# SURFACE PROPERTIES **AND SURFACE CHARACTERIZATION OF BIOMATERIALS**

- Biomaterials "show" to the world (and the biological environment) only surfaces. Atoms and molecules make up the outermost surface of a biomaterial (the interface between the material and the world).

- The success or failure of many biomaterials depends on the physical and chemical characteristics of their surface.

- Surface properties dictate interactions between a material and its environment, and thus indicate whether a permanently implanted material will be tolerated or rejected

Atoms and molecules that reside at the surface of a biomaterial have special reactivity and direct biological response.
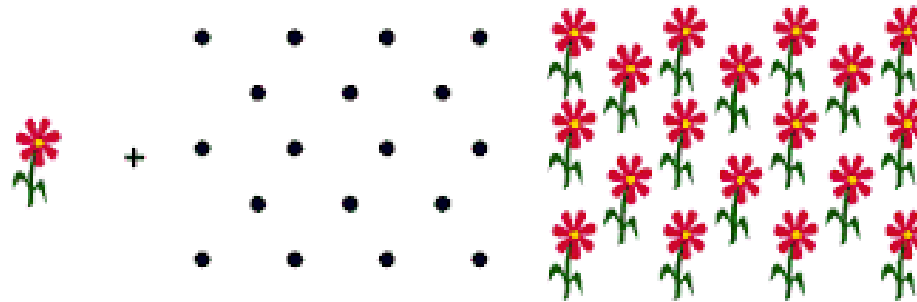
General Surface Considerations

- A two-dimensional representation of a crystal lattice suggesting bonding orbitals (red or pink ovals).

(a)
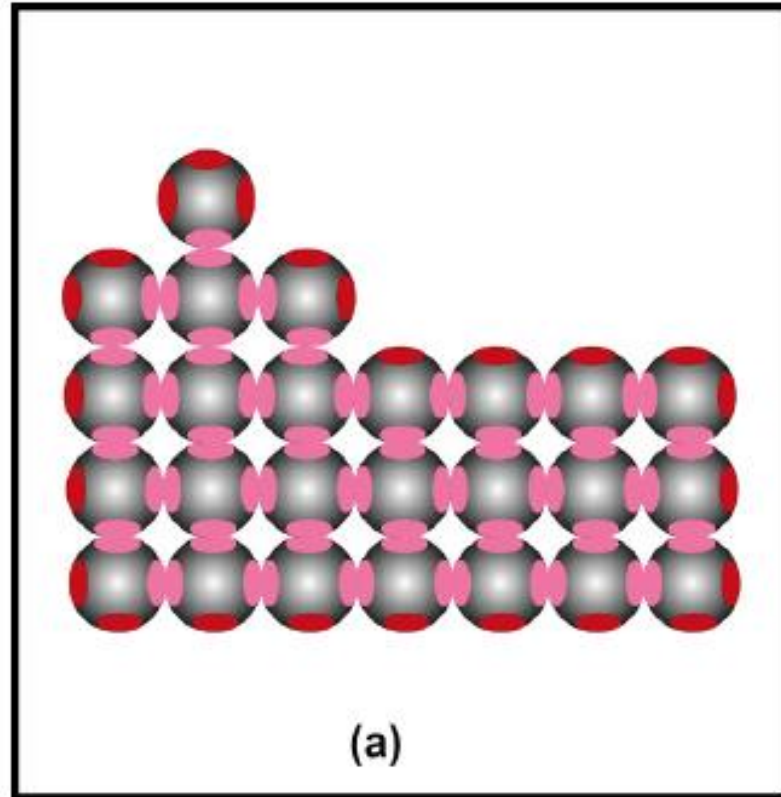
Crystal structure = lattice + basis

o A lattice is a set of regular and periodic geometrical points in space

o A basis is a collection of atoms or molecules at a lattice point

o A crystal is a collection of atoms or molecules arranged at all the lattice points.
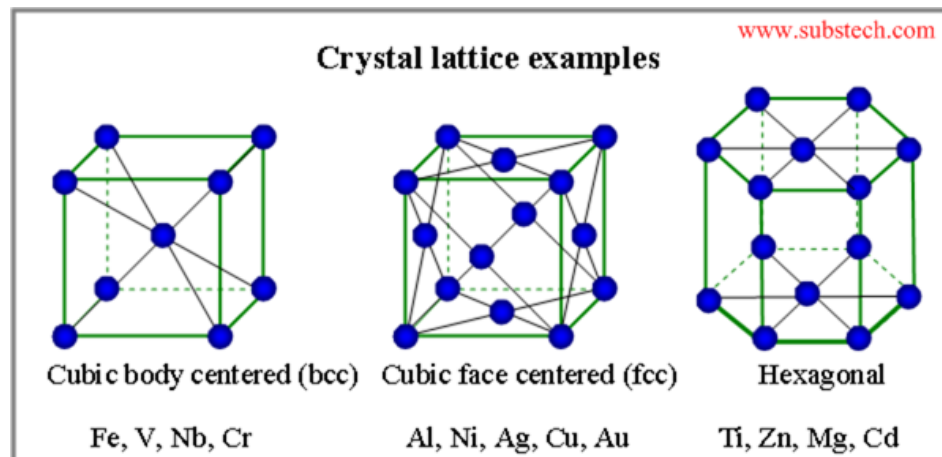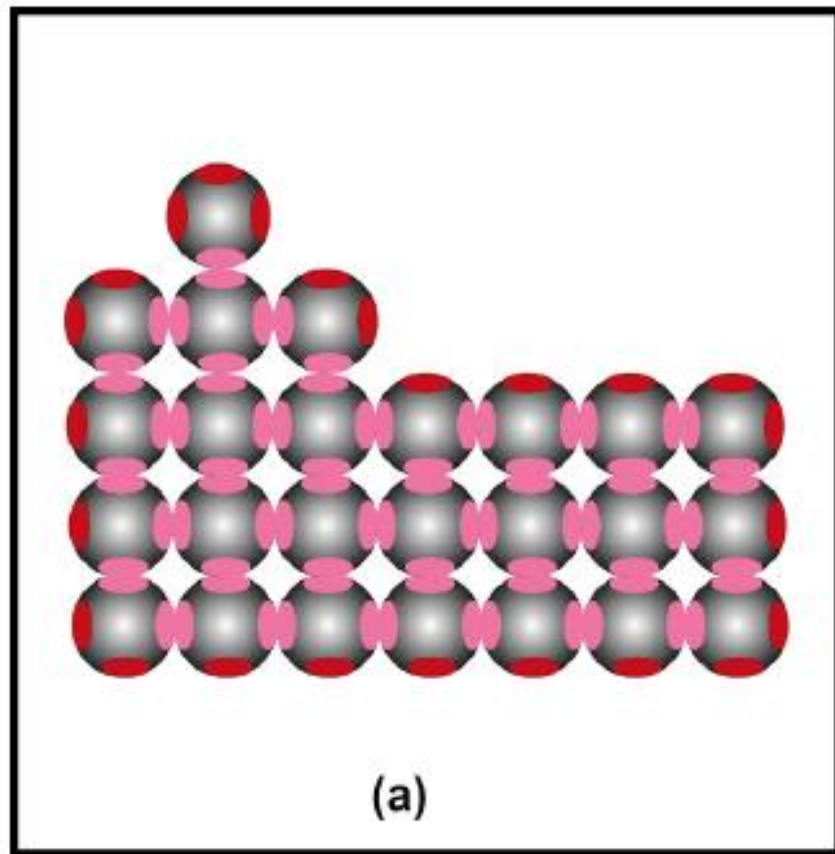
Basis   +   Lattice   =   Crystal

Crystal lattice examples

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Cubic body centered (bcc)   Cubic face centered (fcc)   Hexagonal

Fe, V, Nb, Cr   Al, Ni, Ag, Cu, Au   Ti, Zn, Mg, Cd

(a)

- For atoms in the center (bulk) of the crystal (pink ovals), all binding sites have associations with those of other atoms (sharing electrons).

- At planar exterior surfaces, one of the bonding sites is unfulfilled (red oval).

- At corners, two bonding sites are unfulfilled.

- The single atom on top of the crystal (an adatom) has three unfulfilled valencies.

- If we cleave a "real-world" material (a block of metal from an orthopedic device, for example), we should find

  - hydrocarbon on the outermost layer (perhaps 3 nm, surface energy ~22 ergs/cm^2),
  - polar organic molecules (>1 nm, surface energy ~45 ergs/cm^2)
  - adsorbed water (<1 nm, surface energy ~72 ergs/cm^2)
  - metal oxide (approximately 5 nm, surface energy ~200 ergs/cm^2)
  - the uniform bulk interior (surface energy ~1000 ergs/cm^2).

