BME – 220 Biomaterials

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Surface Characterization of Biomaterials – X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM)

Unit 9

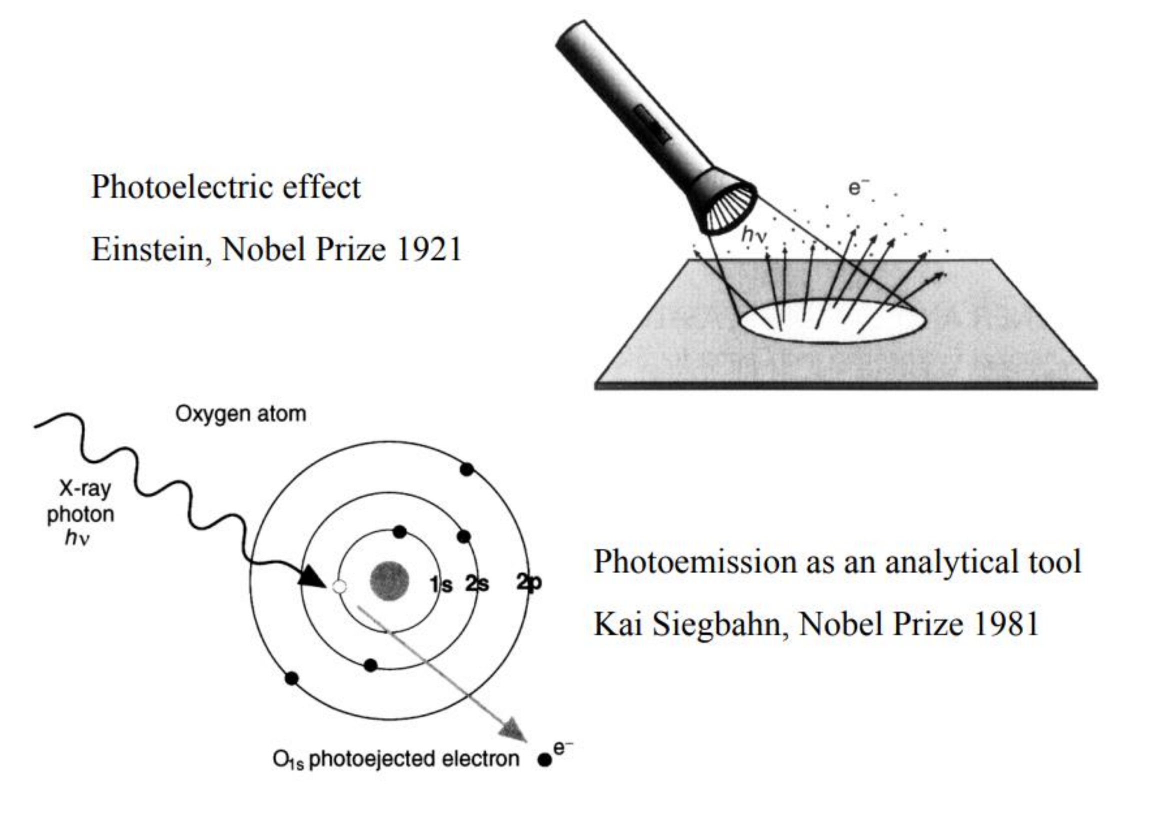
The structure and chemistry of a biomaterial surface greatly dictates the degree of biocompatibility of an implant. Surface characterization is thus a central aspect of biomaterials research.

Surface chemistry can be investigated directly using high vacuum methods:

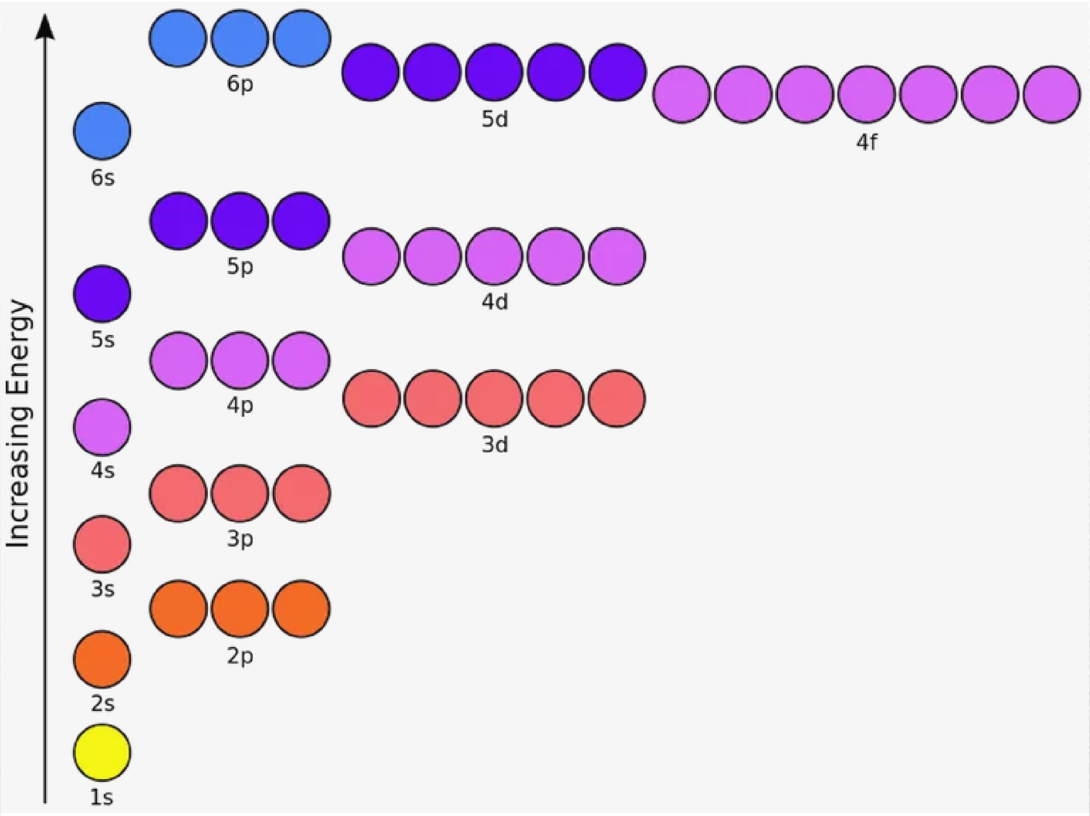
- Electron spectroscopy for Chemical Analysis (ESCA)/X-ray Photoelectron Spectroscopy (XPS)
- Auger Electron Spectroscopy (AES)
- Secondary Ion Mass Spectroscopy (SIMS)

- X-ray Photoelectron Spectroscopy (XPS), also known as Electron Spectroscopy for Chemical Analysis (ESCA) is a widely used technique to investigate the chemical composition of surfaces.
- Secondary electrons ejected by x-ray bombardment from the sample near surface (0.5-10 nm) with characteristic energies
- Analysis of the photoelectron energies yields a quantitative measure of the surface composition

