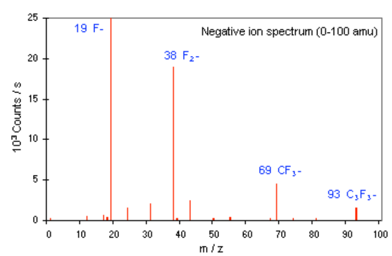


SIMS



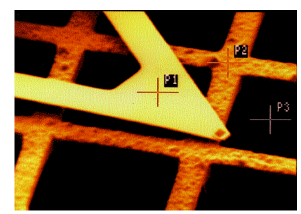
Secondary ions can be atomic and molecular, positive or negative (99% of scattered mass is neutral!)





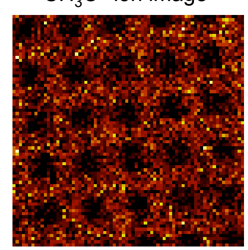
Imaging Modes for ESCA and SIMS

Auger image of AFM cantilever over inorganic pattern – point in the image can be selected for greater analysis

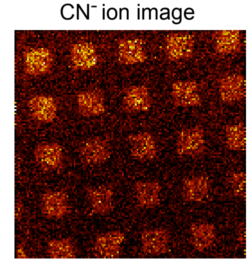


ToF-SIMS Image: Fibronectin Squares on PEO background

CH_3O^+ ion image



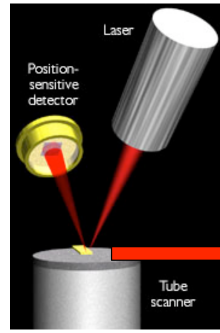
CN^- ion image



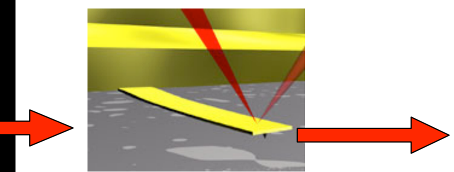
SPM

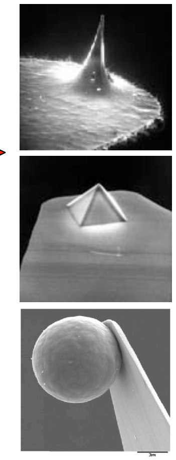
Alias:	Scanning Probe Microscopy, Scanning Force Microscopy
Modes:	Lots: atomic force (AFM), tunneling (STM), magnetic, kelvin probe, electrostatic, acoustical, calorimetry
Probe:	Cantilever tip – single atom!
Signal:	Position of tip, etc
Information:	Topography, etc
Sample:	Just about anything
Principle:	Raster a small tip over the surface to collect data and reconstruct image
Depth:	5 or less Å
Spatial Resolution:	as low as 1 Å^2
Sensitivity:	Atomic sensitivity
Relative Cost:	Moderate to expensive
Other:	Near-field Scanning Optical Microscopy (NSOM)
	Tip functionalization (change force regime or promote specific binding)

Instrument

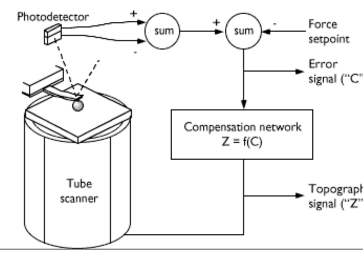


As the cantilever moves the position of the reflected laser beam on the detector changes





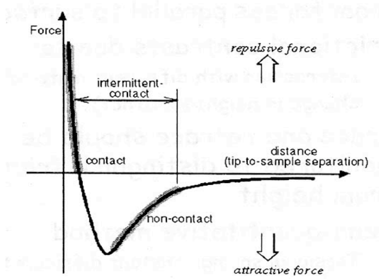
It is necessary to "close" the control loop to maintain the signal



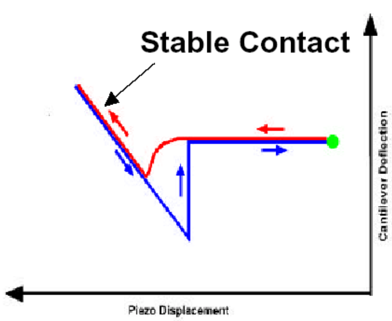
AFM

We will only consider atomic force microscopy (AFM) in this introduction as it is a widely used technique.