hydrocarbon   adsorbed H$_2$O   bulk
polar organics   metal oxide

(b)
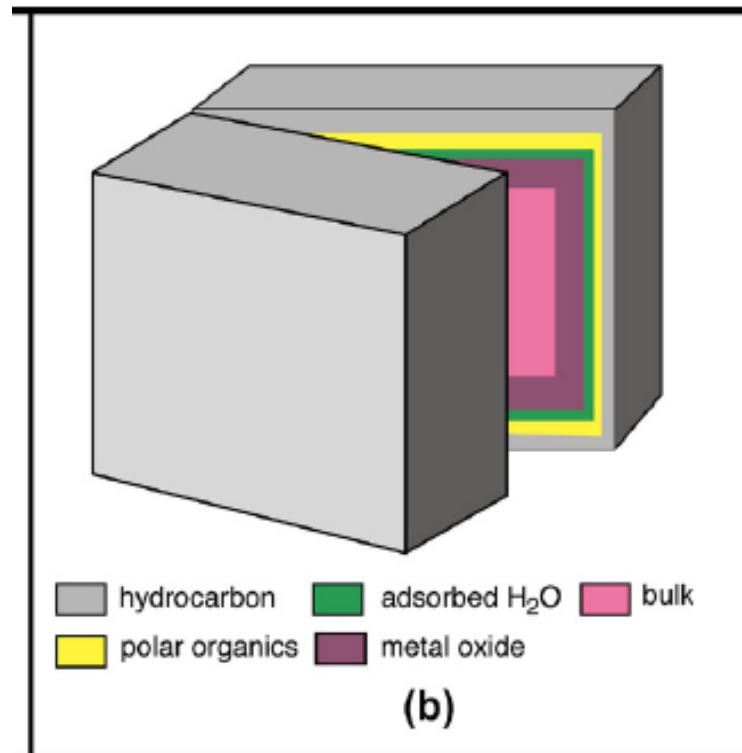
- The movement of atoms and molecules near the surface in response to the outside environment is often highly significant.
- In response to a hydrophobic environment (e.g., air), more hydrophobic (lower energy) components may migrate to the surface of a material – a process that reduces interfacial energy
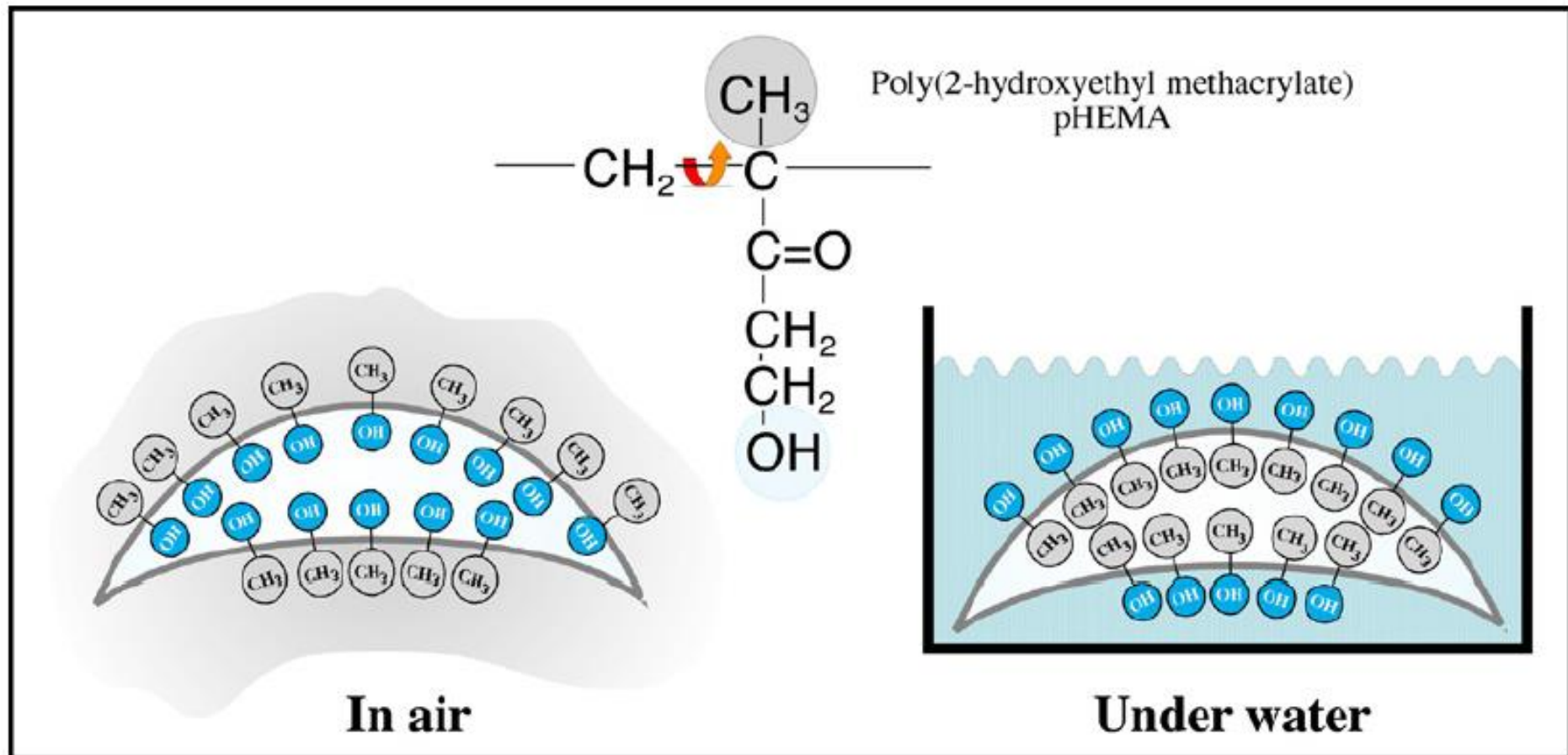
FIGURE I.1.5.2 Many materials can undergo a reversal of surface structure when transferred from air into a water environment. In this schematic illustration, a hydroxylated polymer (for example a pHEMA contact lens) exhibits a surface rich in methyl groups (from the polymer chain backbone) in air, and a surface rich in hydroxyl groups under water. This has been observed experimentally, see Ratner et al. (1978). *J Appl Polym Sci*, 22, 643; Chen et al. (1999). *J Am Chem Soc*, 121(2); 446.

Five Points About Surfaces

1. Surfaces have unique reactivity
2. The surface is inevitably different from the bulk
3. The mass of material that makes up the surface zone is very small
4. Surfaces readily contaminate
5. Surface molecules can exhibit considerable mobility.

Definition

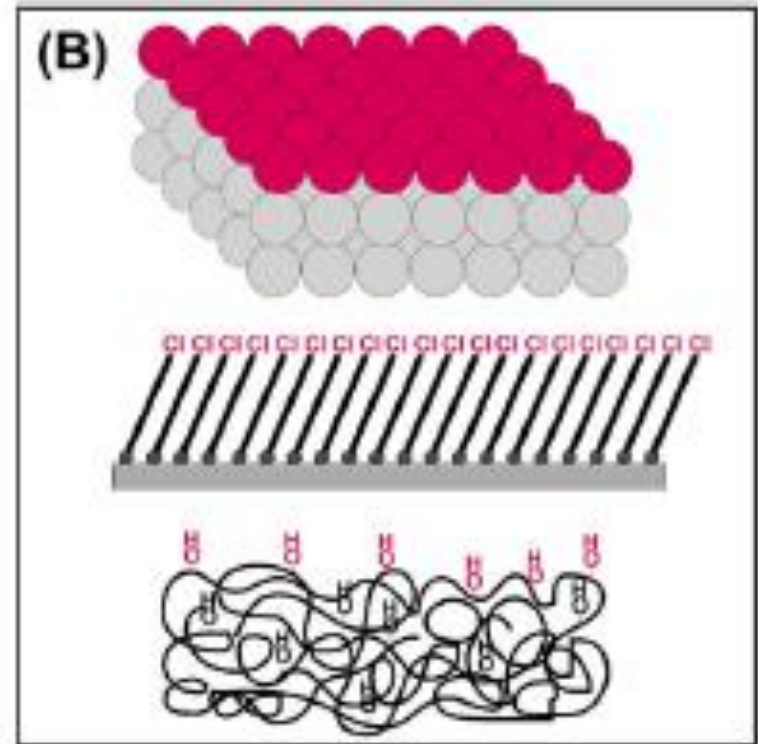- When we say "surface," a question that immediately comes to mind is: "how deep into the material does it extend?"

  - **the surface is the zone where the structure and composition differs from the average (bulk) composition and structure.**

What Surface Properties are we Interested in?
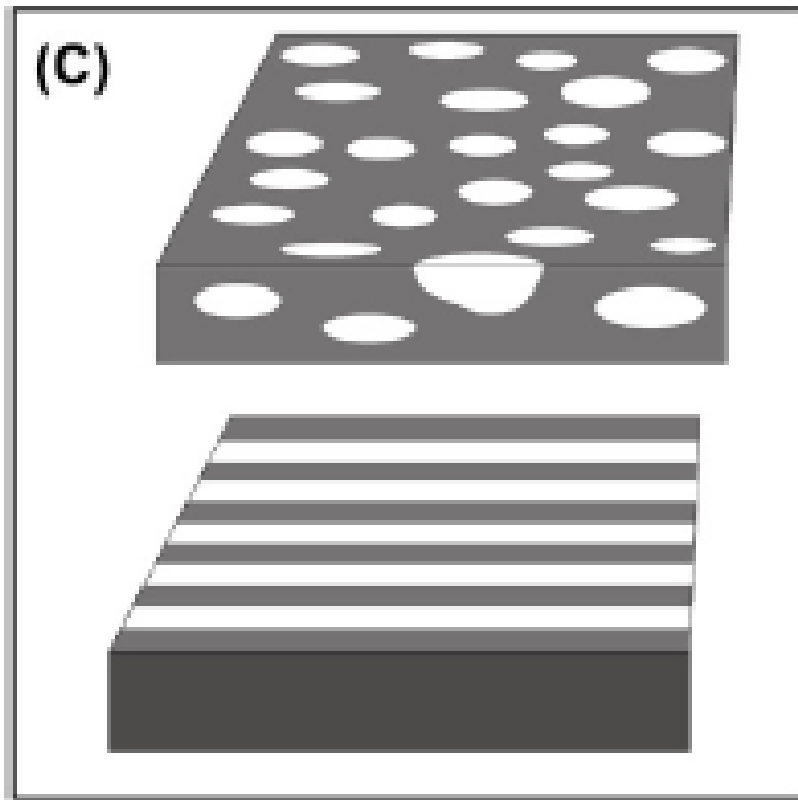
- A surface is fully described by many parameters
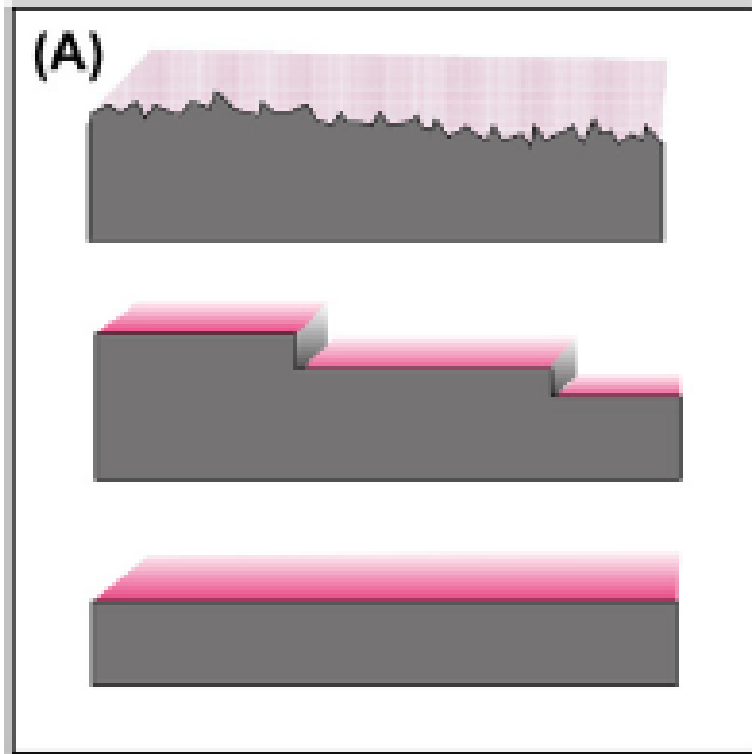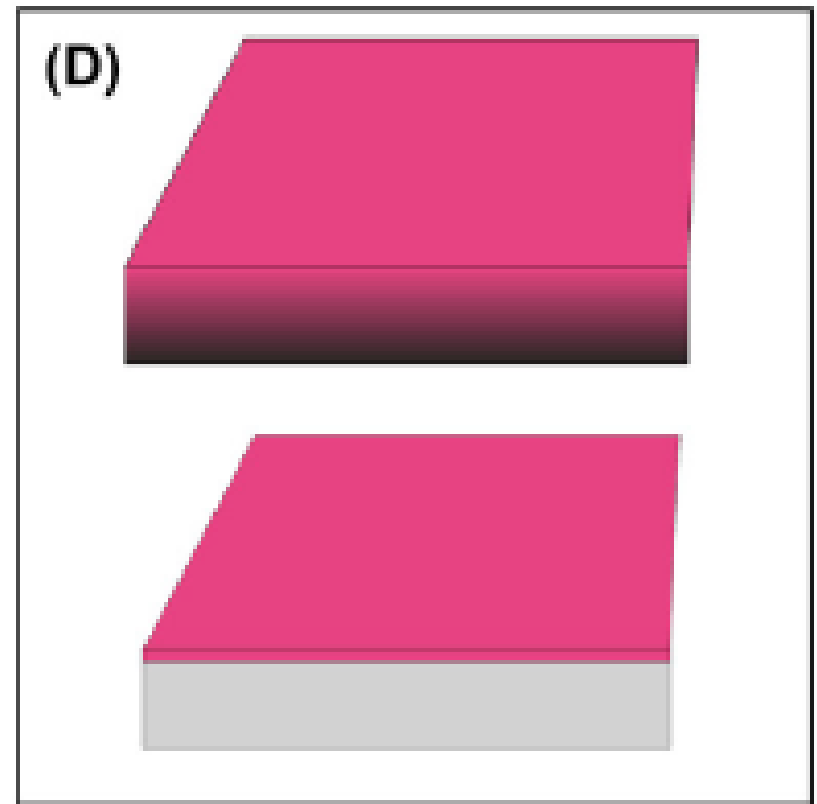
(A) Surfaces can be rough, stepped or smooth.