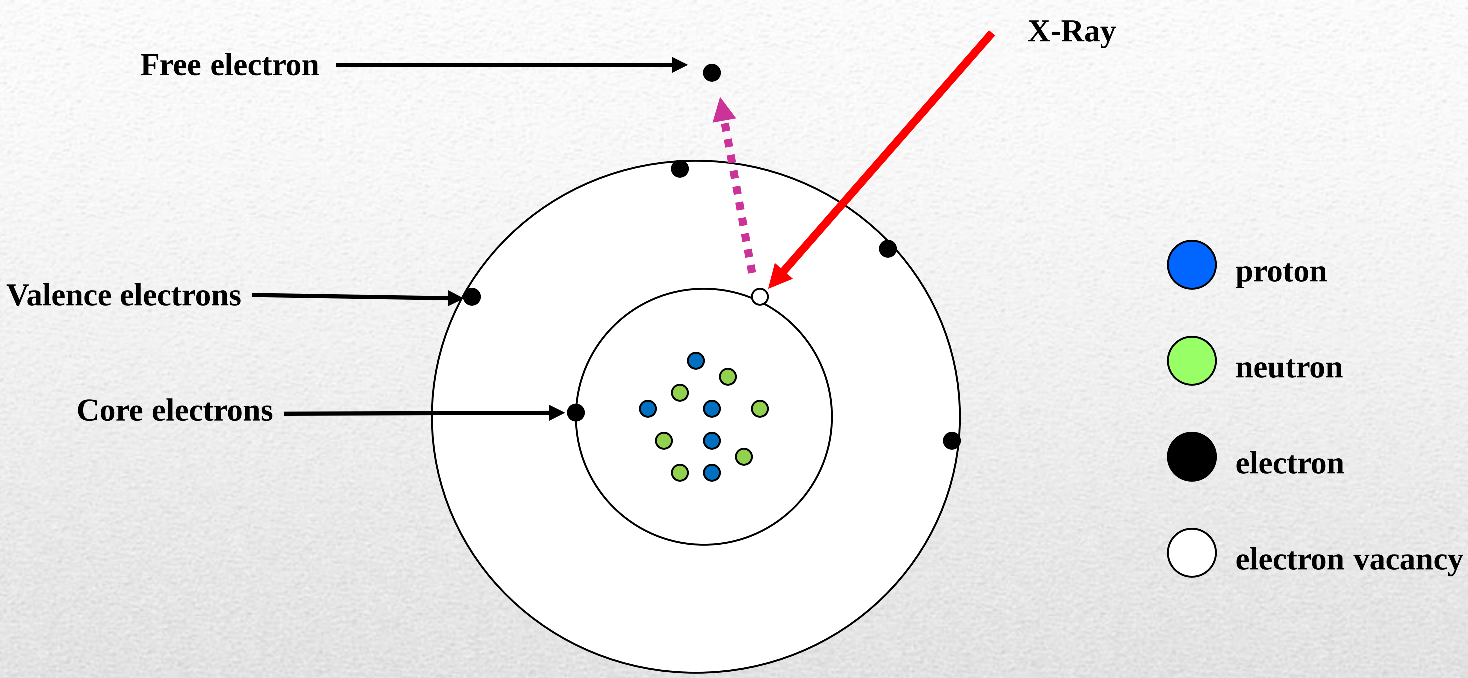
XPS/ESCA



Photoelectric Effect

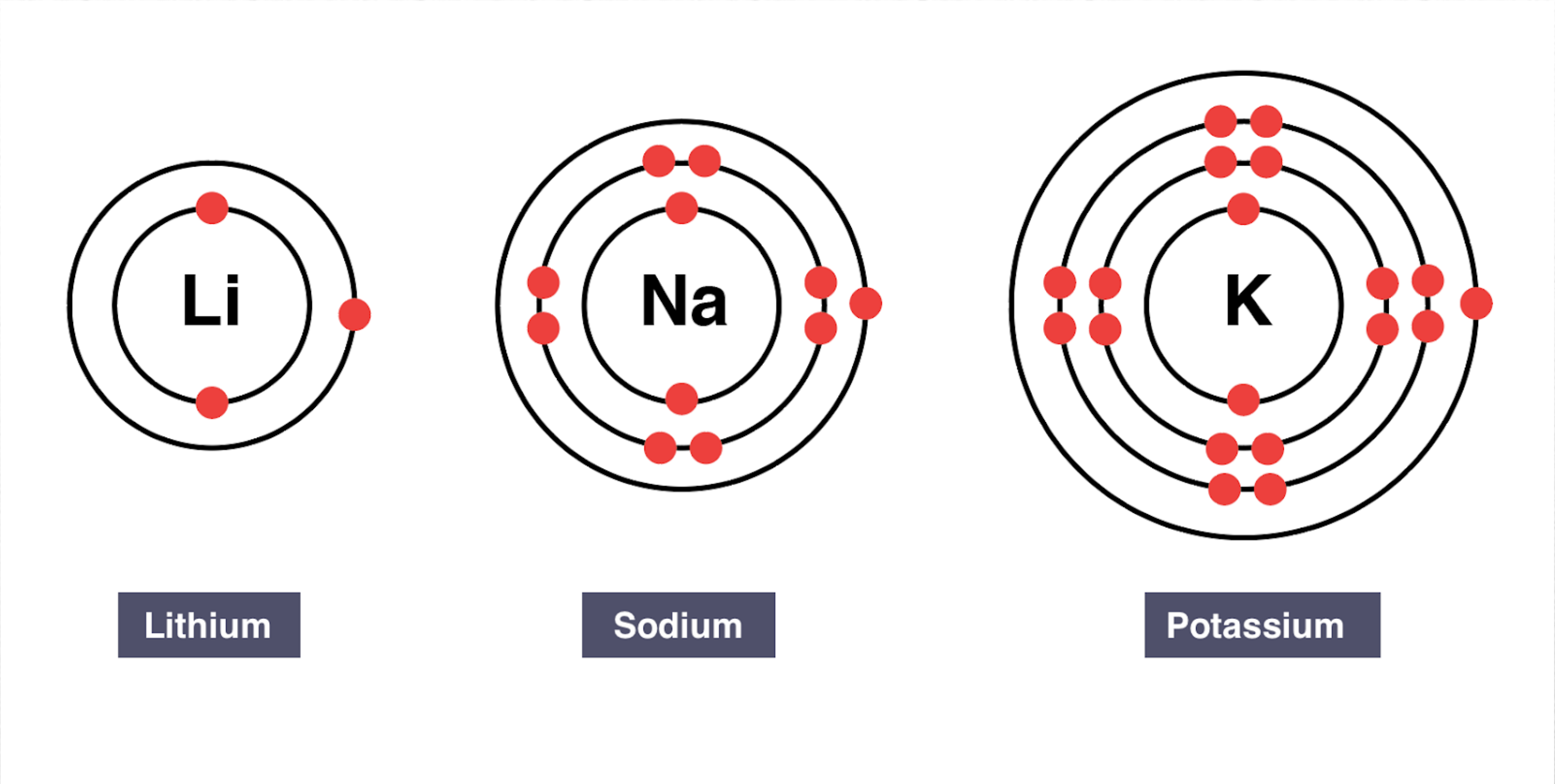


Electron Configuration

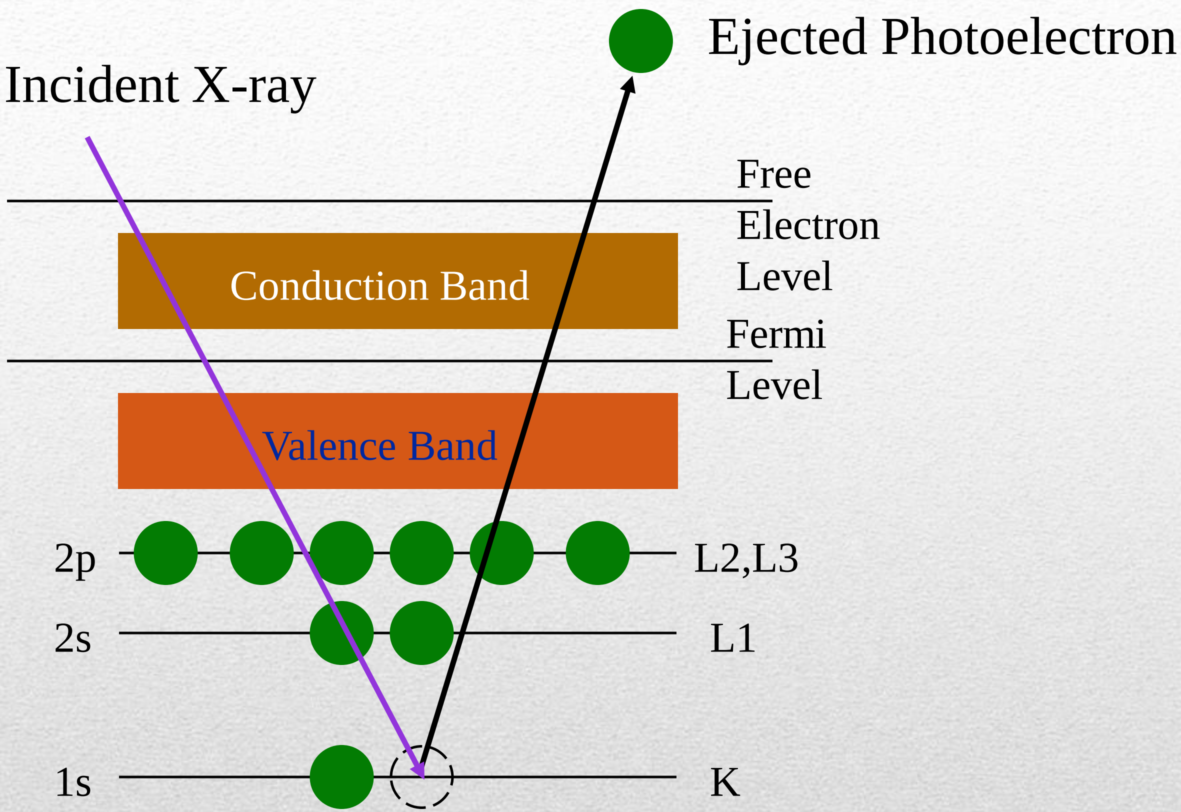


The core electrons respond very well to the X-Ray energy

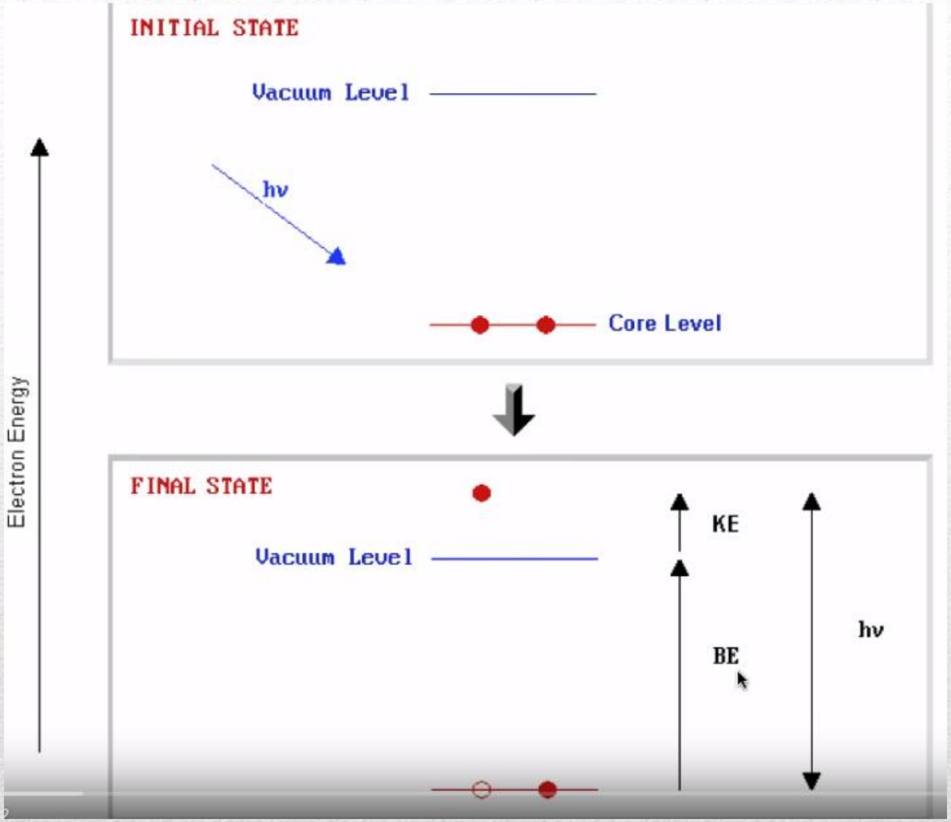
Electron Configuration



Electron Configuration


Ejected Photoelectron
Incident X-ray
Free
Electron
Level
Conduction Band
Fermi
Level
Valence Band
2p
L2,L3
2s
L1
1s
K

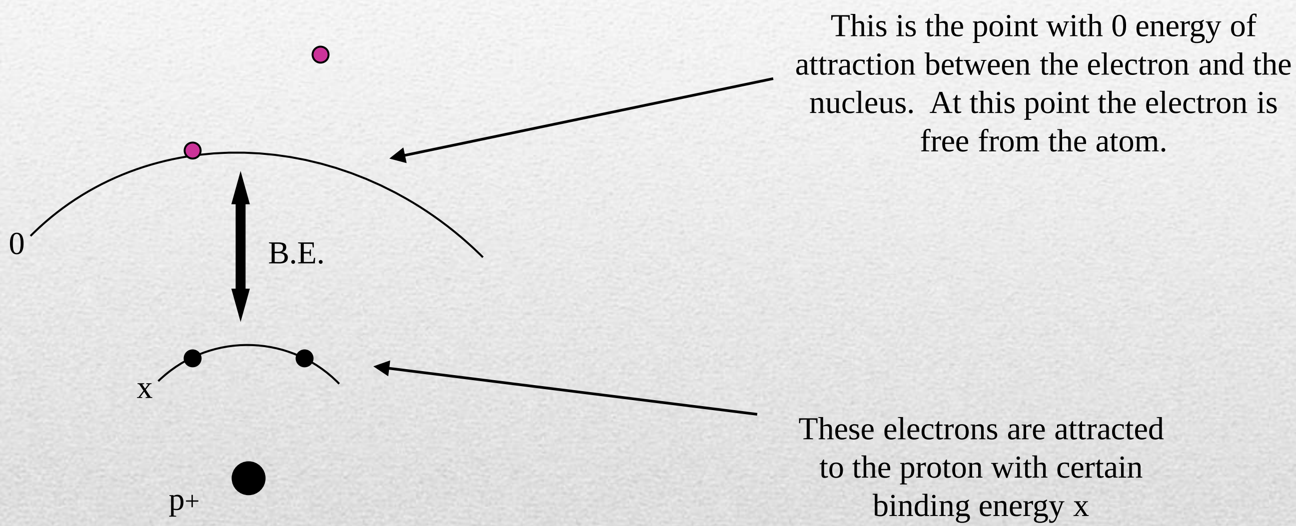
XPS/ESCA


INITIAL STATE
Vacuum Level
hv
Core Level
Electron Energy
FINAL STATE
Vacuum Level
KE
BE
hv

XPS/ESCA

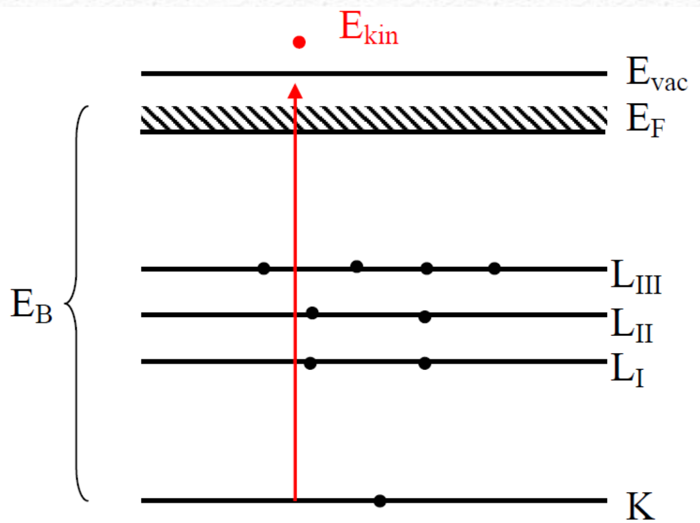
Binding Energy

The Binding Energy (BE) is characteristic of the core electrons for each element. The BE is determined by the attraction of the electrons to the nucleus. If an electron with energy x is pulled away from the nucleus, the attraction between the electron and the nucleus decreases and the BE decreases. Eventually, there will be a point when the electron will be free of the nucleus.



Binding Energy

Photoelectron binding energy is characteristic of the element and bonding environment



Chemical analysis!

Binding energy = incident x-ray energy – photoelectron kinetic energy

$$E_B = h\nu - E_{kin}$$

$$\boxed{KE = h\upsilon - BE - \phi_{spec}}$$

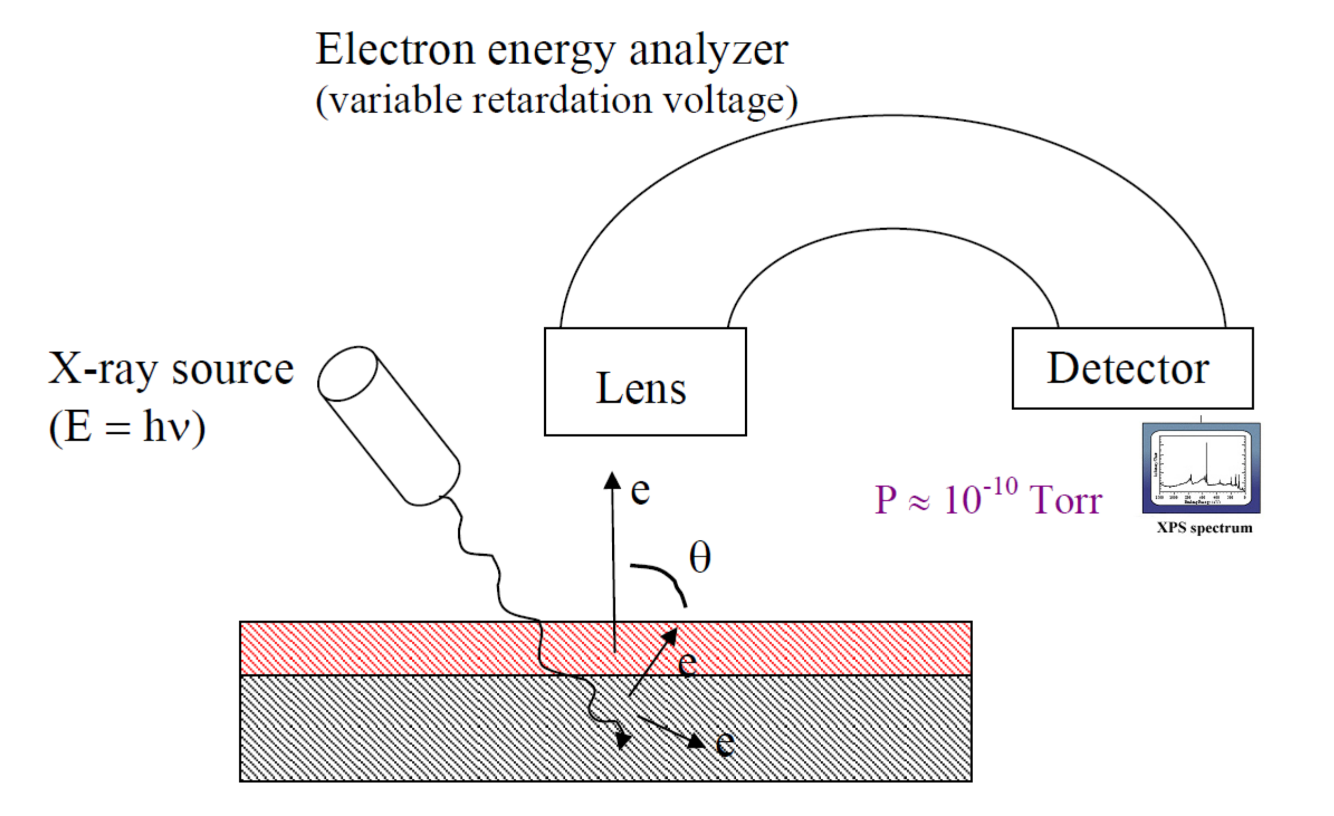
KE	Kinetic Energy (measure in the XPS spectrometer)
$h\upsilon$	photon energy from the X-Ray source (controlled)
ϕ_{spec}	spectrometer work function. It is a few eV, it gets more complicated because the materials in the instrument will affect it. Found by calibration.
BE	is the unknown variable

Binding Energy

$$KE = h\upsilon - BE - \phi_{spec}$$

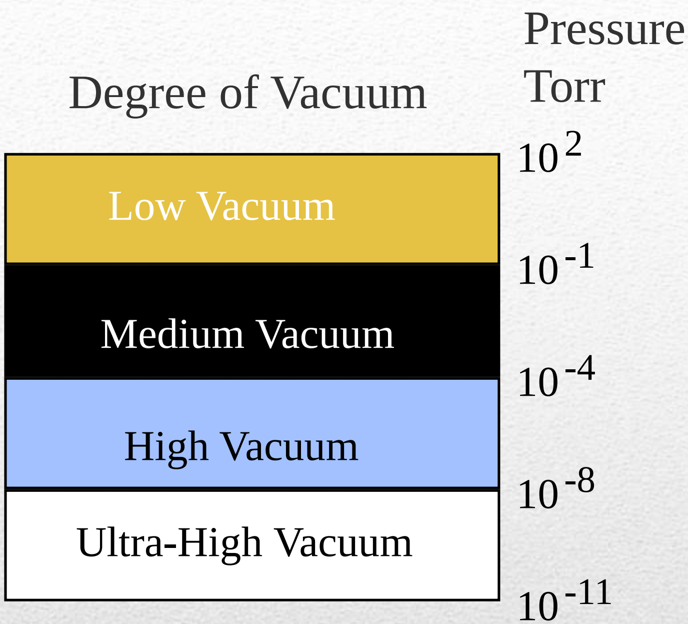
- The equation will calculate the energy needed to get an e^- out from the surface of the solid.
- Knowing KE, $h\upsilon$ and ϕ_{spec} the BE can be calculated.