Remember Lennard-Jones:



Thus one can stably locate the tip "in contact" with the surface

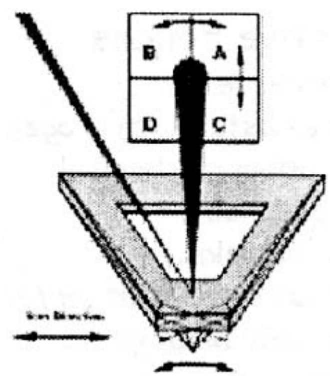


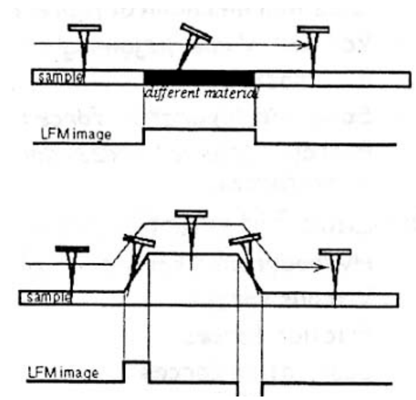
Main AFM modes: Contact, Non-Contact, Tapping, Adhesion Force, Lateral Force

DLVO theory (among others) is used to quantify "force curve" data

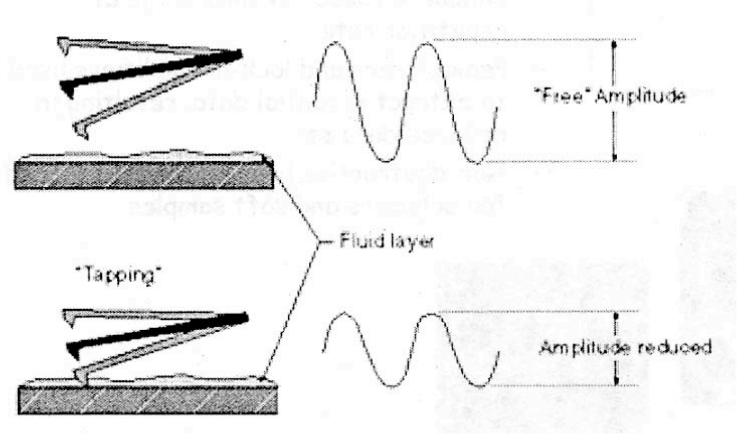
approach → retraction →

AFM Lateral Force

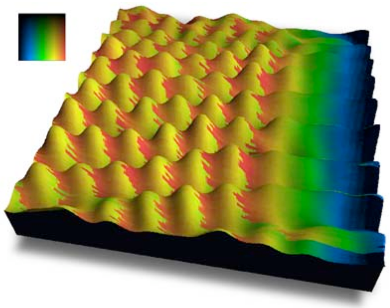




AFM Tapping Mode

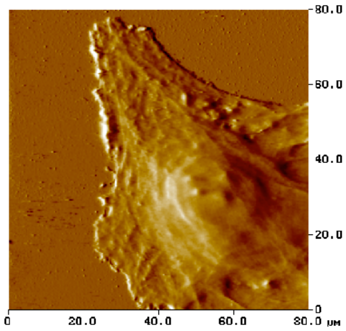


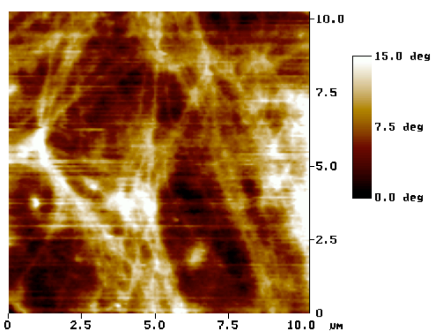
Nice, Pretty Pictures



2.5 x 2.5 nm simultaneous topographic and friction image of highly oriented pyrolytic graphic (HOPG). The bumps represent the topographic atomic corrugation, while the coloring reflects the lateral forces on the tip. The scan direction was right to left.

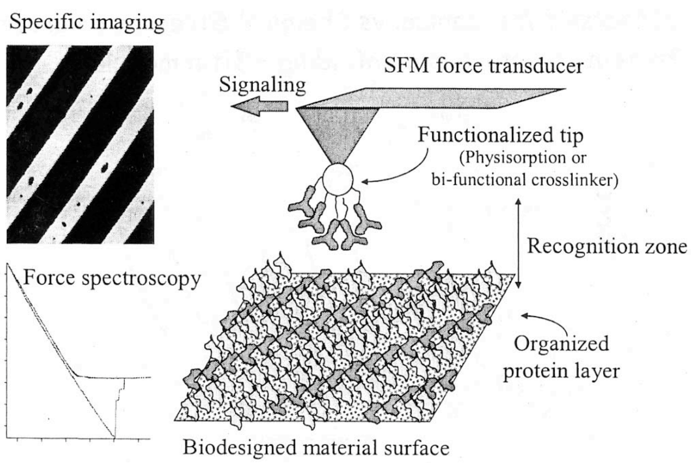
Nice, Pretty Pictures





AFM topography and phase mode images of live cells

SFM: Functional Imaging



AFM – Caution!

There is a great tendency to “see what you want” to in AFM images, although multimode operation helps to reduce interpretations. This technique has not-so-obvious limitations:

- Tip contamination
- Piezo non-linearities and drift
- Tips are rarely characterized for spring constant, geometry
- Artifacts (double tip)
- topography / phase convolution in lateral force
- “near sightedness”