(B) Surfaces can be comprised of different chemistries

(C) Surfaces may be structurally or compositionally inhomogeneous in the plane of the surface such as phase-separated domains or micro-contact printed lanes.

(D) Surfaces may be inhomogeneous with depth into the specimen or simply overlayered with a thin film.
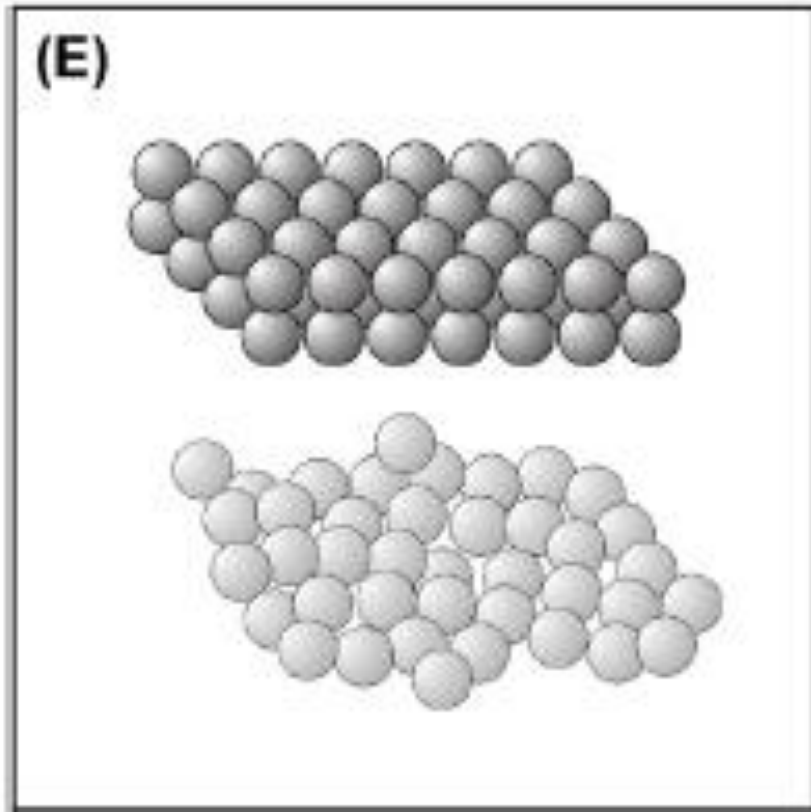
(E) Surfaces may be highly crystalline or disordered.
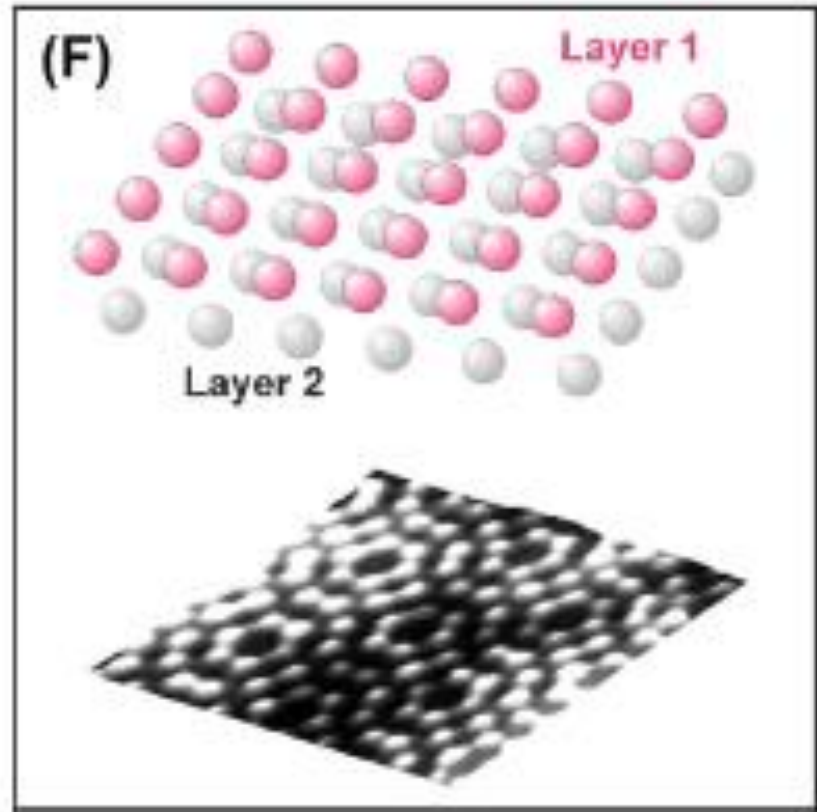
(F) Crystalline surfaces are found with many organizations such as a silicon (100) unreconstructed surface or a silicon (111) (7 × 7) reconstructed surface.

- The more of these parameters we measure, the more we can piece together a complete description of the surface.

- A complete characterization requires a series of techniques to examine the many facets that contribute to the surface properties.

- Unfortunately, we cannot yet specify which parameters are most important for understanding specific biological responses to surfaces.

- Studies have been published on the importance of
    o roughness
    o patterns
    o wettability
    o surface mobility
    o chemical composition
    o electrical charge
    o crystallinity
    o modulus
   o Heterogeneity

on biological reaction.

- We cannot be certain which surface factors are predominant in each situation so variable or variables must be independently measured and correlated.
- For this purpose; we use surface analysis techniques