Binding Energy



Electron energy analyzer
(variable retardation voltage)
X-ray source
(E = hν)
Lens
Detector
e
θ
e
e
P ≈ 10^-10 Torr
XPS spectrum

XPS/ESCA

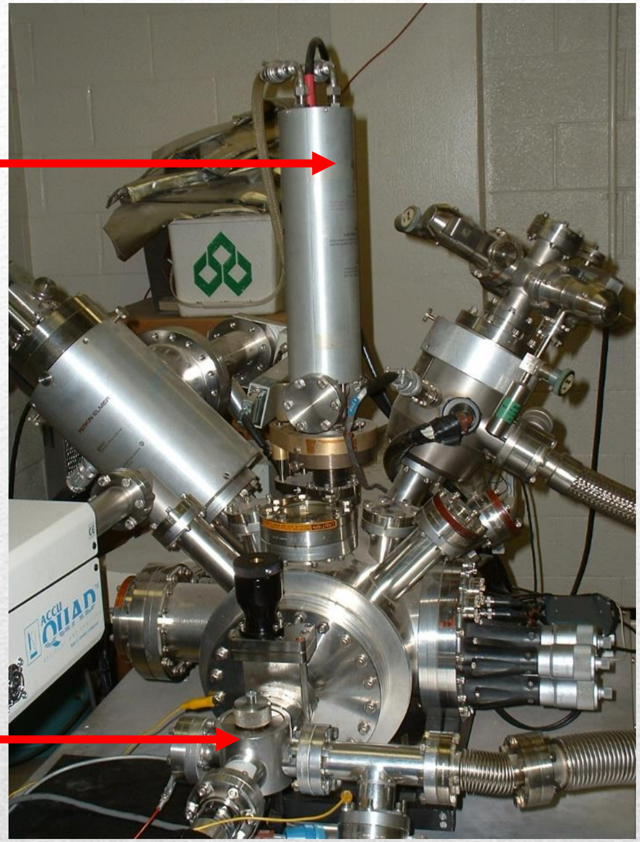


- Remove adsorbed gases from the sample.
- Eliminate adsorption of contaminants on the sample.
- Increase the mean free path for electrons, ions and photons.

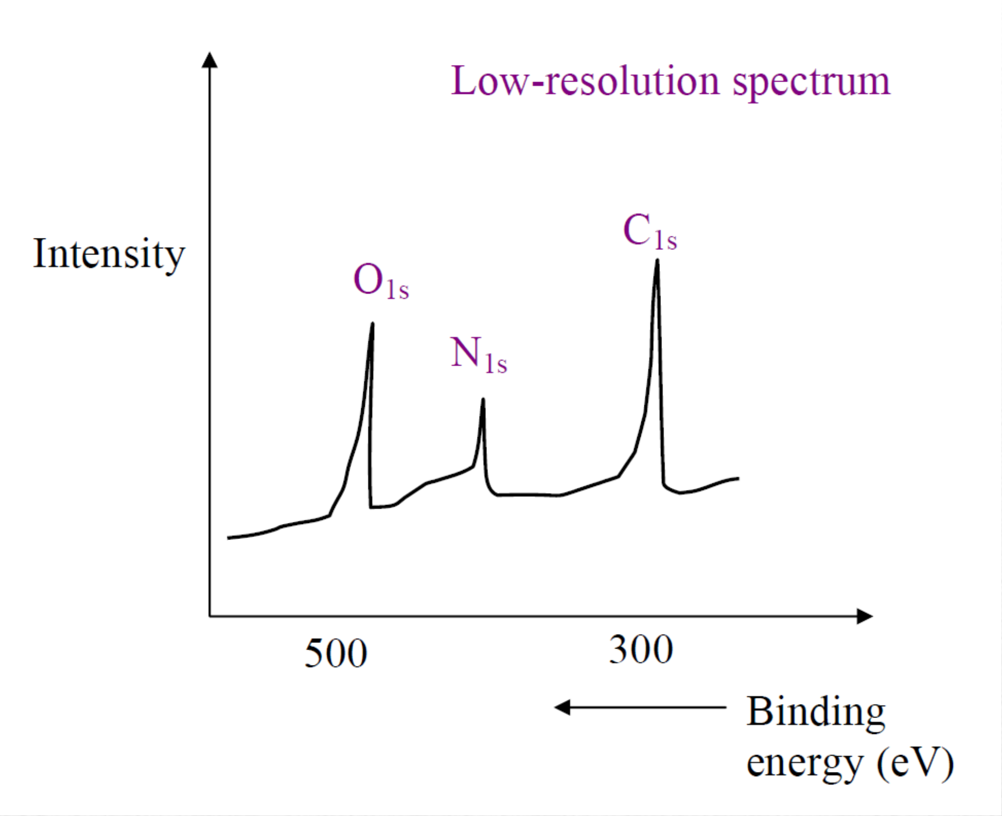
XPS/ESCA

X-Ray Source

Sample introduction Chamber

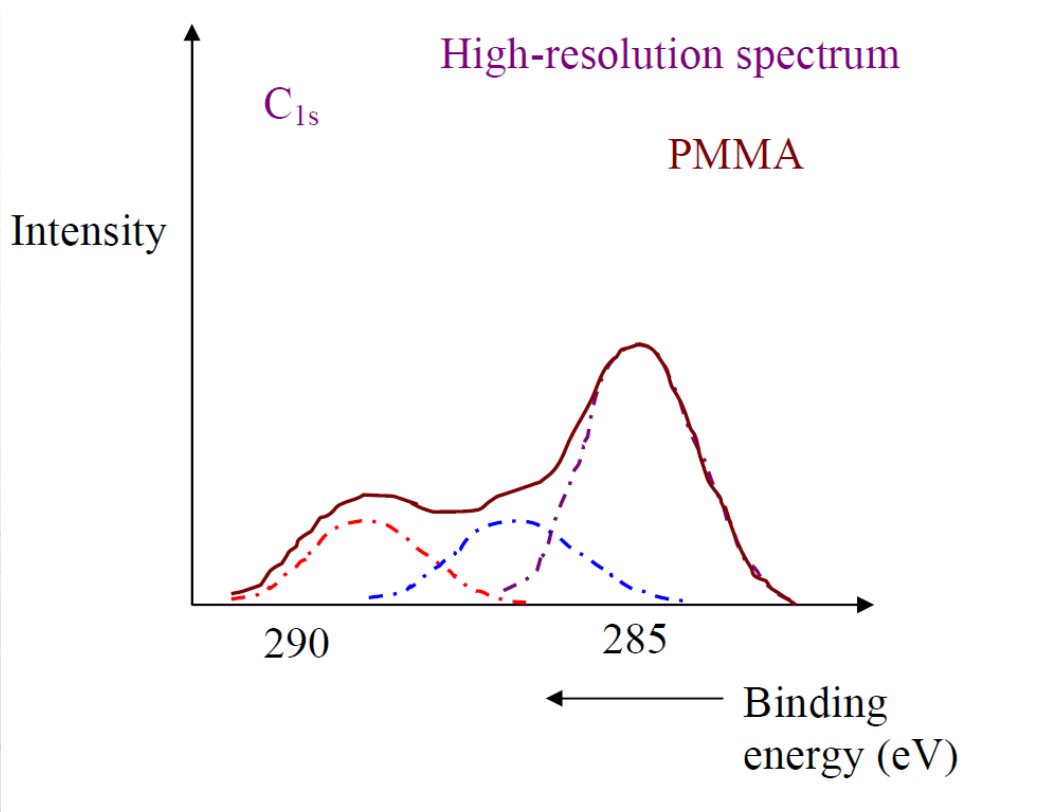


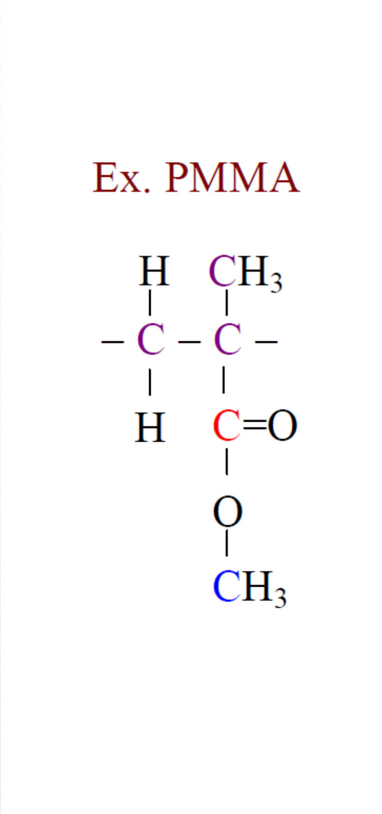
XPS/ESCA



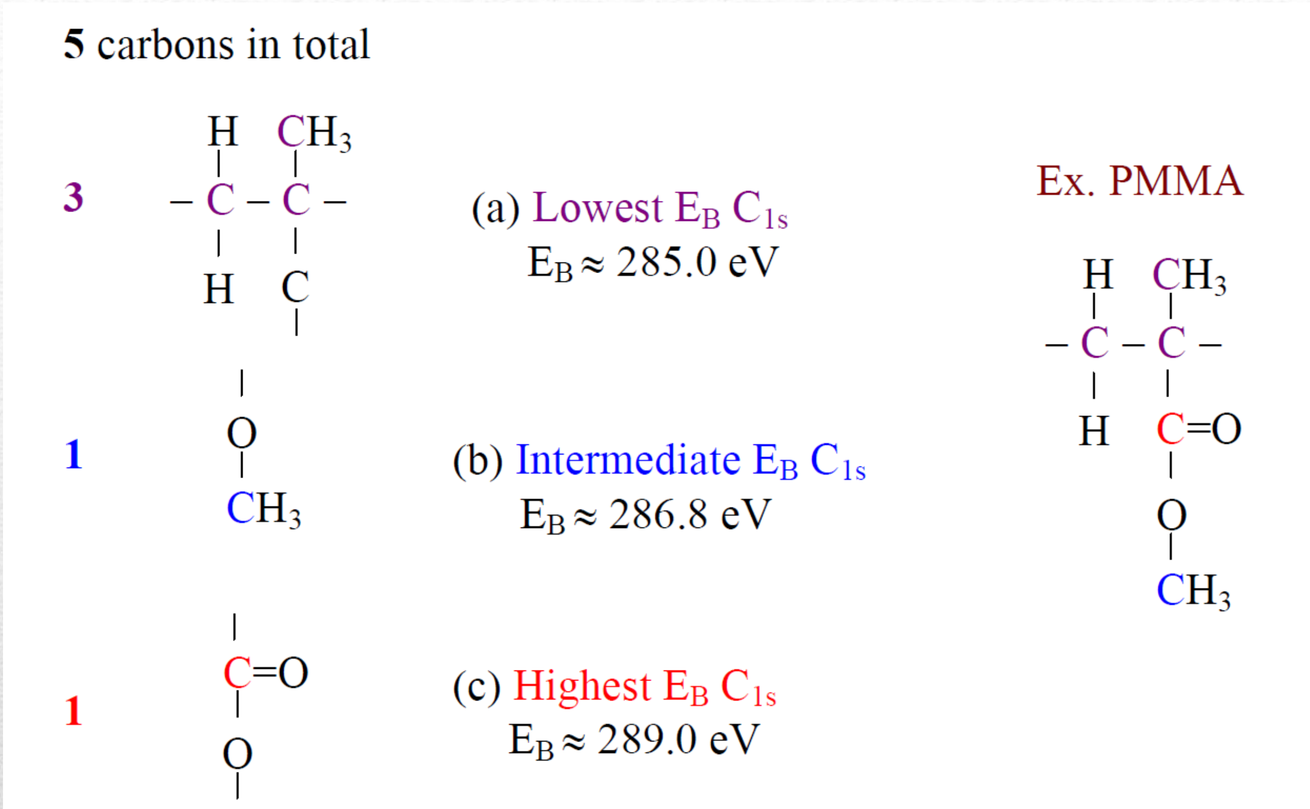
- Area under peak $I_i \propto$ number of electrons ejected (& atoms present)
- Only electrons in the near surface region escape without losing energy by inelastic collision
- Sensitivity: depends on element. Elements present in concentrations >0.1 atom% are generally detectable (H & He undetected)

Quantitative Elemental Analysis





Quantitative Elemental Analysis



Quantitative Elemental Analysis

Electronegative oxygen “robs” valence electrons from carbon
(electron density higher toward O atoms)

⇩

Carbon core electrons held “tighter” to the + nucleus
(less screening of + charge)

⇩

Slight shift to higher C_{1s} binding energy

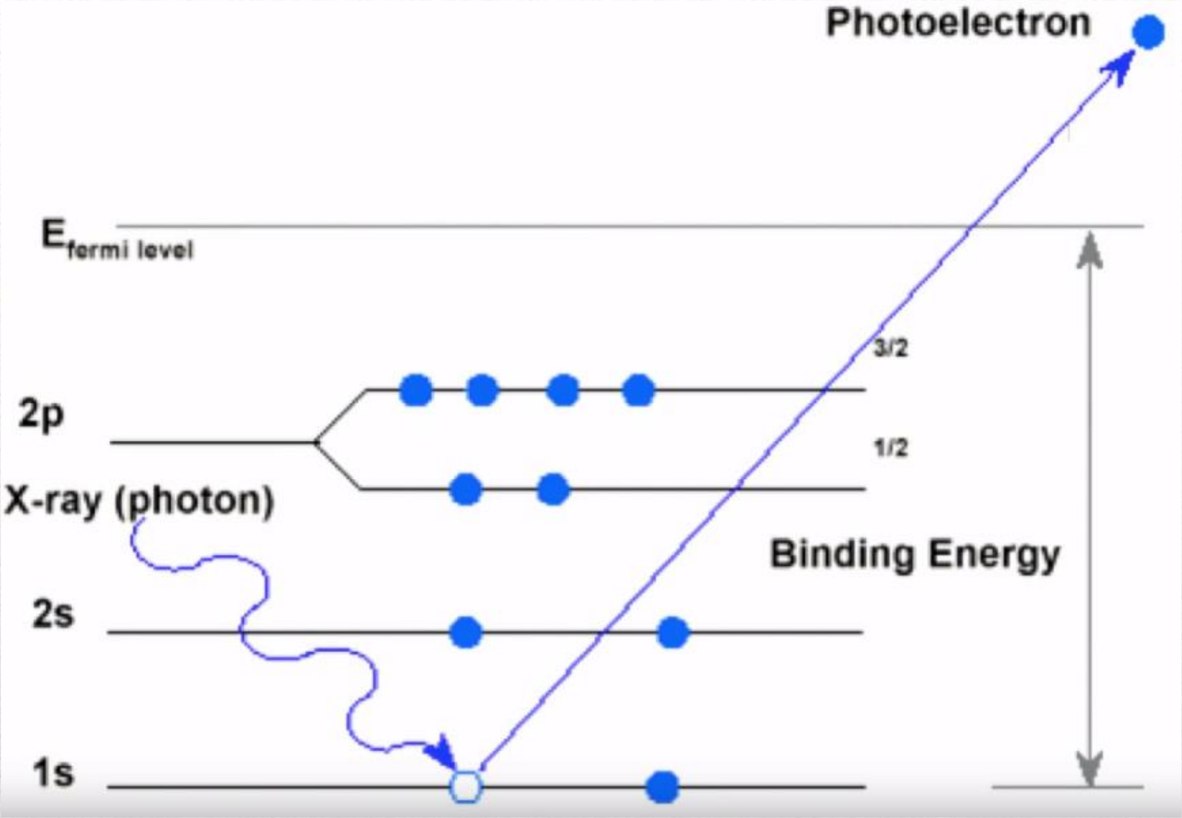
Similarly, different oxidation states of metals can be distinguished.