SURFACE ANALYSIS TECHNIQUES: PRINCIPLES AND METHODS

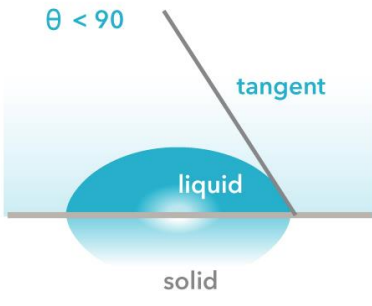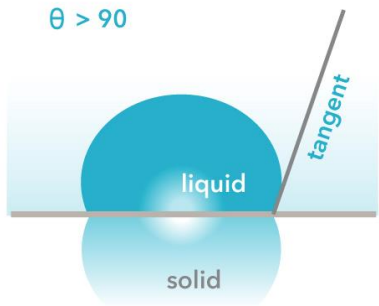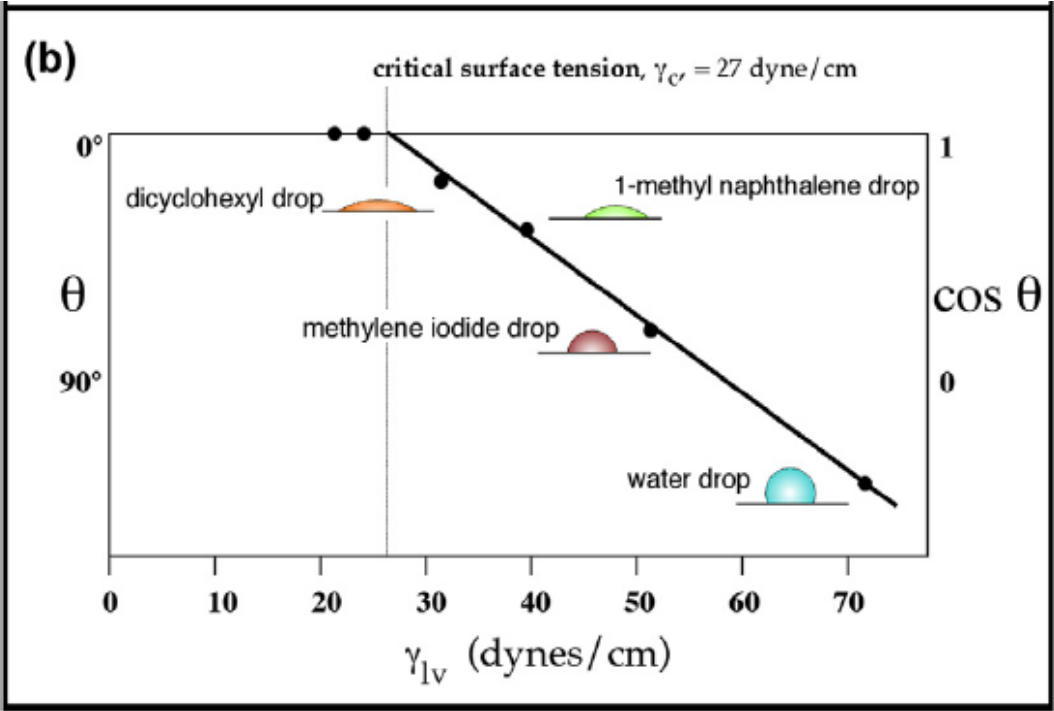| TABLE I.1.5.1	Common Methods to Characterize Biomaterial Surfaces				
Method	Principle	Depth Analyzed	Spatial Resolution	Analytical Sensitivity	Cost
Contact Angles	Liquid wetting of surfaces is used to estimate the energy of surfaces	3–20 Å	1 mm	Low or high depending on the chemistry	$
ESCA (XPS)	X-rays induce the emission of electrons of characteristic energy	10–250 Å	10–150 μm	0.1 atom %	$$$
Auger Electron Spectroscopy*	A focused electron beam stimulates the emission of Auger electrons	50–100 Å	100 Å	0.1 atom %	$$$
SIMS	Ion bombardment sputters secondary ions from the surface	10 Å–1 μm**	100 Å	Very high	$$$
FTIR-ATR	IR radiation is adsorbed and excites molecular vibrations	1–5 μm	10 μm	1 mole %	$$
STM	Measurement of the quantum tunneling current between a metal tip and a conductive surface	5 Å	1 Å	single atoms	$$
SEM	Secondary electron emission induced by a focused electron beam is spatially imaged	5 Å	40 Å typically	High, but not quantitative	$$

➢ **Contact Angle Method**
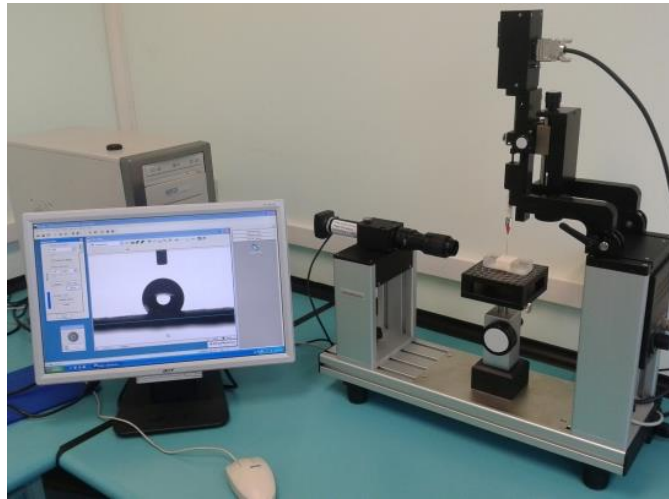
- A drop of liquid sitting on a solid surface represents a simple, but potentially powerful, method to probe surface properties.
- The phenomenon of the contact angle can be explained as a balance between the force with which the molecules of the drop liquid are being attracted to each other (a cohesive force) and the attraction of the liquid molecules for the surface (an adhesive force).
- An equilibrium is established between surface tension forces contracting a liquid drop to a spherical shape and forces interacting the drop with the surface.
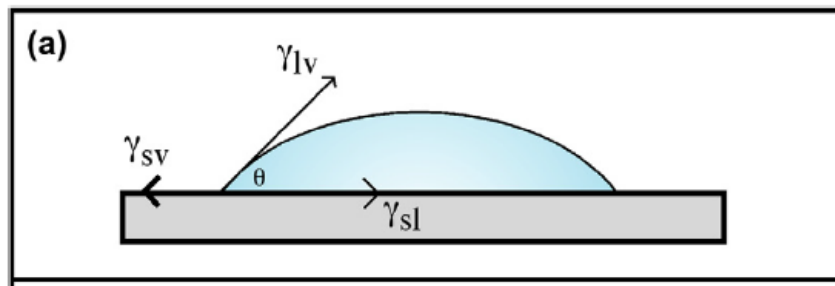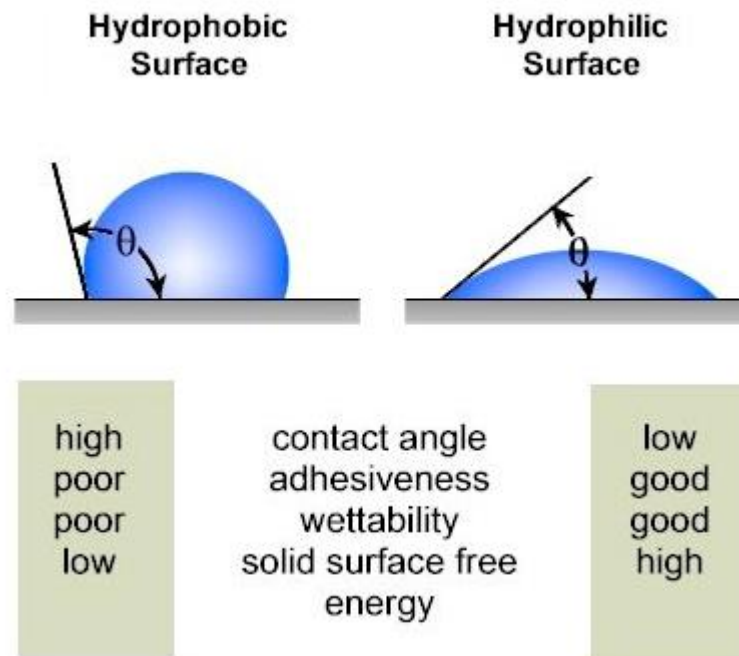

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos\theta$$

The force balance between the liquid–vapor surface tension (γ_{lv}) of a liquid drop and the interfacial tension between a solid and the drop (γ_{sl}), manifested through the contact angle (θ) of the drop can be used to quantitatively characterize the surface–vapor interfacial tension (γ_{sv}).

- The Zisman method permits a critical surface tension value, an approximation to the solid surface tension, to be measured.

- Drops of liquids of different surface tensions are placed on the solid, and the contact angles of the drops are measured.

(b) critical surface tension, $\gamma_{c'} = 27$ dyne/cm

θ

$0°$ — 1

dicyclohexyl drop

1-methyl naphthalene drop

methylene iodide drop

$90°$ — 0 — $\cos \theta$

water drop

$0 \quad 10 \quad 20 \quad 30 \quad 40 \quad 50 \quad 60 \quad 70$

γ_{lv} (dynes/cm)

$\theta > 90$ $\theta < 90$

tangent   tangent

liquid   liquid

solid   solid

	contact angle	
high	adhesiveness	low
poor	wettability	good
poor	solid surface free	good
low	energy	high
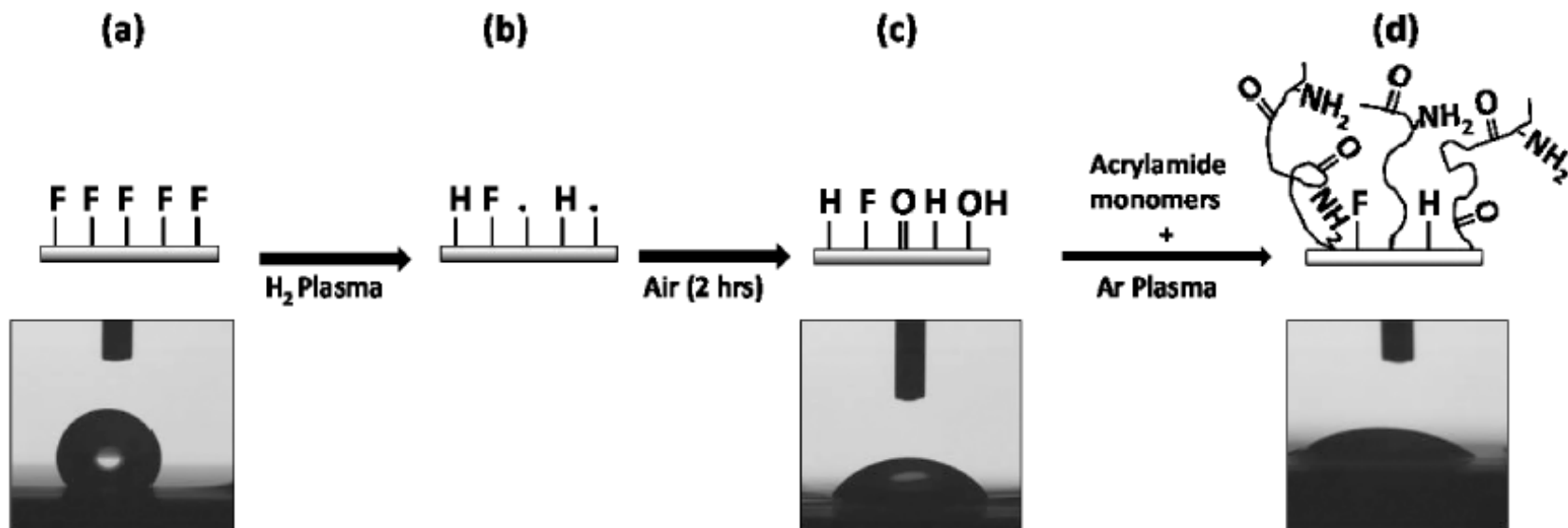
- Some critical surface tensions for common materials

TABLE I.1.5.2	Critical Surface Tension Values for Common Polymeric Materials Calculated from Contact Angle Measurements
Material	**Critical Surface Tension (dynes/cm)**
Polytetrafluoroethylene	19
Poly(dimethyl siloxane)	24
Poly(vinylidine fluoride)	25
Poly(vinyl fluoride)	28
Polyethylene	31
Polystyrene	33
Poly(2-hydroxyethyl methacrylate)	37
Poly(vinyl alcohol)	37
Poly(methyl methacrylate)	39
Poly(vinyl chloride)	39
Polycaproamide (nylon 6)	42
Poly(ethylene oxide)-diol	43
Poly(ethylene terephthalate)	43
Polyacrylonitrile	50

Contact angles directly measure surface wettability, and indirectly probe surface energy, roughness, heterogeneity, contamination, and molecular mobility.

- Contact angle measurements provide a "first line" characterization of materials and can be performed in any laboratory.

- Contact angle measurements provide unique insight into how the surface will interact with the external world. However, in performing such measurements, a number of concerns must be addressed to obtain meaningful data

TABLE I.1.5.3	Concerns in Contact Angle Measurement
• The measurement is operator dependent (for manual, goniometer instruments)	
• Surface roughness influences the results	
• Surface heterogeneity influences the results	
• The liquids used are easily contaminated (typically reducing their γ_{lv})	
• Liquid evaporation and temperature changes can impact measurement	
• The liquids used can reorient the surface structure	
• The liquids used can absorb into the surface, leading to swelling	
• The liquids used can dissolve the surface	
• Few sample geometries are appropriate for contact angle measurement	
• Information on surface structure must be inferred from the data obtained	

➢ **Electron Spectroscopy for Chemical Analysis**

- Electron spectroscopy for chemical analysis (ESCA) provides a comprehensive qualitative and quantitative overview of a surface

- In contrast to the contact angle technique, ESCA requires complex, expensive apparatus and demands considerable training to perform the measurements
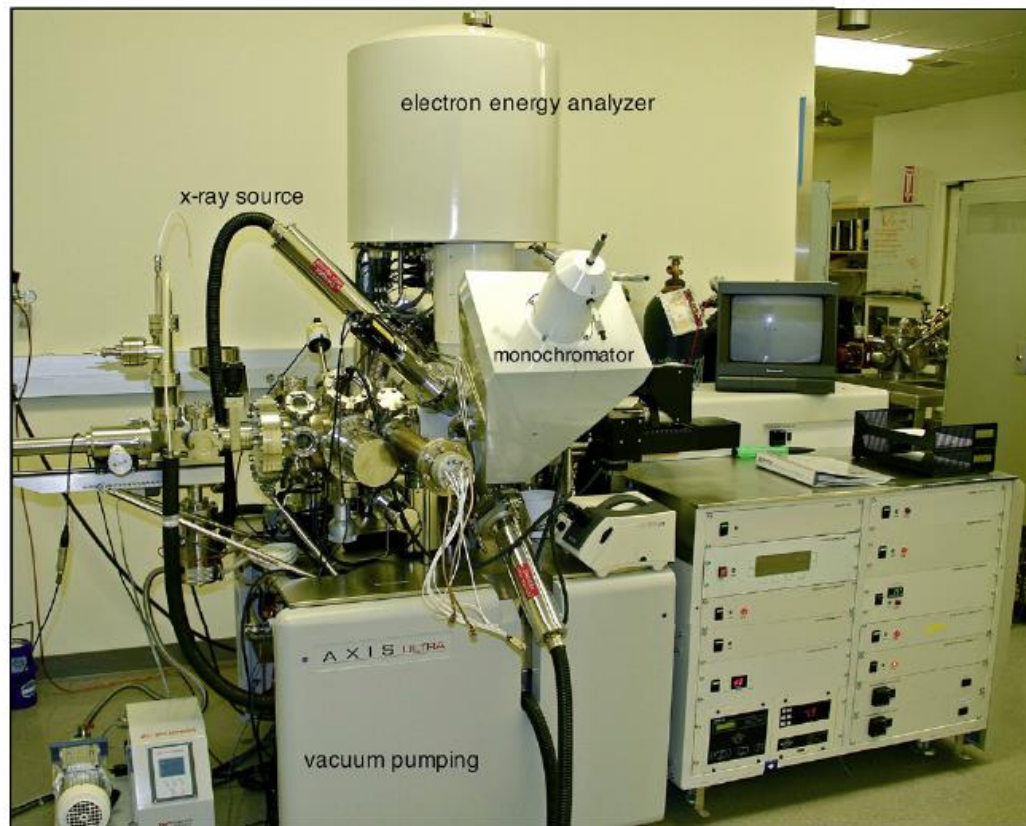
FIGURE I.1.5.6 (a) Photograph of a contemporary ESCA instrument (photo by Kratos Analytical Corp.).

- The ESCA method (also called X-ray photoelectron spectroscopy, XPS) is based upon the photoelectric effect, properly described by Einstein in 1905.
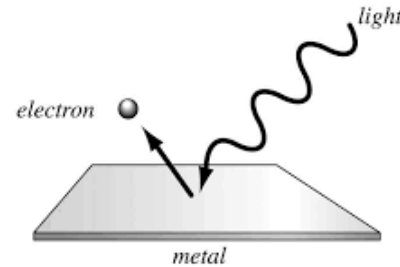
- X-rays are focused upon a specimen. The interaction of the X-rays with the atoms in the specimen causes the emission of core level (inner shell) electrons.

- The energy of these electrons is measured and their values provide information about the nature and environment of the atom or atoms from which they came.

- The basic energy balance describing this process is given by the relationship:

$$BE = h\nu - KE$$

   where

   - BE is the energy binding the electron to an atom (the value desired)

   - KE is the kinetic energy of the emitted electron (the value measured in the ESCA spectrometer)

   - $h\nu$ is the energy of the X-rays, a known value.
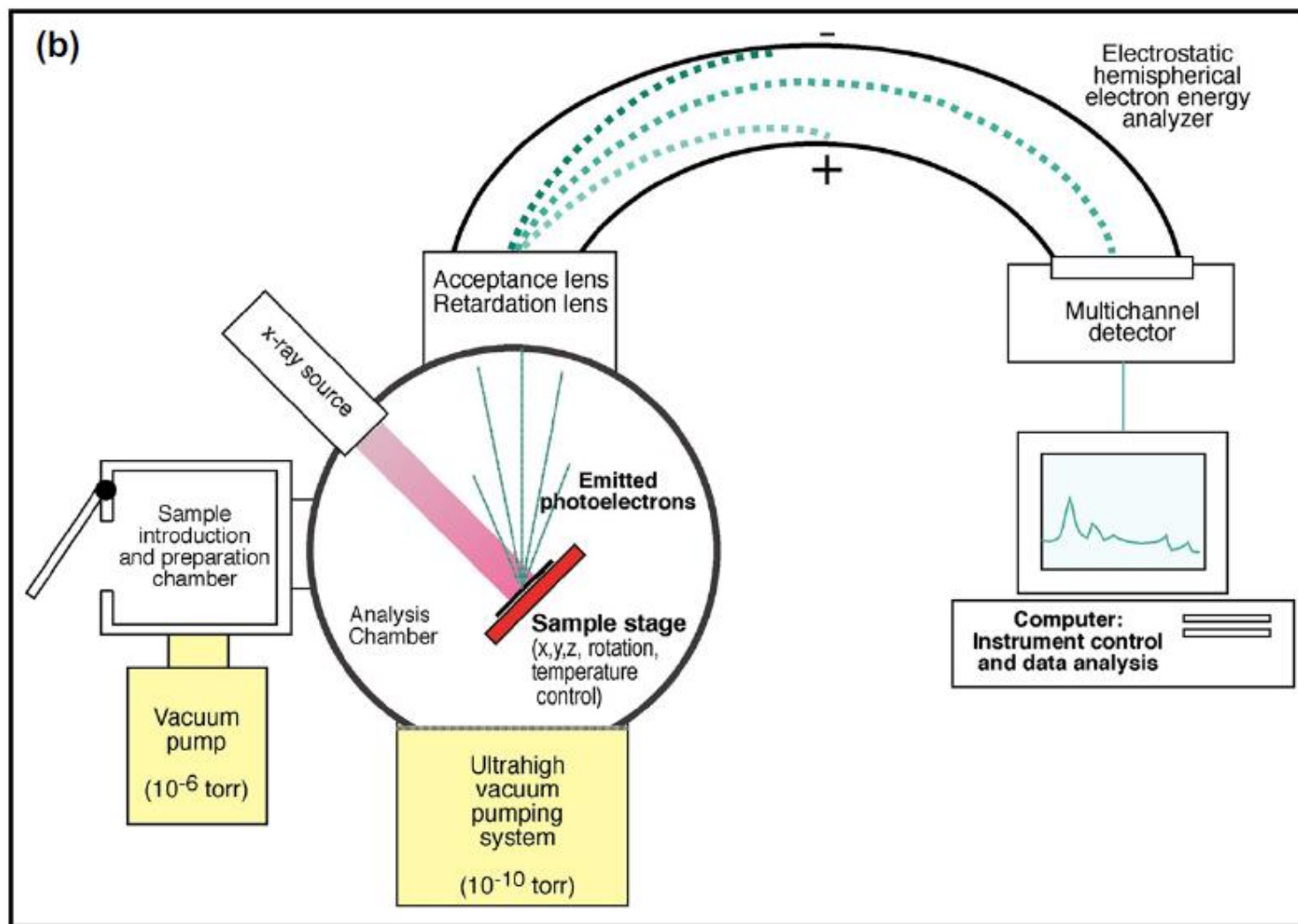
(b) Schematic diagram of a monochromatized ESCA instrument.

- types of information about the nature of a surface that can be obtained by using ESCA

TABLE I.1.5.4	Information Derived From an ESCA Experiment

In the outermost 100 Å of a surface, ESCA can provide:

- Identification of all elements (except H and He) present at concentrations >0.1 atomic %
- Semiquantitative determination of the approximate elemental surface composition (±10%)
- Information about the molecular environment (oxidation state, bonding atoms, etc.)
- Information about aromatic or unsaturated structures from shake-up $\pi^* \leftarrow \pi$) transitions
- Identification of organic groups using derivatization reactions
- Nondestructive elemental depth profiles 100 Å into the sample and surface heterogeneity assessment using angular-dependent ESCA studies and photoelectrons with differing escape depths
- Destructive elemental depth profiles several thousand angstroms into the sample using argon etching (for inorganics)
- Lateral variations in surface composition (spatial resolution 8–150 µm, depending upon the instrument)
- "Fingerprinting" of materials using valence band spectra and identification of bonding orbitals
- Studies on hydrated (frozen) surfaces

ESCA analyzes to approximately 10 nm and gives information on elements present, their concentrations and their bonding environments.