Ex. Fe FeO Fe_3O_4 Fe_2O_3

$\longrightarrow$

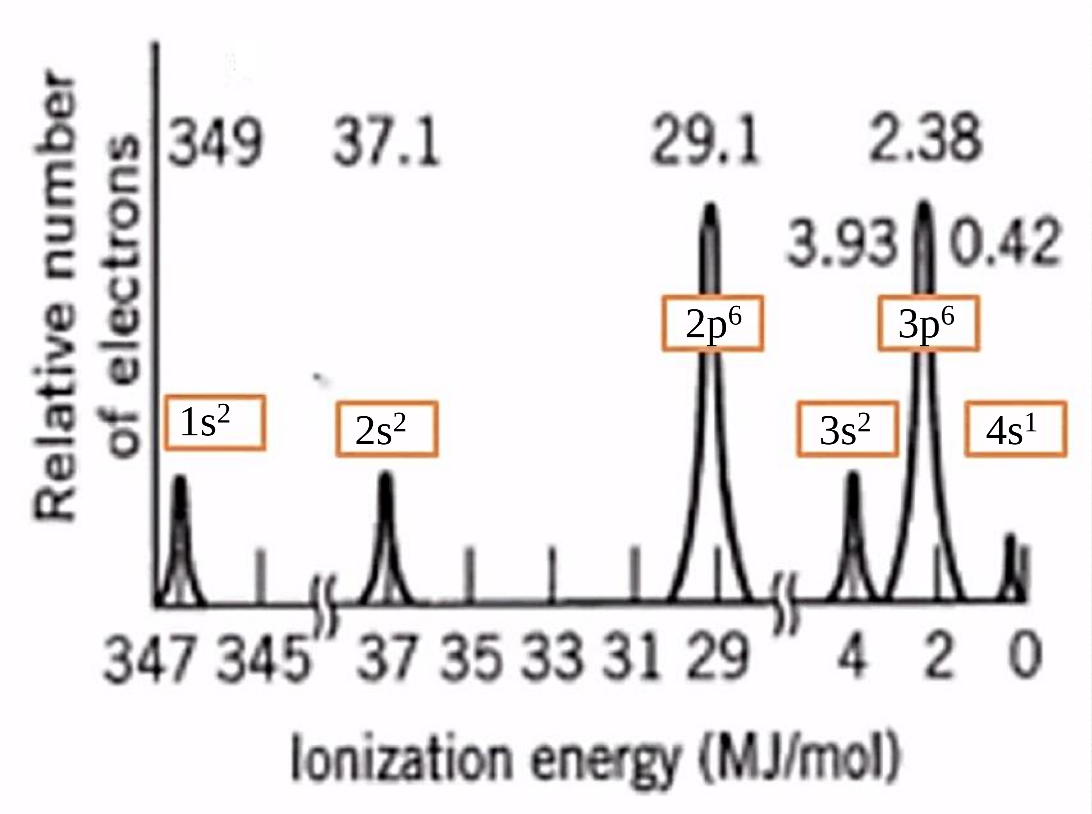
Fe_{2p} binding energy

Quantitative Elemental Analysis

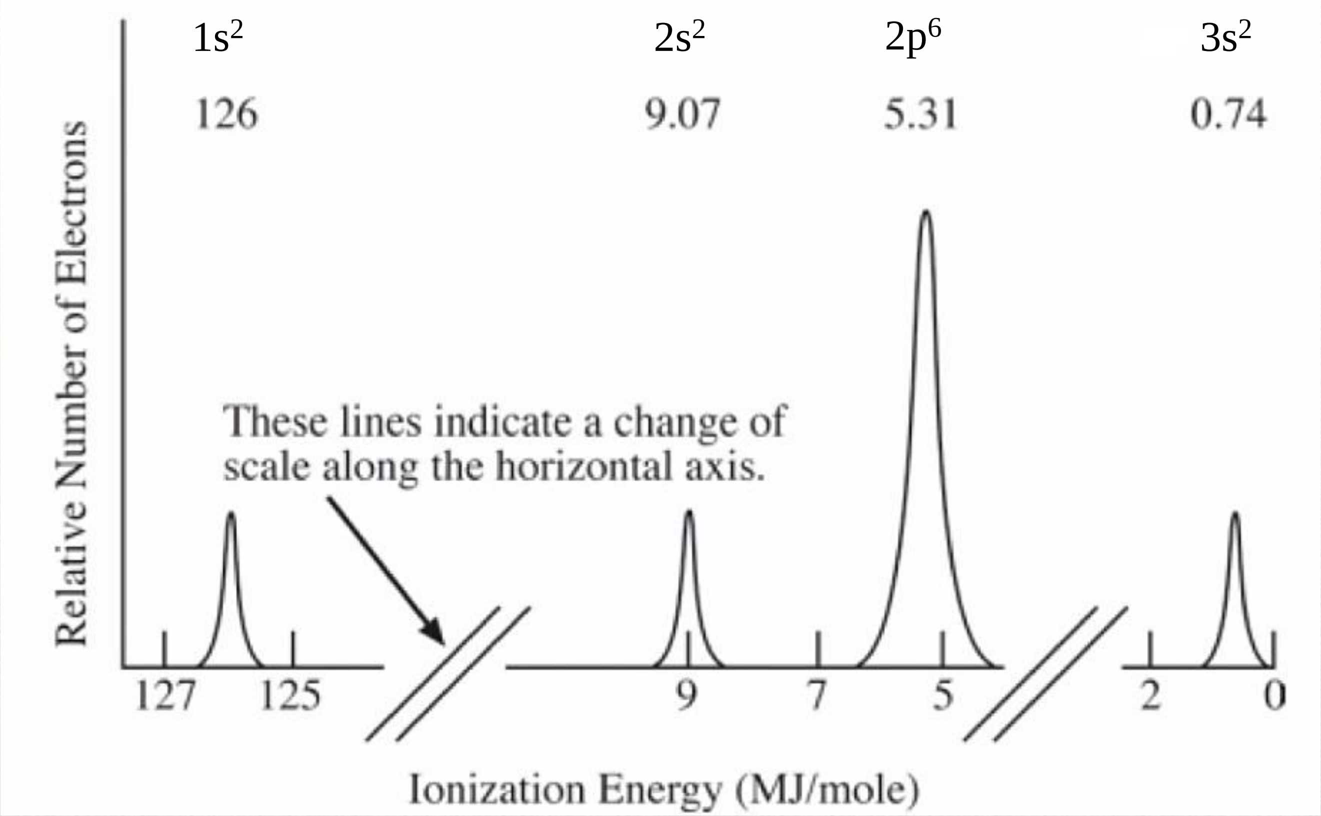


The more energy it takes to knock out an electron, the less KE it has.