- surface sensitivity of ESCA
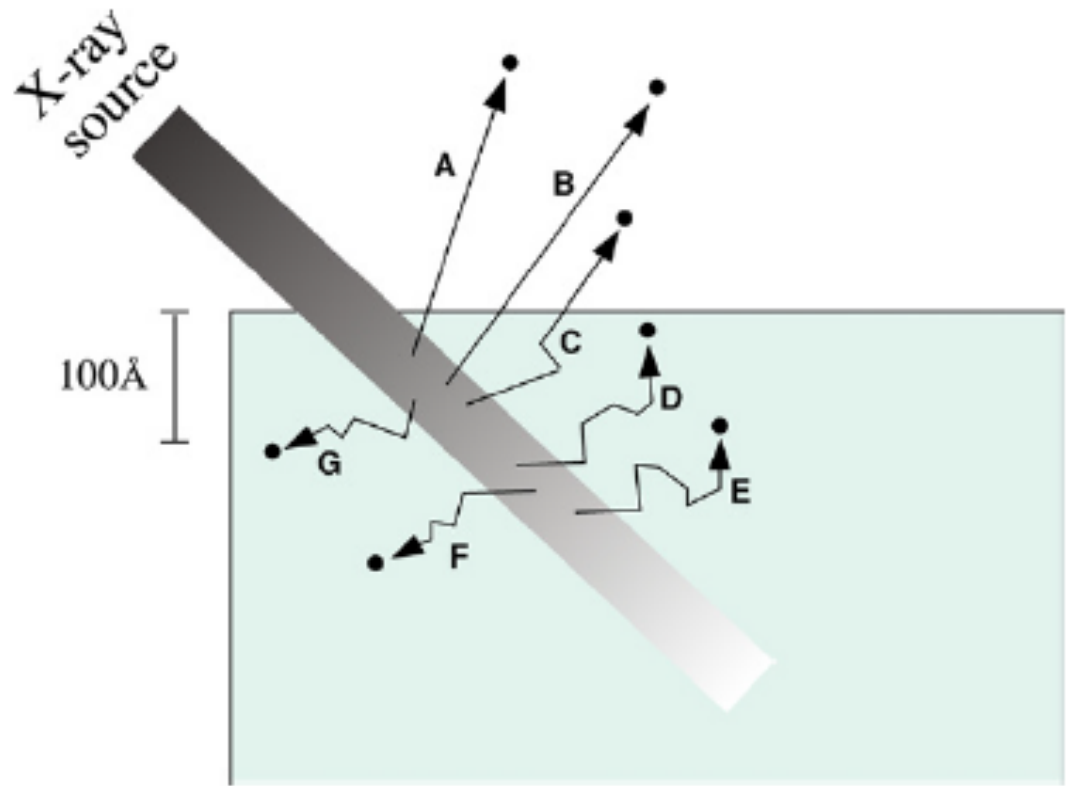
FIGURE I.1.5.7 ESCA is a surface-sensitive method. Although the X-ray beam can penetrate deeply into a specimen, electrons emitted deep in the specimen (D, E, F, G) will lose their energy in inelastic collisions and never emerge from the surface. Only those electrons emitted near the surface that lose no energy (A, B) will contribute to the ESCA signal used analytically. Electrons that lose some energy, but still have sufficient energy to emerge from the surface (C) contribute to the background signal.

- **ESCA has many advantages, and a few disadvantages, for studying biomaterials**

  ➢ **The advantages include**

  o high information content ,
  o surface localization of the measurement (outermost 8–10 nm),
  o speed of analysis,
  o the ability to analyze most samples with no special specimen preparation.

    ▪ This is particularly important since biomedical devices (or parts of devices) can often be inserted, as fabricated and sterilized, directly in the analysis chamber for study.
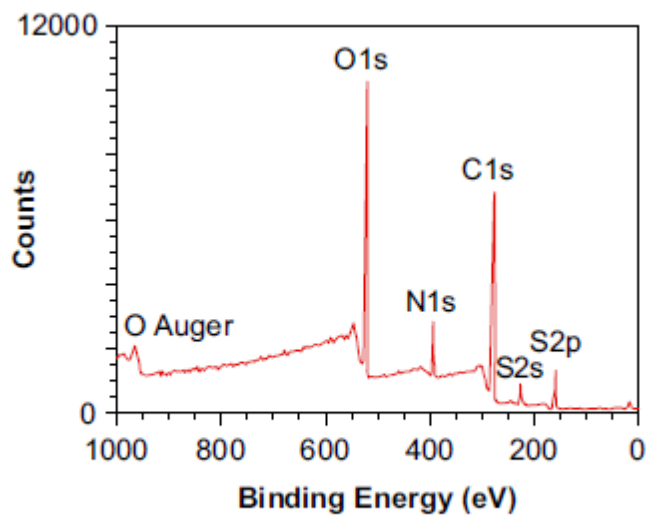
  ➢ **The disadvantages include**

  o the need for vacuum compatibility (i.e., no outgassing of volatile components)
  o the vacuum environment and its impact on the specimen (particularly for hydrated specimens)
  o the possibility of sample damage by X-rays if long analysis times are used
  o the need for experienced operators
 o the cost associated with this complex instrumentation

- **The use of ESCA ; a brief example.**

First, a wide scan is made in which the energies of all emitted electrons over a 1000 eV range are detected

Then, narrow scans are made in which each of the elements detected in the wide scan is examined in higher resolution

FIGURE I.1.5.8 ESCA wide scan of a surface-modified poly(methyl methacrylate) ophthalmologic device.
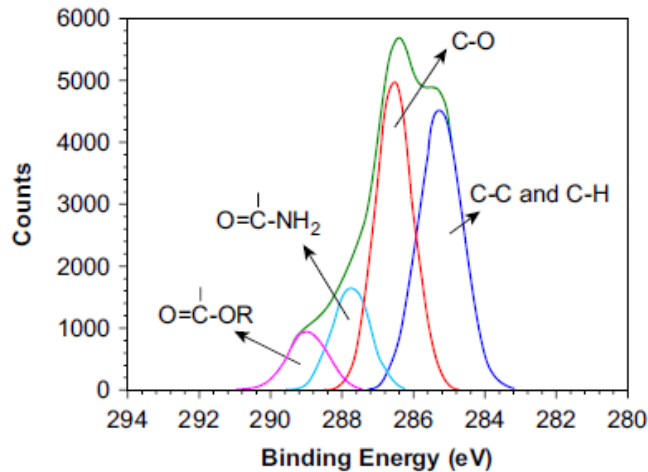
FIGURE I.1.5.9 The carbon 1s narrow scan ESCA spectrum of a surface-modified poly(methyl methacrylate) ophthalmologic device. Narrow scan spectra can be generated for each element seen in low energy resolution mode in Figure I.1.5.8.

- From the wide scan, we learn that the specimen contains carbon, oxygen, nitrogen, and sulfur. The presence of sulfur and nitrogen is unexpected for PMMA. We can calculate atomic percentage composition from the wide scan spectral data. The sample surface contains 58.2% carbon, 27.7% oxygen, 9.5% nitrogen, and 4.5% sulfur.

- The narrow scan for the carbon region (C1s spectrum) suggests four classes of compounds: hydrocarbons; carbons singly bonded to oxygen (the predominant species); carbons in amide-like molecular environments; and carbons in carboxylic acid or ester environments

Secondary Ion Mass Spectrometry

- SIMS produces a mass spectrum of the outermost 1–2 nm of a surface.
- Like ESCA, it requires complex instrumentation and an ultrahigh vacuum chamber for the analysis. However, it provides unique information that is complementary to ESCA, and greatly aids in understanding surface composition.

- Some of the analytical capabilities of SIMS are

| TABLE I.1.5.5	Analytical Capabilities of SIMS	
	Static SIMS	Dynamic SIMS
Identify hydrogen and deuterium	✓	✓
Identify other elements (often must be inferred from the data)	✓	✓
Suggest molecular structures (inferred from the data)	✓	—
Observe extremely high mass fragments (proteins, polymers)	✓	—
Detection of extremely low concentrations	✓	✓
Depth profile to 1 μm into the sample	*	✓
Observe the outermost 1–2 atomic layers	✓	—
High spatial resolution (features as small as approximately 400 Å)	✓	✓
Semiquantitative analysis (for limited sets of specimens)	✓	—
Useful for polymers	✓	—
Useful for inorganics (metals, ceramics, etc.)	✓	✓
Useful for powders, films, fibers, etc.	✓	✓

*Cluster ion sources allow depth profiling with static-SIMS-like information content.

- In SIMS analysis, a surface is bombarded with a beam of accelerated ions. The collision of these ions with the atoms and molecules in the surface zone can transfer enough energy that they sputter* from the surface into the vacuum phase.

- In SIMS, positive ions (cesium, gallium, bismuth, C60-buckyballs, and cluster ions are commonly used) that are accelerated at the surface to be analyzed with energies of 5000–20,000 eV. The particles ejected from the surface are positive and negative ions (secondary ions), radicals, excited states, and neutrals. Only the secondary ions are measured in SIMS.

- In ESCA, the energy of emitted particles (electrons) is measured. SIMS measures the mass of emitted ions (more rigorously, the ratio of mass to charge, m/z) using a time-of-flight (TOF) mass analyzer, magnetic sector analyzer or, in older instruments, a quadrupole mass analyzer.

* Sputtering is a term used to describe the mechanism in which atoms are ejected from the surface of a material when that surface is bombarded by sufficiency energetic particles.
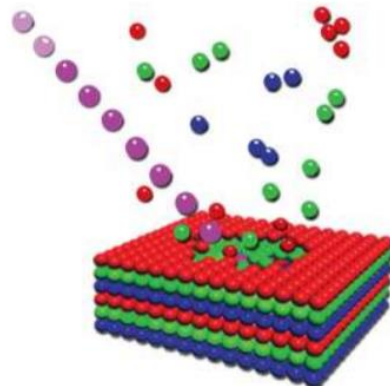
- There are two modes for SIMS analysis, **dynamic and static**.