Quantitative Elemental Analysis

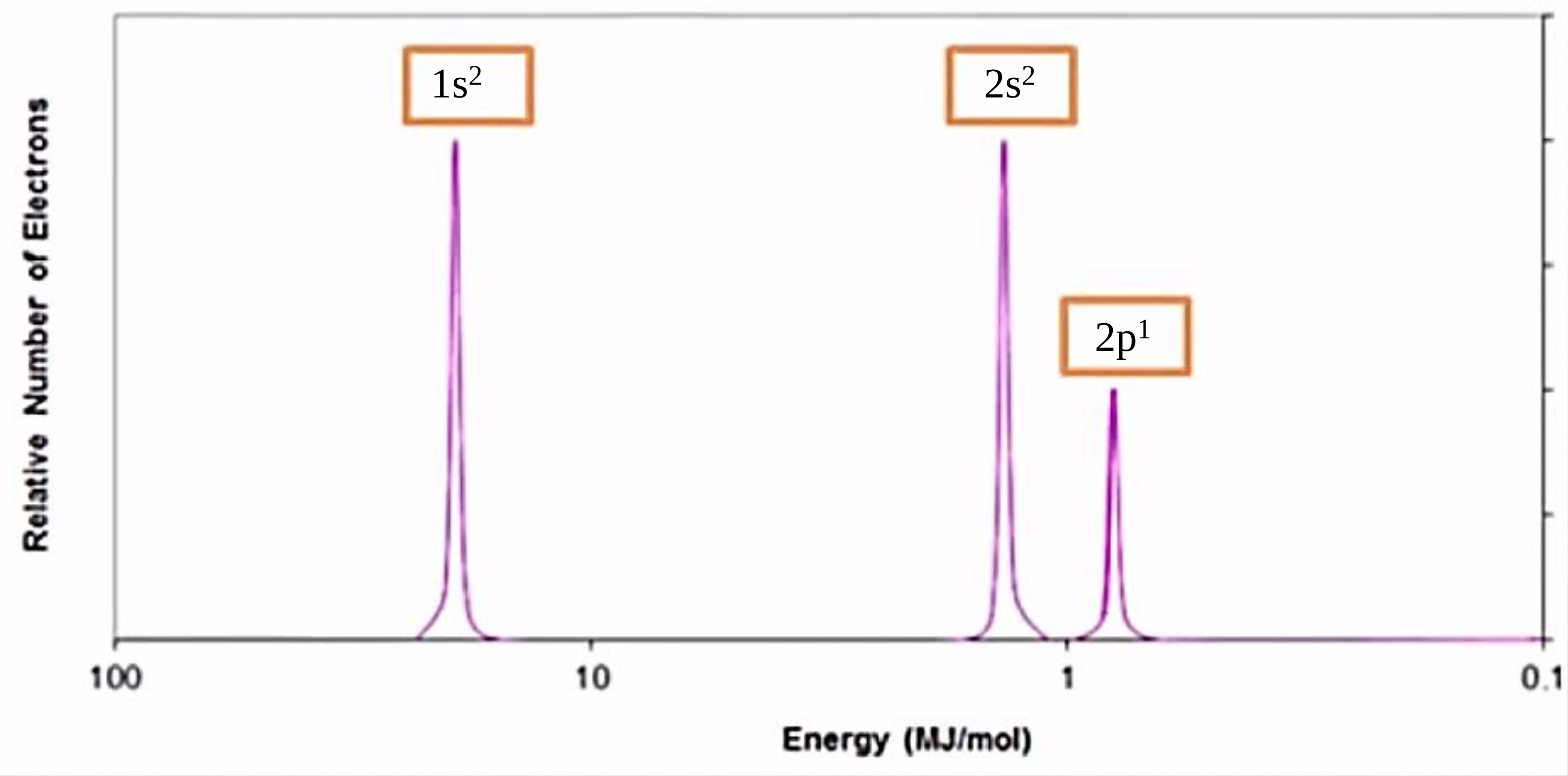


Interpreting the Spectrum



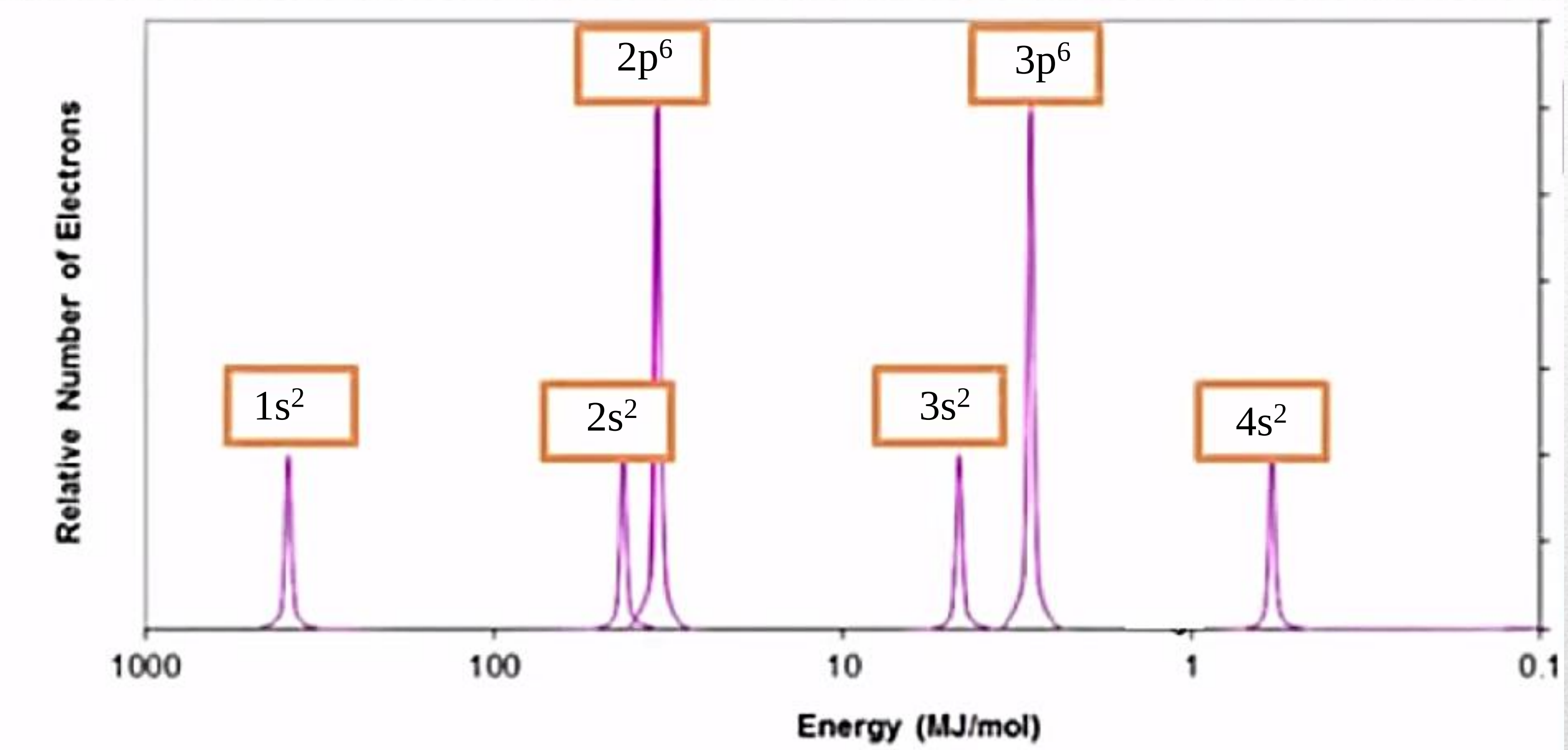
Mg

Determine the Element



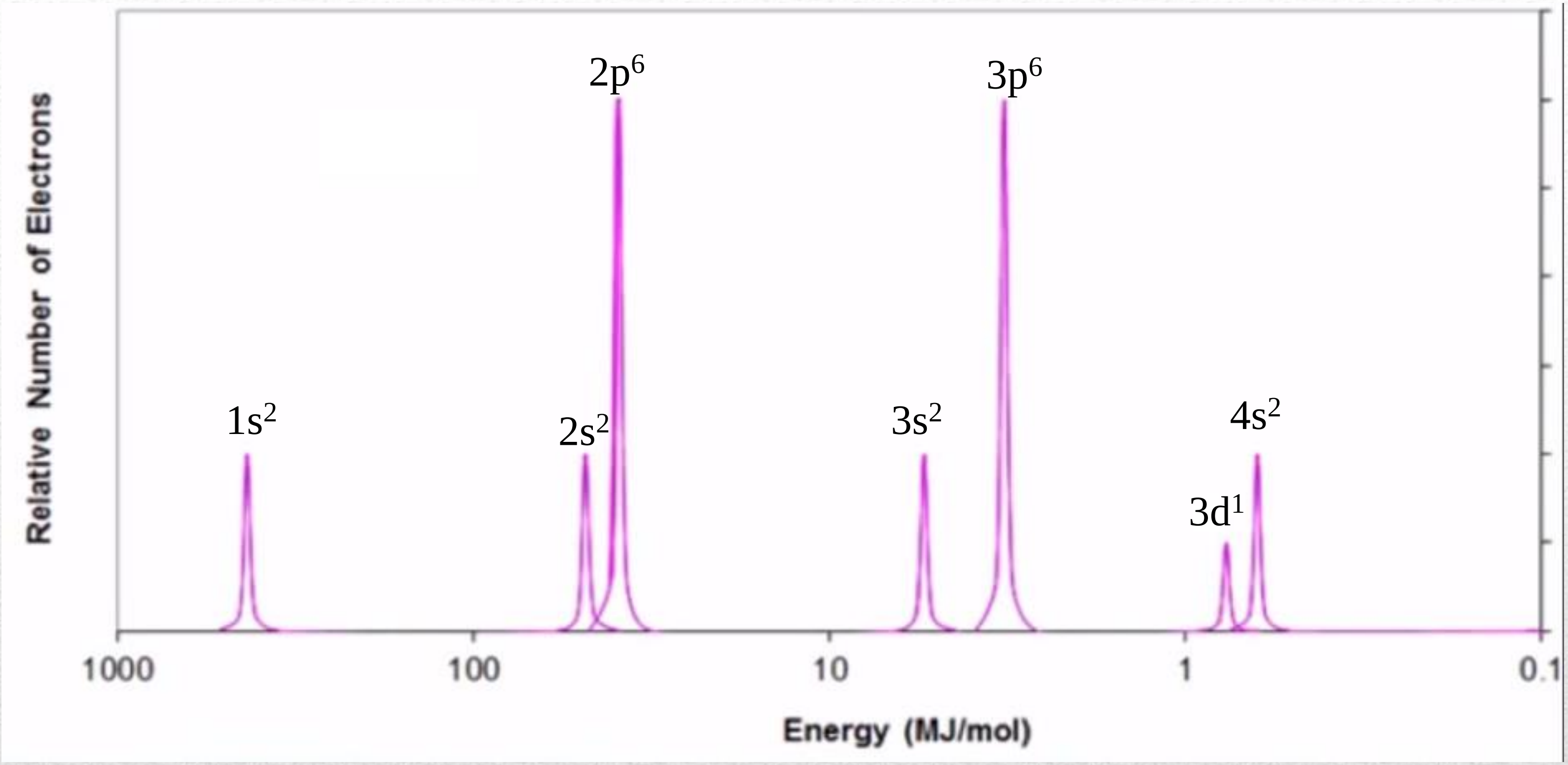
Boron

Determine the Element



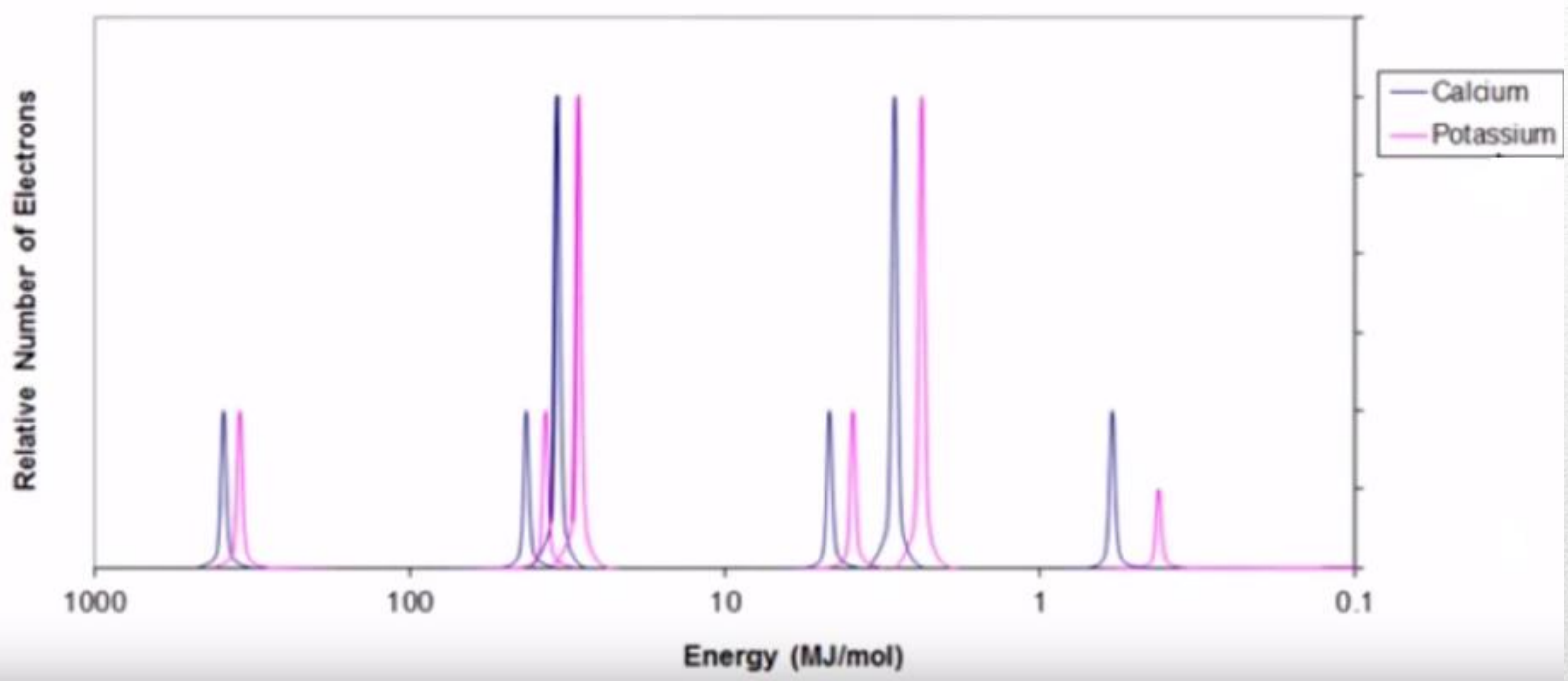
Ca

Determine the Element



Photoelectron spectrum of Scandium. Atomic number 21.

Single Spectrum



Dual Spectra

The Periodic Table

1 H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	57-71	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	89-103	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og
		57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu	
		89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr	

- 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 one common technique:

Atomic Force Microscopy (AFM)

- The biologic response to implanted biomaterials is largely determined by the molecular interactions that occur at the interface between the material and host. Protein adsorption, surface-induced thrombosis and cell - surface interactions are well-known examples.
- Atomic force microscopy (AFM) provides unique opportunities for visualizing these and other surface-dependent cellular and molecular interactions in three dimensions on a nanometer (nm) scale in aqueous environments.

Why AFM?

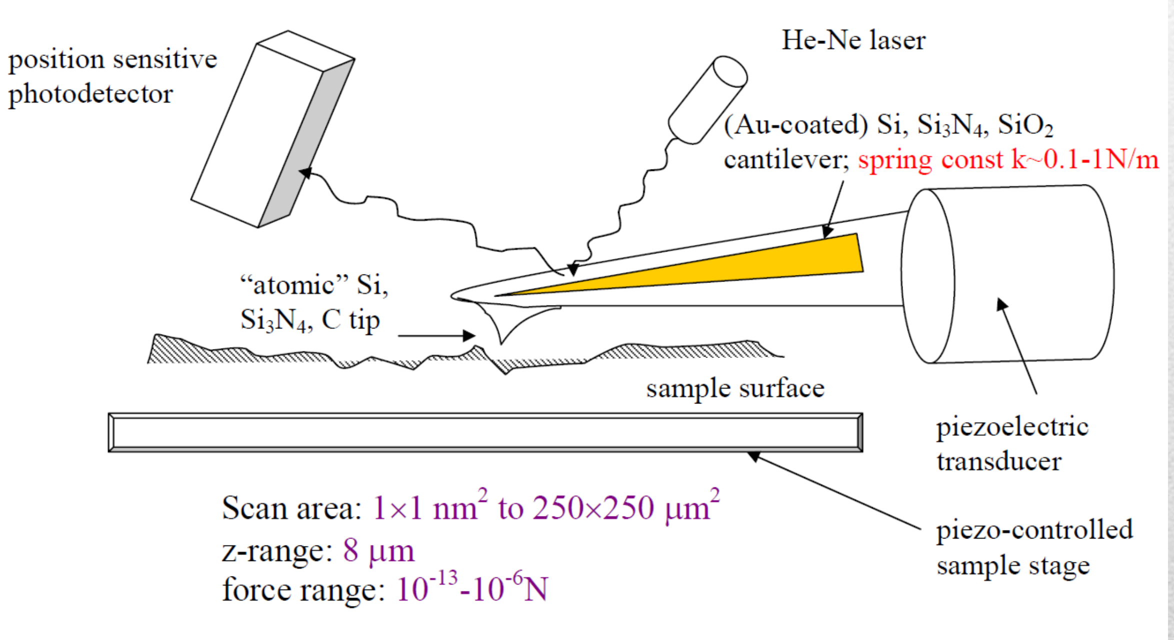
- Unprecedented access to the details of structure and function of biological interactions at surfaces is provided by AFM. Under optimum conditions (hard, smooth samples) AFM is capable of resolving surface detail down to the atomic level
- The nature of AFM data collection, which employs a small probe interacting with the sample, also means that AFM can be used to obtain sensitive measurements of the intermolecular forces (sub-nanoNewton, nN) and the sample's surface properties (mechanical, physical) under aqueous conditions.

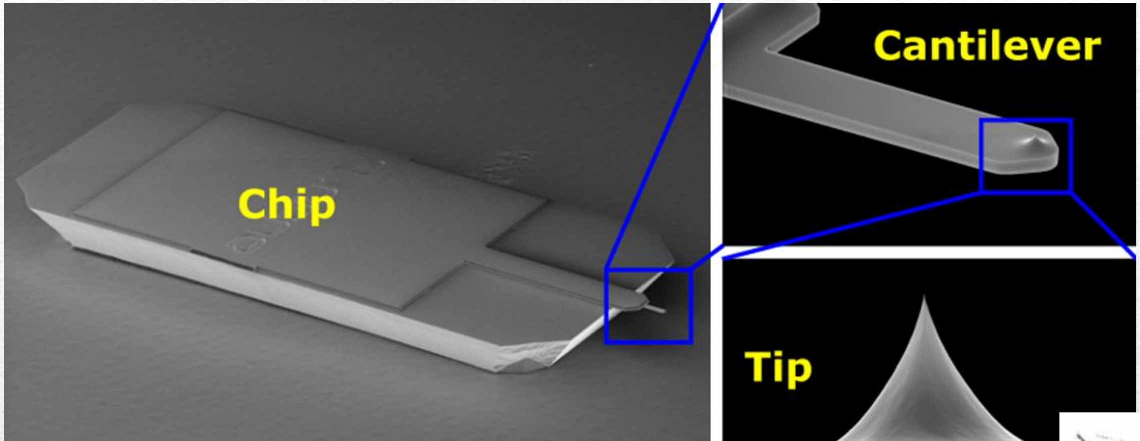
Why AFM?

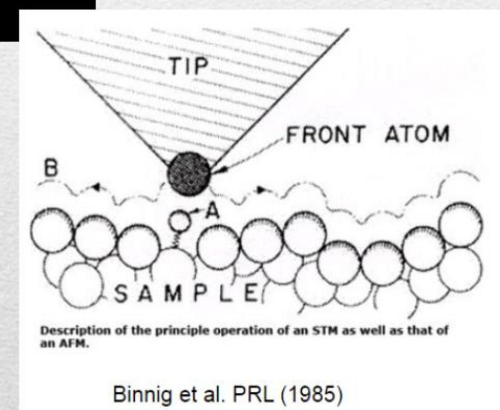
AFM

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

Piezoelectric transducer: When mechanical stress or forces are applied to some materials along certain planes, they produce electric voltage. This electric voltage can be measured easily by the voltage measuring instruments, which can be used to measure the stress or force.

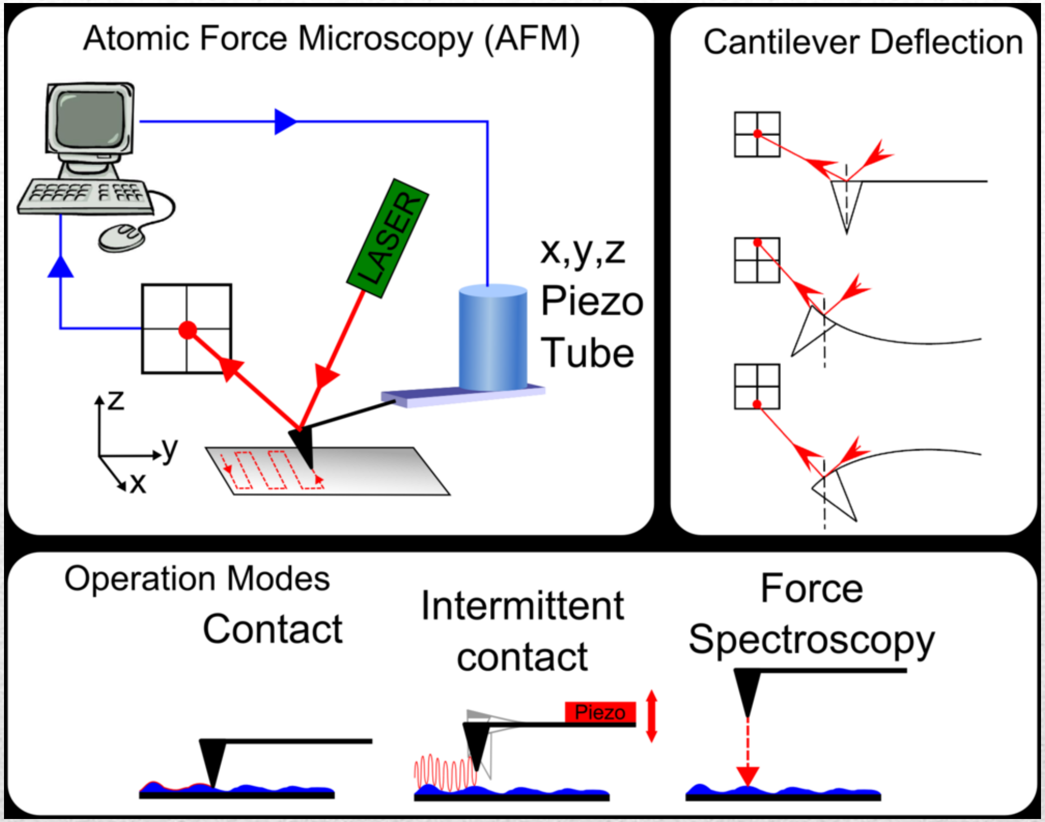




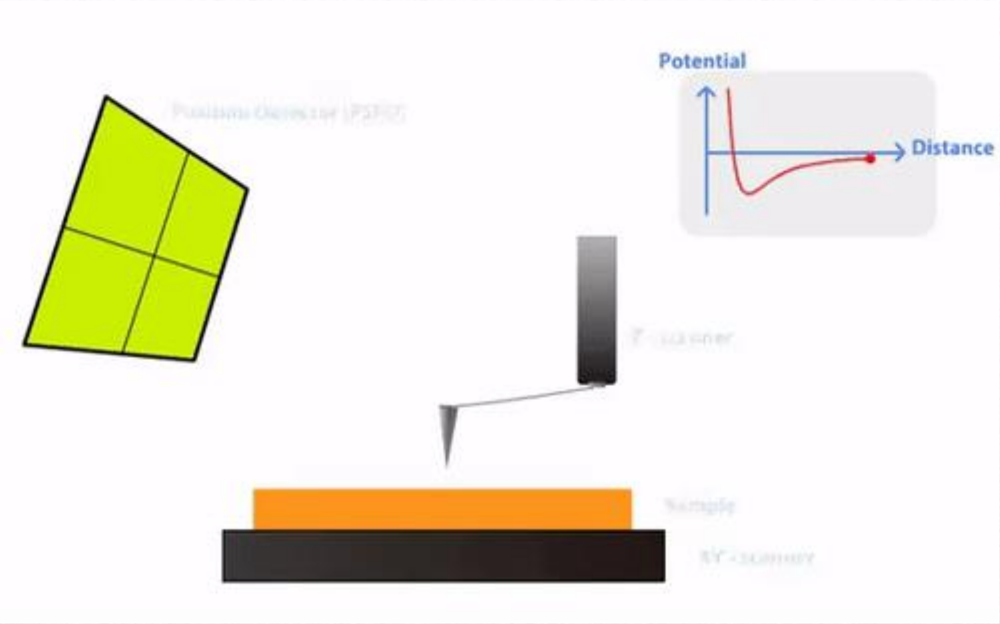


Cantilever - a projecting beam supported at only one end

AFM - Cantilever

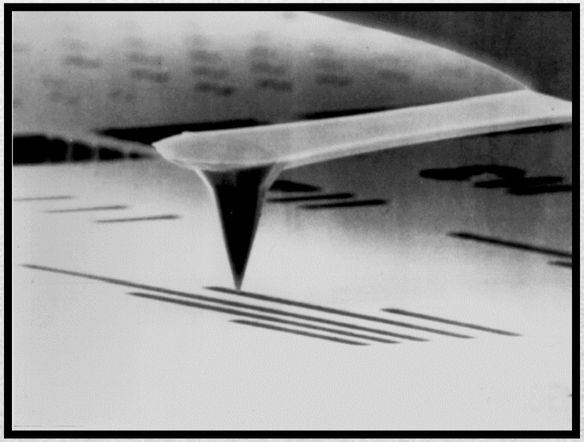


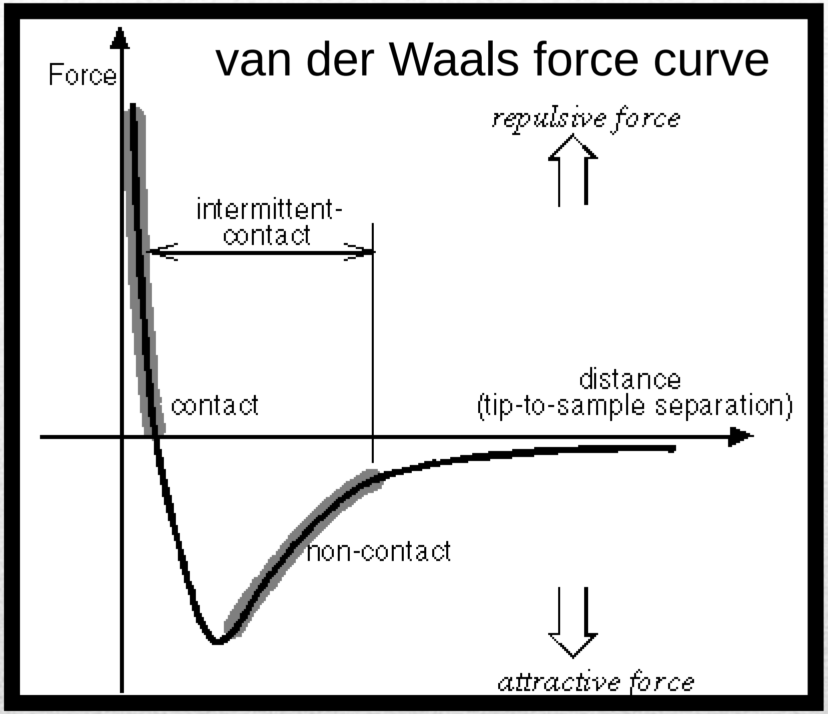
AFM – Working Principle



AFM – Working Principle

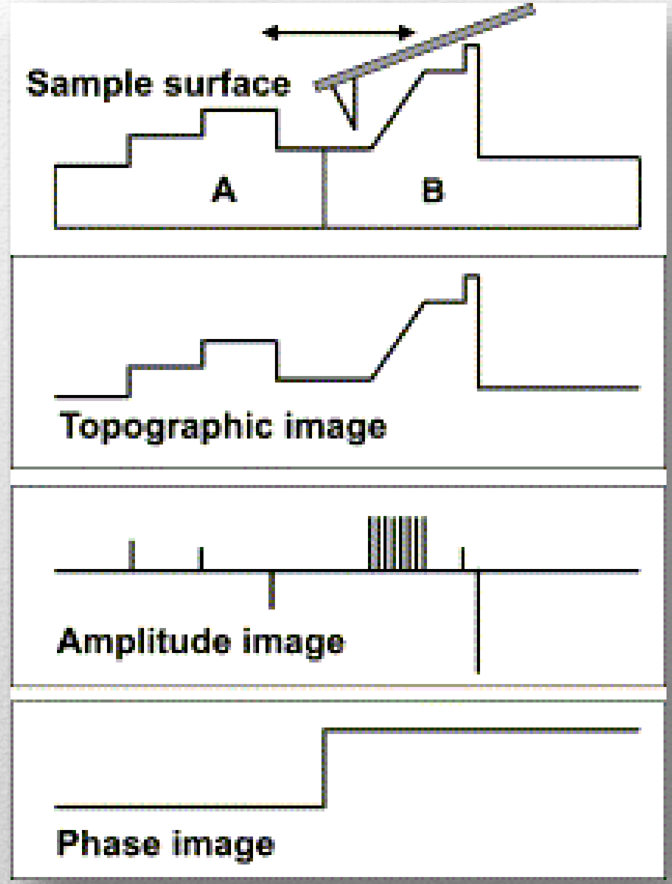
- The AFM brings a probe in close proximity to the surface
- The force is detected by the deflection of a spring, usually a cantilever (diving board)
- Forces between the probe tip and the sample are sensed to control the distance between the tip and the sample.





AFM – Working Principle

Commercial AFM

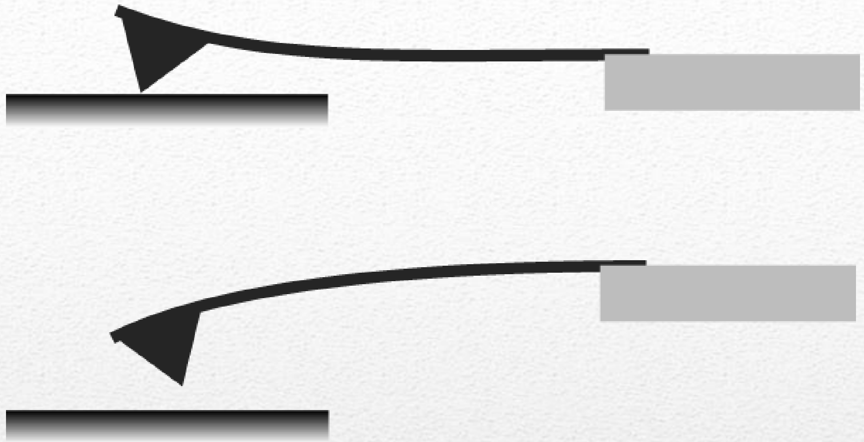


Sketch demonstrating AFM imaging modes. The AFM tip scans a sample in tapping mode. The sample consists of two regions with different stiffnesses A and B. The topographic image gives information about the three-dimensional sample profile whereas the amplitude image is edge sensitive. The different stiffnesses of the regions A and B are reflected by the two different phase signals observed.

AFM – Imaging Output

Jandt. (2001). Surface Science, 491(3), 303-332.

General Operation Modes



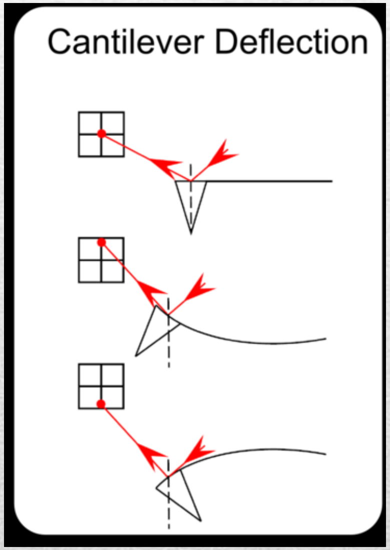
Repulsive (contact)

- At short probe-sample distances, the forces are repulsive

Attractive Force (non-contact)

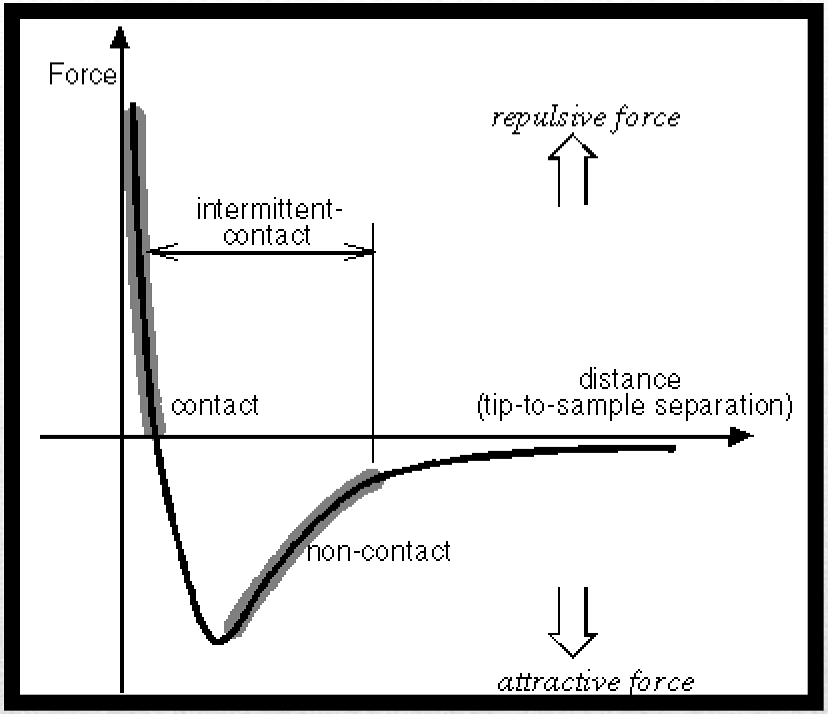
- At large probe-sample distances, the forces are attractive

The AFM cantilever can be used to measure both attractive force mode and repulsive forces.



Contact Mode

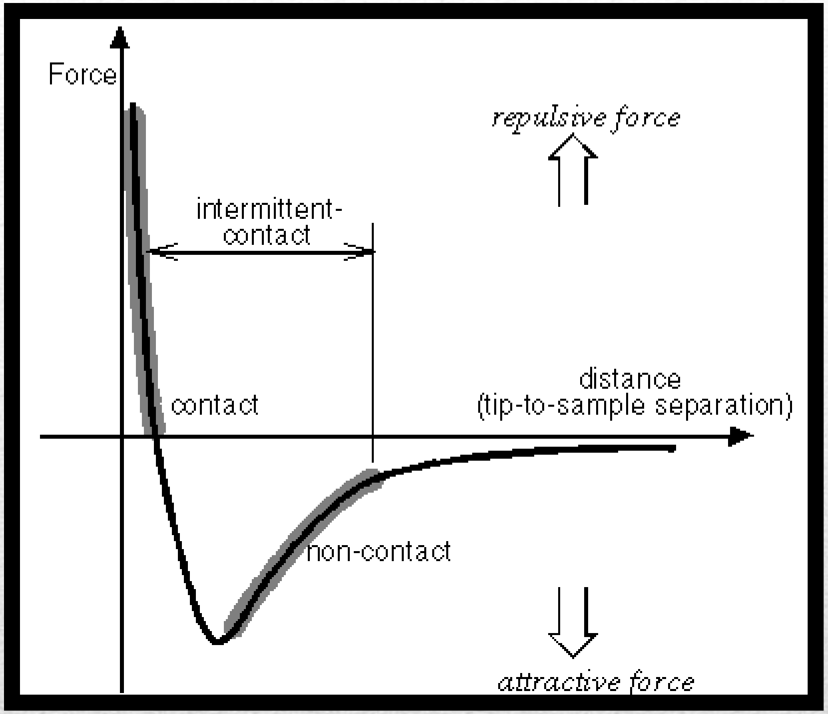
- Contact mode operates in the repulsive regime of the van der Waals curve
- Tip attached to cantilever with low spring constant (lower than effective spring constant binding the atoms of the sample together).
- In ambient conditions there is also a capillary force exerted by the thin water layer present
(2-50 nm thick).



van der Waals force curve

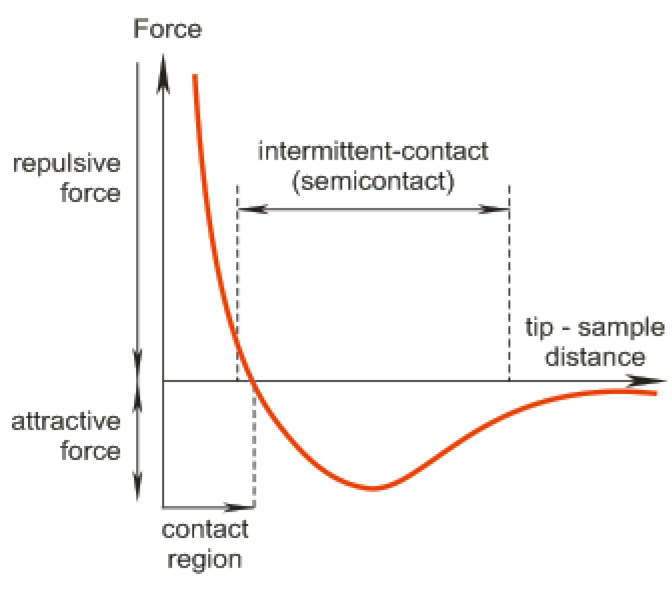
Non-Contact Mode

- Uses attractive forces to interact surface with tip
- Operates within the van der Waal radii of the atoms
- Oscillates cantilever near its resonant frequency (~ 200 kHz) to improve sensitivity
- Advantages over contact: no lateral forces, non-destructive/no contamination to sample.