- Dynamic SIMS uses high ion doses over a given analysis time. Depending on the primary ion used, for example, cesium or gallium, and its energy, the high-flux ion beam can destroy organic samples and relevant organic fragments, and predominantly atomic fragments (e.g., C−, CH−, O−, OH−, Na+, etc.) will be detected.

- Static SIMS, in contrast, induces minimal surface destruction. The ion dose is adjusted so that during the period of the analysis less than 10% of one monolayer of surface atoms is sputtered.
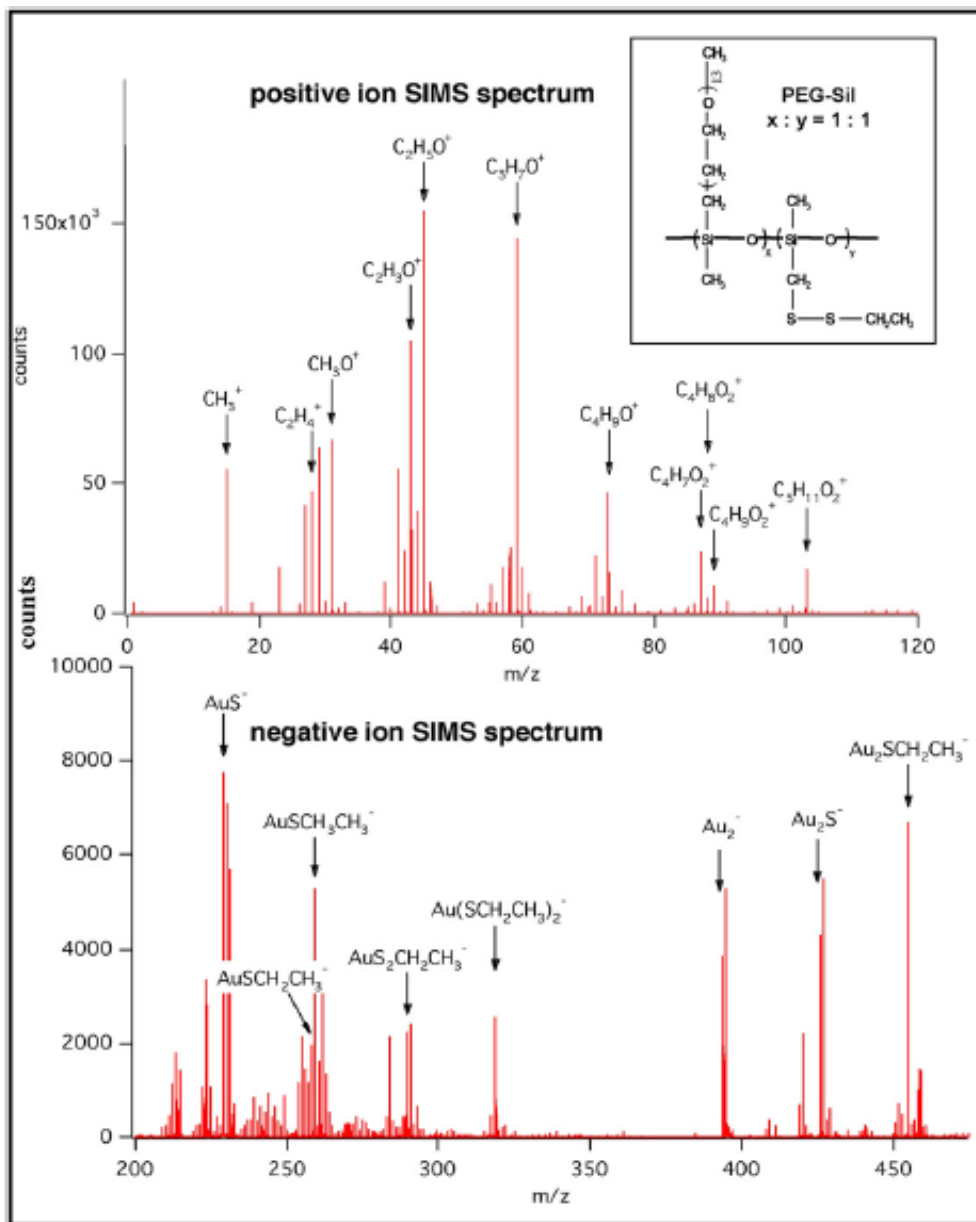
Static SIMS provides qualitative information on the atomic and molecular composition in the outermost 1–2 nm of surface with high analytical sensitivity and excellent *x,y* spatial resolution.

FIGURE I.1.5.10 Static positive and negative ion SIMS spectra of a poly(ethylene glycol)–poly(dimethyl siloxane) copolymer containing disulfide side groups on a gold surface. The primary peaks are identified. The low mass region of the negative ion spectrum offers little insight into the polymer structure, but the high mass region is rich in information. In this case, the low mass positive spectrum is rich in information. Further details on this class of polymers can be found in *Macromolecules*, 27, 3053 (1994). (Figure supplied by D. Castner.)

➤ **Scanning Electron Microscopy**

- Scanning electron microscopy (SEM) images of surfaces have great resolution and depth of field, with a three-dimensional quality.

- SEM functions by focusing and rastering a relatively <u>high-energy electron beam</u> (typically, 5–100 keV) on a specimen that is under vacuum.

- Low-energy secondary electrons (1–20 eV) are emitted from each spot where the focused electron beam makes an impact.

- The intensity of the secondary electron emission is a function of the atomic composition of the sample and the geometry of the features under observation.

- The image of the surface is spatially reconstructed on a phosphor screen (or CCD detector) from the intensity of the secondary electron emission at each point.

- Nonconductive materials observed in the SEM are typically coated with a thin, to minimize negative charge accumulation from the electron beam.

- This metal layer is always thick enough (>200 Å) so that the electrons emitted from the sample beneath cannot penetrate.

- Therefore, in SEM analysis of nonconductors, the surface of the metal coating is being monitored.

- If the metal coat is truly conformal, a good representation of the surface geometry will be conveyed. However, the specimen surface chemistry no longer influences secondary electron emission.

SEM provides a high resolution image of the surface. On insulating materials, metallic coating is required and the image is actually of the coating surface, not the underlying material.
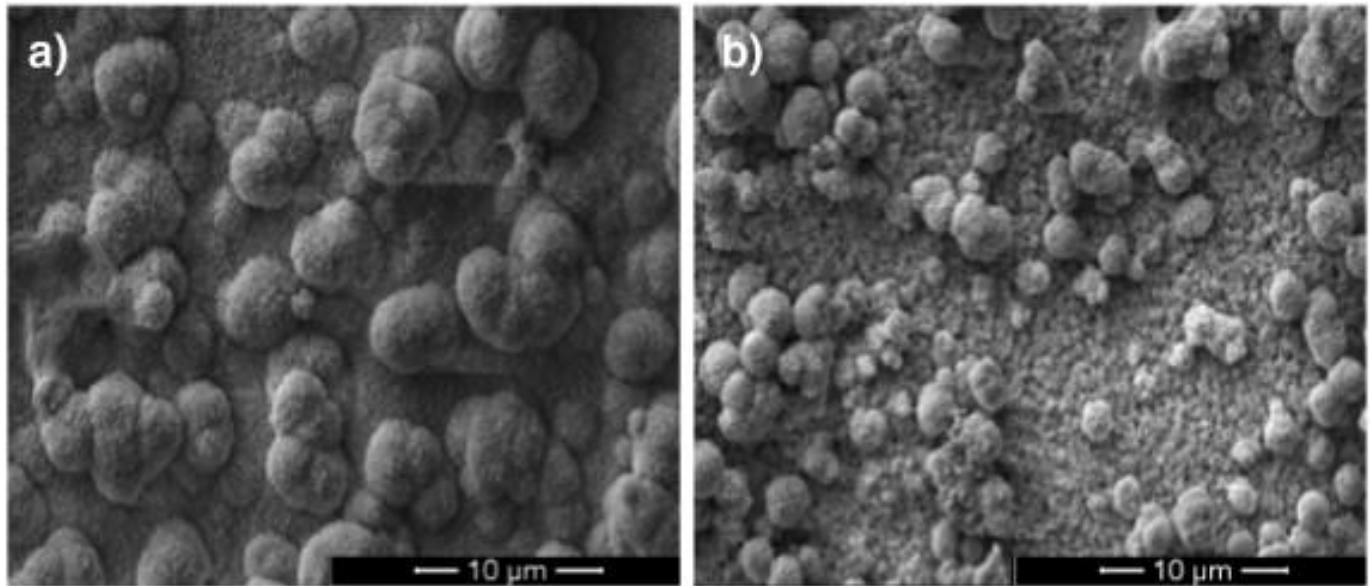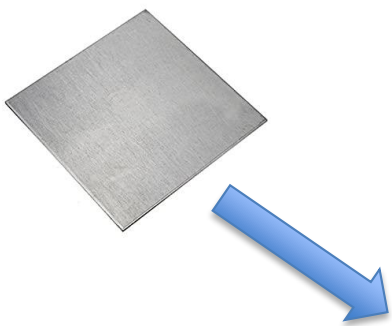
SEM micrographs of (a) (Ti,Mg)N and (b) TiN coatings after 5X SBF tests.

- SEM is an important corroborative method to use in conjunction with other surface analysis methods.

  - Surface roughness and texture can have a profound influence on data from ESCA, SIMS, and contact angle determinations.

  - SEM provides important information in the interpretation of data from these methods.

➢ **Infrared Spectroscopy**

- Infrared spectroscopy provides information on the bond vibrations of molecular species.

- It is a widely used analytical method that can reveal information on specific chemistries and the orientation of structures.

- Fourier transform infrared (FTIR) spectrometry offers outstanding signal-to-noise ratio (S/N) and spectral accuracy.