van der Waals force curve

- Contact mode (short-range)
- Tapping mode
- Phase imaging
- Force modulation mode
- Non-contact Mode



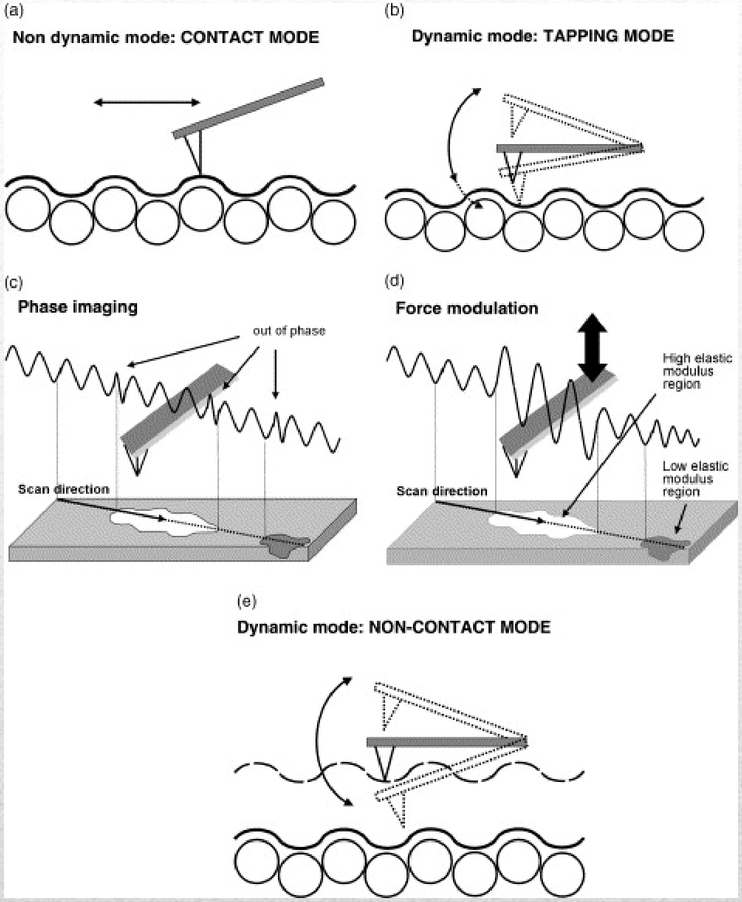
Operation Modes

Contact mode (short-range)

- Tiny cantilever deflections detected by photodiode array
- Tip rastered over sample surface at fixed force (via photodetector-piezo feedback loop) generates topographical image ⇒ analogous to stylus on a record player
- Good for hard samples; *can drag soft materials!*

Contact Mode

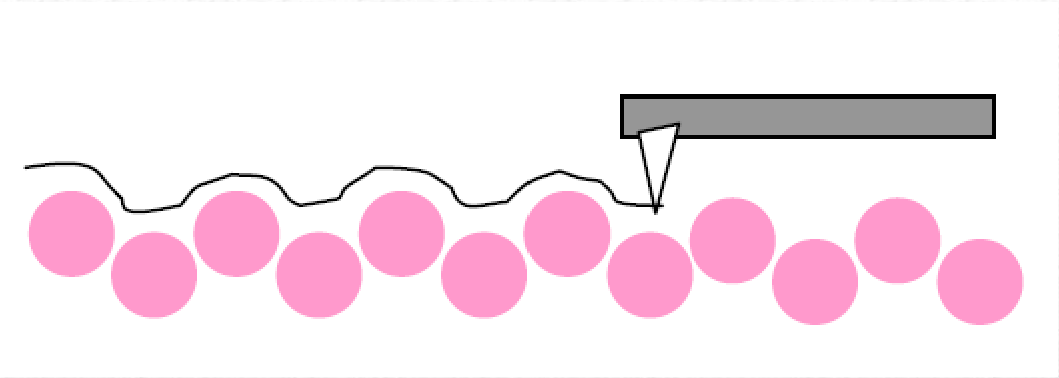
(a) In contact mode the tip is in permanent contact with the sample surface. Owing to the permanent tip–sample contact the shear forces applied to the sample during scanning are significant and potentially damaging to weakly bound molecules such as proteins adsorbed on biomaterials. Nevertheless, contact mode enables extreme high resolution images.

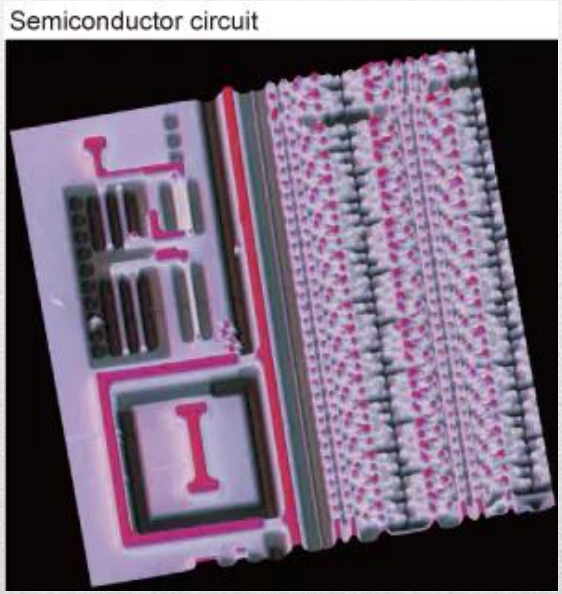


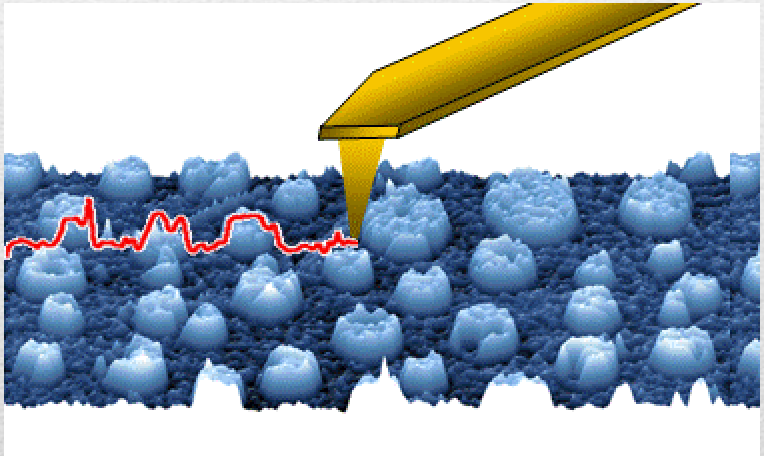
Contact Mode

Contact Mode

- Force applied: nN
- x-y resolution: 1Å (1 x 10^{-10} m)
- z resolution: < 1Å







Hitachi High-Technologies

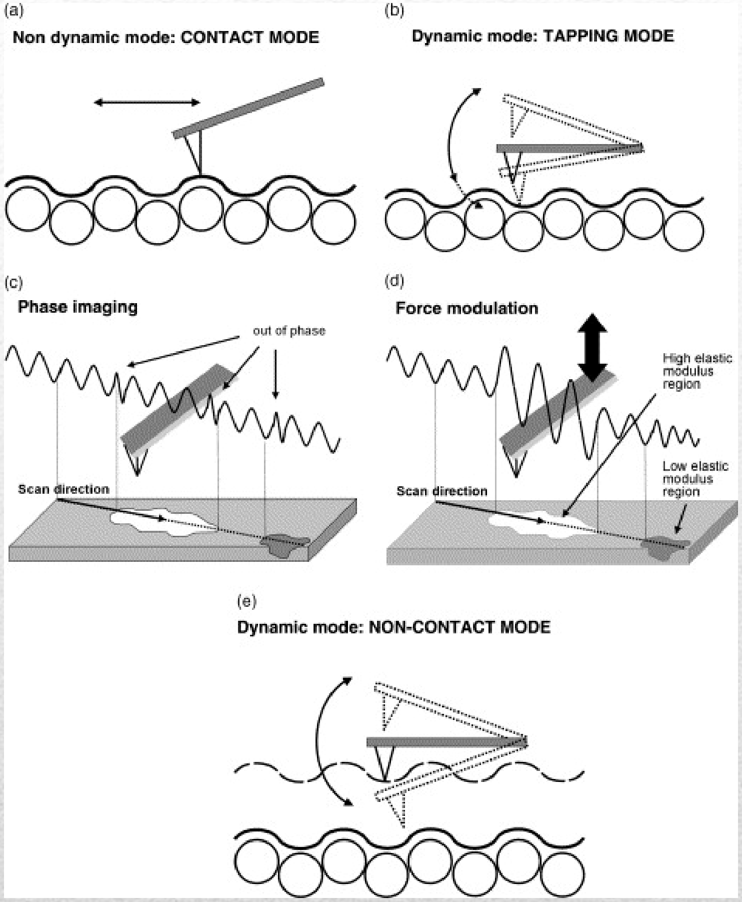
Tapping mode

- Tip oscillates in z-axis at high resonance frequency (ω) (~50-500 kHz in air, 10 kHz in fluids) with intermittent sample contact $\Rightarrow$ 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 $\Rightarrow$ "height" data
- Commonly used for soft samples, aqueous environments

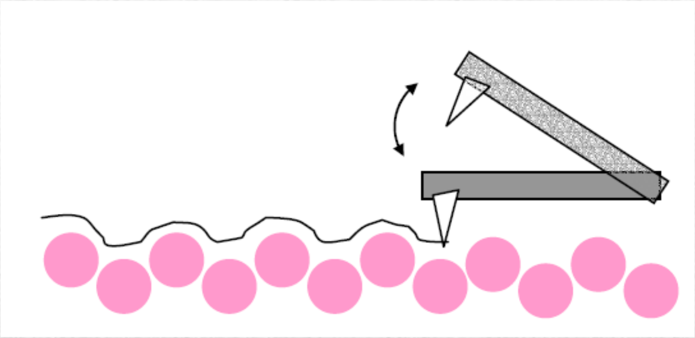
Tapping Mode

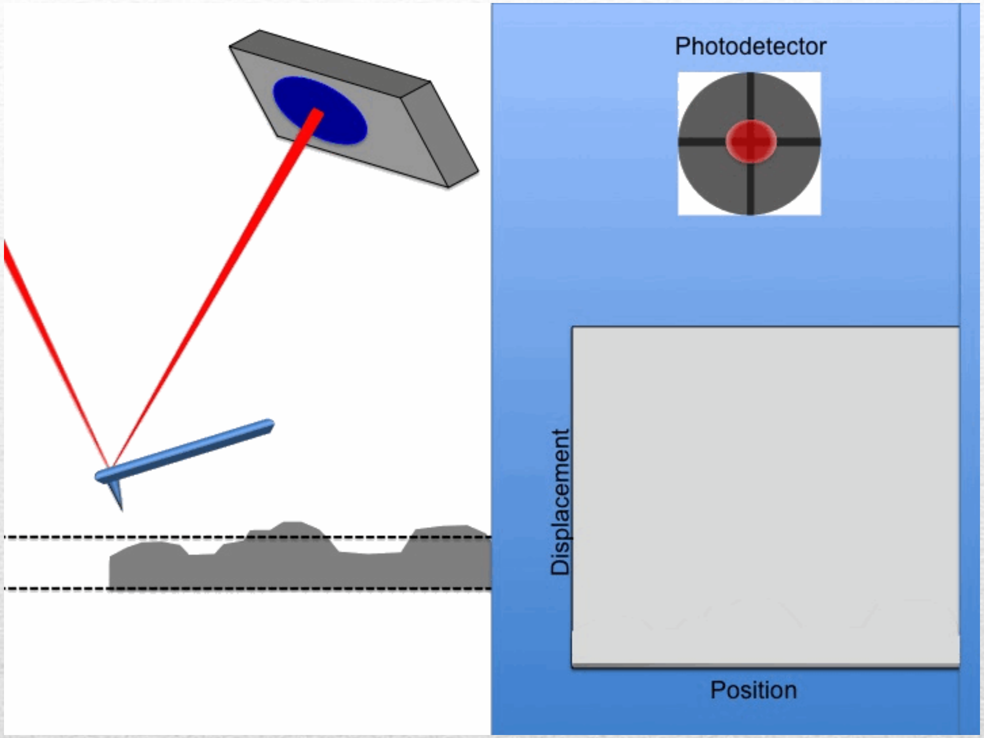
(b) Shows the AFM tapping mode, which uses a tip oscillating at typically 25 kHz. Since the tip is not in contact with the sample during lateral movement during scanning, shear forces applied to the sample by the tip are negligible. Similar to the contact mode, the tapping mode provides information about the sample topography.



Tapping Mode

- x-y resolution: 1-2 nm





Tapping Mode

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

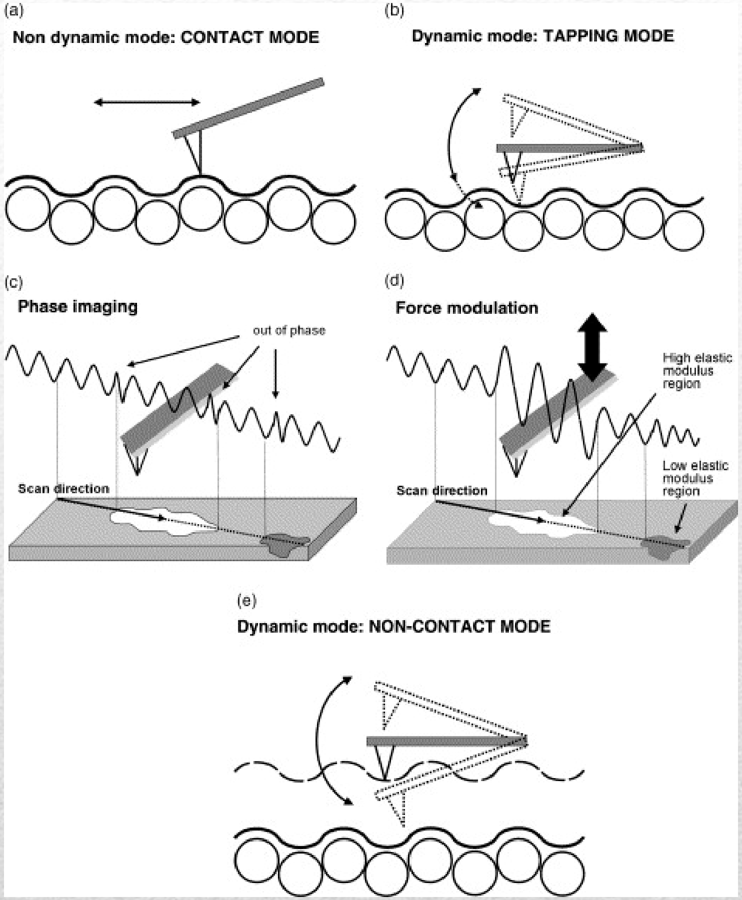
oscillation amplitude attenuation ⇒ “height” data

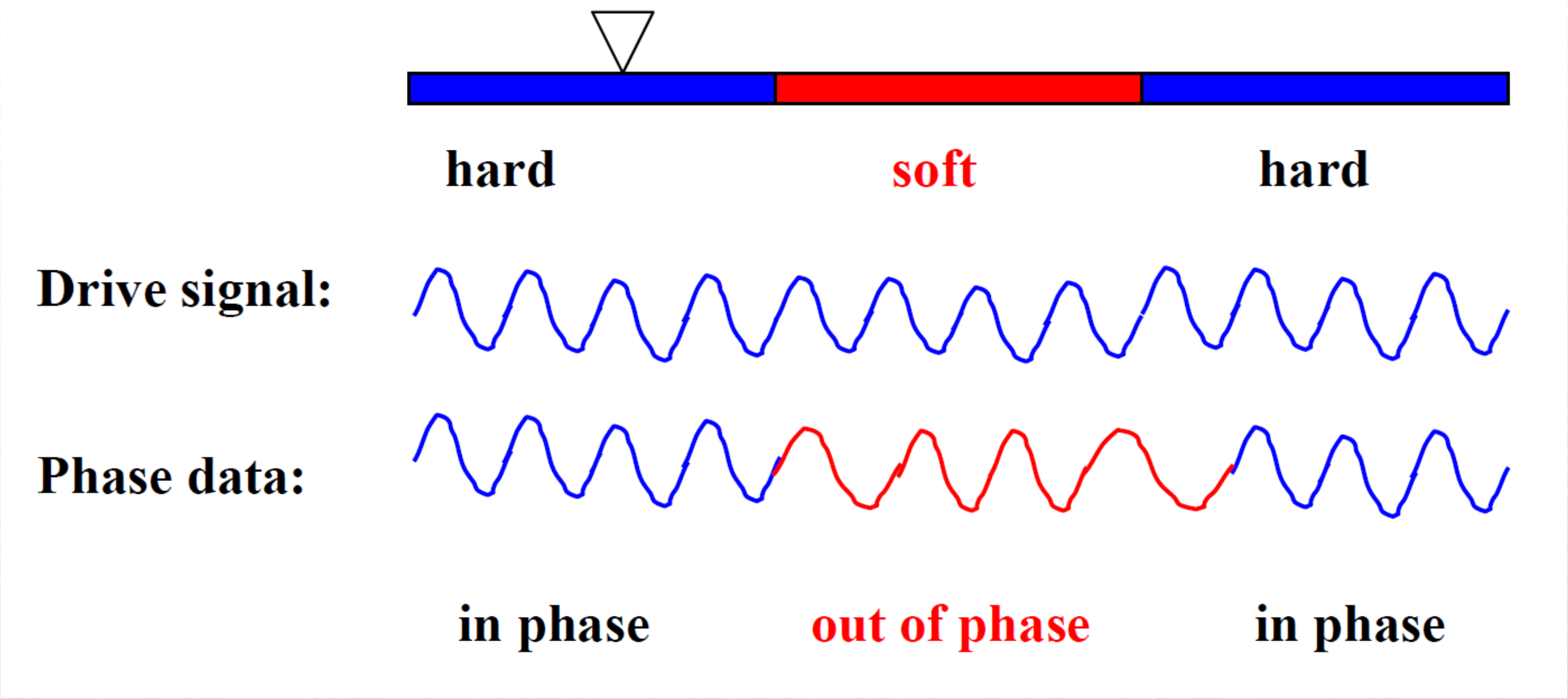
oscillation phase-shift ⇒ “elasticity” map

Phase Imaging Mode

Phase Imaging Mode

In AFM phase imaging shown in (c) variations in materials viscoelasticity lead to different phase lags of the cantilever oscillation, relative to the signal sent to the cantilever's piezooscillation driver. This phase lag is simultaneously monitored by the AFM control electronics, recorded and transformed into AFM images. AFM phase imaging gives non-quantitative information about hardness and elasticity of samples.





Phase Imaging Mode

Force modulation mode

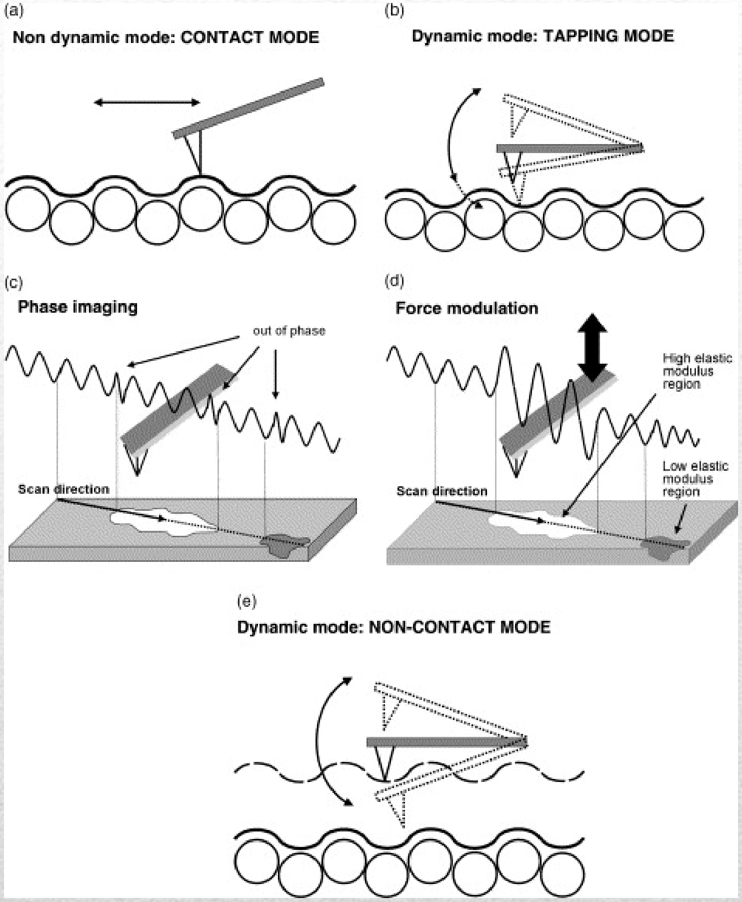
- Tip oscillates in z-axis at $\omega < \omega_o$ (cantilever resonance frequency), making intermittent sample contact; ω ~3-120kHz.
- Interactions between tip and sample cause amplitude attenuation
- Contact force applied to sample is modulated, giving elasticity information

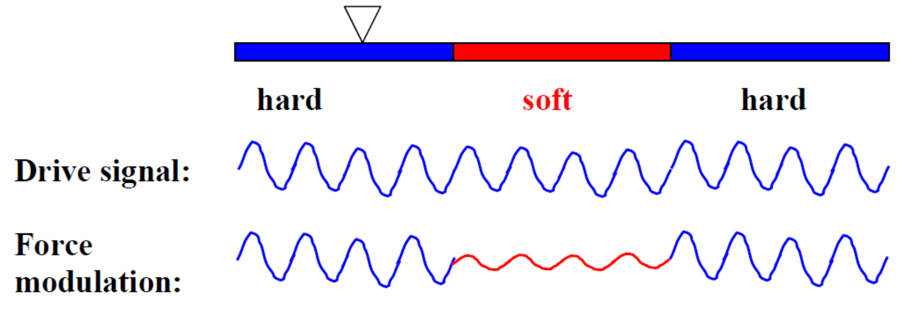
cantilever deflection amplitude $\Rightarrow$ "elasticity" map

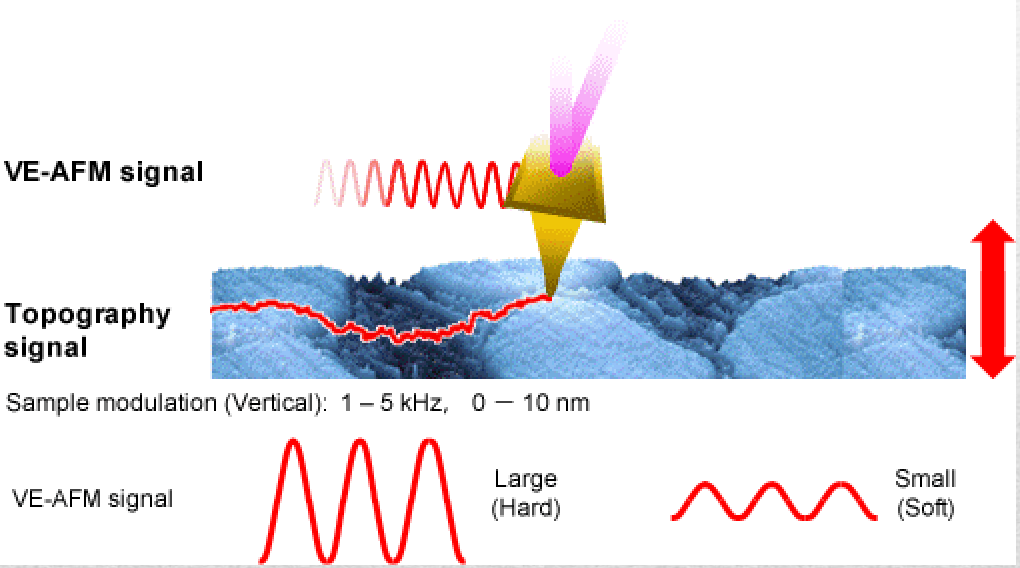
Force Modulation Mode

Force Modulation Mode

(d) shows the force modulation mode (FMM). Compared to tapping mode, in this mode an additional sinusoidal modulation is applied to the cantilever while the tip scans the surface. Thus, the contact force applied to the sample is modulated. From the RMS amplitude of deflection of the cantilever, information about the mechanical properties (stiffness) of the sample can be obtained with a lateral resolution of about 10 nm or better.

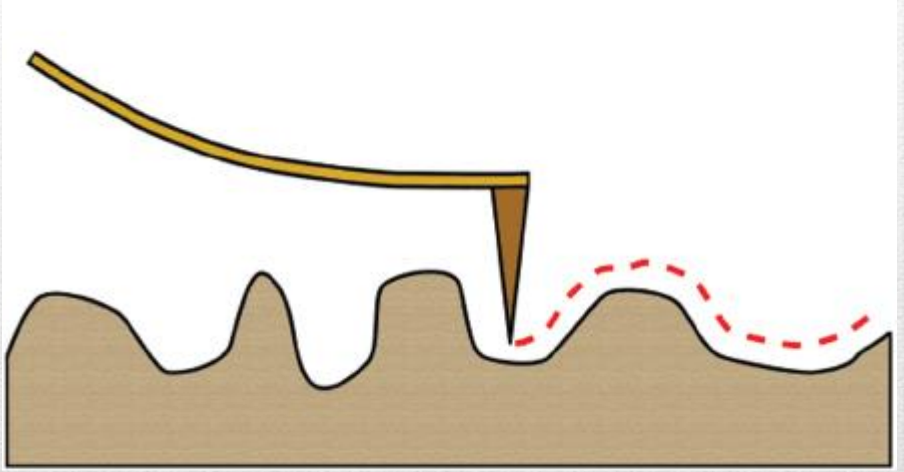






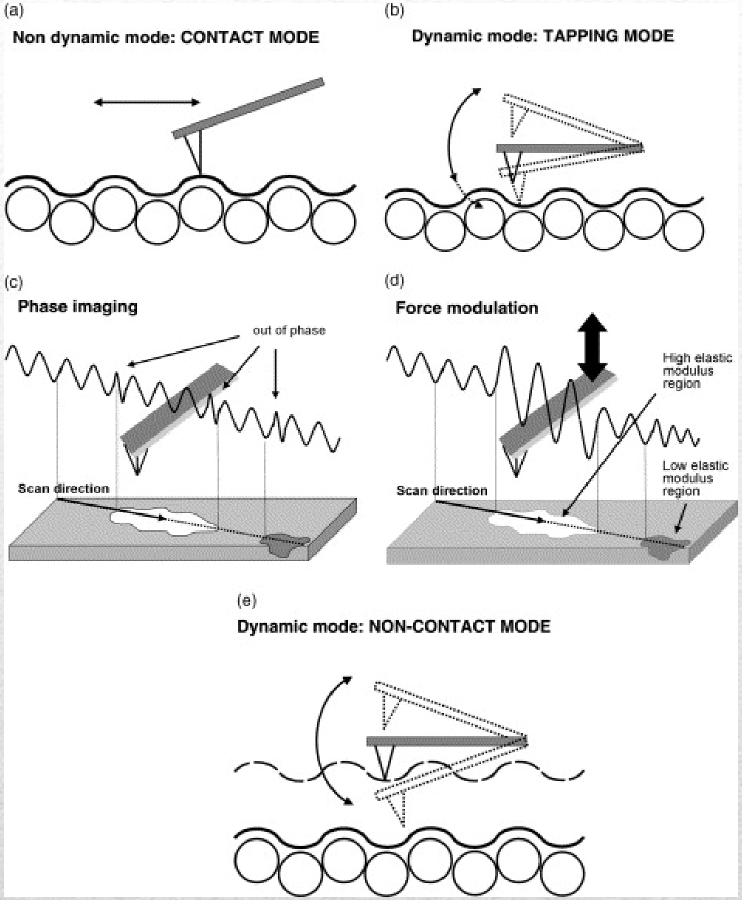
Force Modulation Mode

- The cantilever tip is placed at the attractive force region (i.e., attractive van der Waals forces), and force gradients are detected.
- The attractive forces are usually small compared to repulsive forces.
- Oscillation near resonance frequency *without* tip-surface contact (typical F <1 pN)
- Force gradients from surface interactions shift resonance frequency
- Force gradients used to map secondary interactions (difficult in fluids due to damping; good for soft samples)



Non-Contact Mode

In non-contact AFM modes shown in (e), the cantilever tip is placed at the attractive force region (i.e., attractive van der Waals forces), and force gradients are detected. The attractive forces are usually small compared to repulsive forces. The force gradients can be detected either from shifts in the resonance frequency of the cantilever or the amplitude and the phase of the cantilever. The advantages of these approaches are the high sensitivity of gradient measurements and that small forces are applied to the sample.



Non Contact Mode

Biomaterials-relevant AFM Studies

- Protein adsorption
- Cell membrane proteins
- Initiation of clot formation
- Ligand-receptor interactions
- Cell adhesion
- Surface topography
- Surface elasticity
- Protein structure

Applications of AFM