- Surface FTIR methods couple the infrared radiation to the sample surface to increase the intensity of the surface signal and reduce the bulk signal
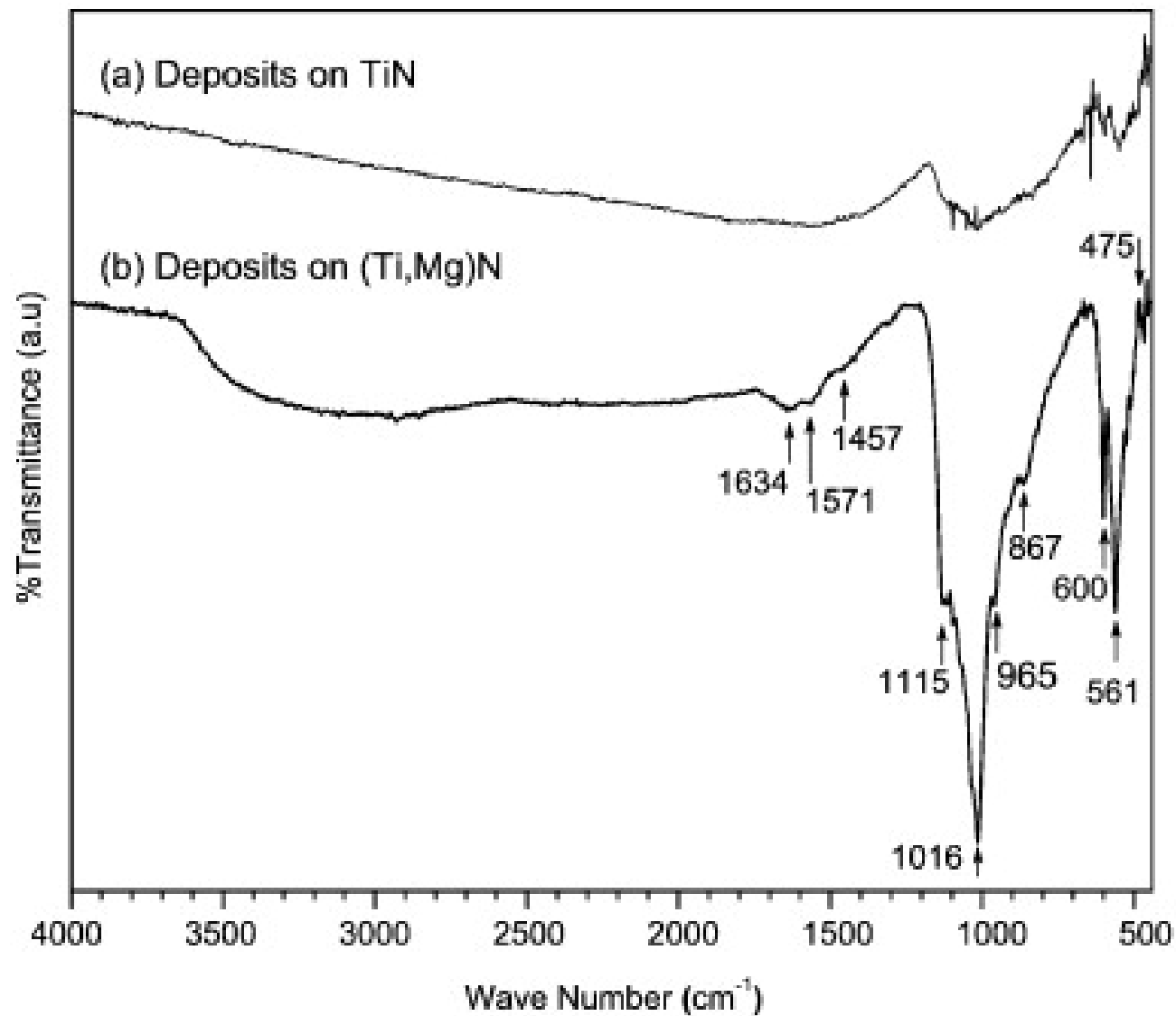
- Some of these sampling modes, and their characteristics
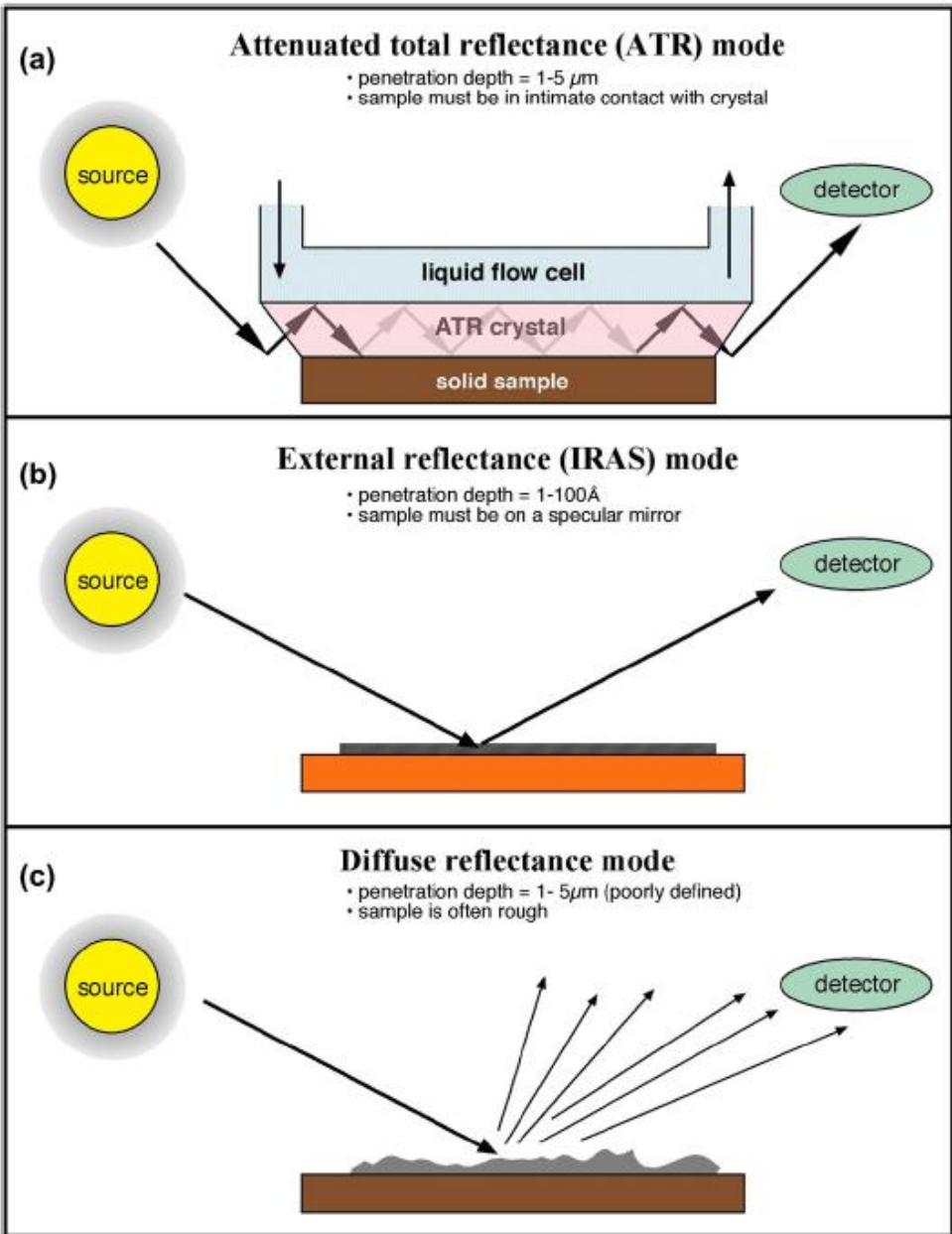
ATR-IR permits detailed molecular analysis of the outermost 1–5 microns of a sample.

FIGURE I.1.5.12 Three surface-sensitive infrared sampling modes. (a) Attenuated total reflectance infrared (ATR-IR); (b) infrared reflection absorption spectroscopy (IRAS); (c) diffuse reflectance infrared spectroscopy.

FTIR spectra of (a) TiN and (b) (Ti,Mg)N coatings after Mg-free 5X SBF tests.

➢ Scanning Tunneling Microscopy (STM)

- The STM capitalizes on quantum tunneling to generate an atom-scale, electron density image of a surface.
- A metal tip terminating in a single atom is brought within 5–10 Å of an electrically conducting surface. At these distances, the electron cloud of the atom at the "tip of the tip" will significantly overlap the electron cloud of an atom on the surface.
- If a potential is applied between the tip and the surface, an electron tunneling current will be established
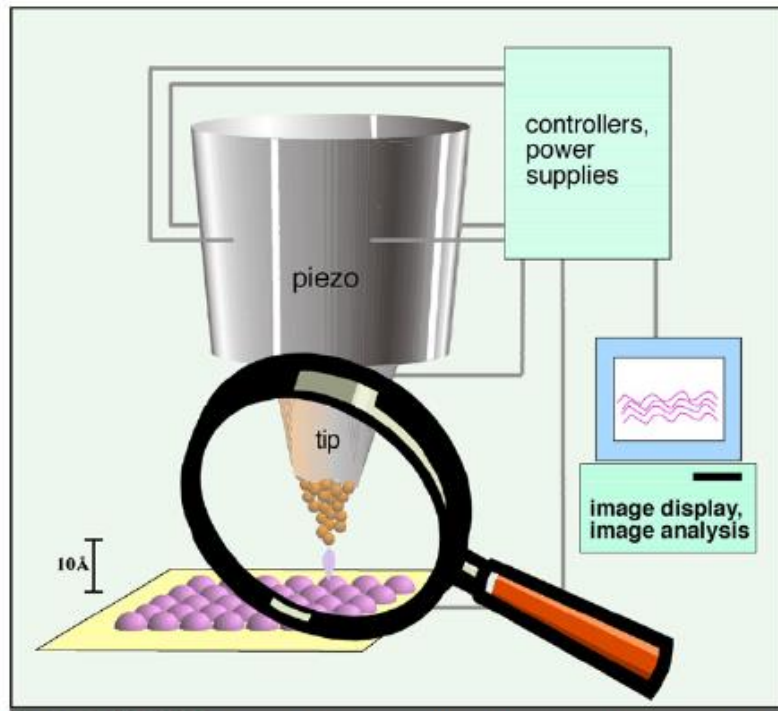
FIGURE I.1.5.13 Schematic diagram illustrating the principle of the scanning tunneling microscope – a tip terminating in a single atom permits localized quantum tunneling current from surface features (or atoms) to tip. This tunneling current can be spatially reconstructed to form an image.
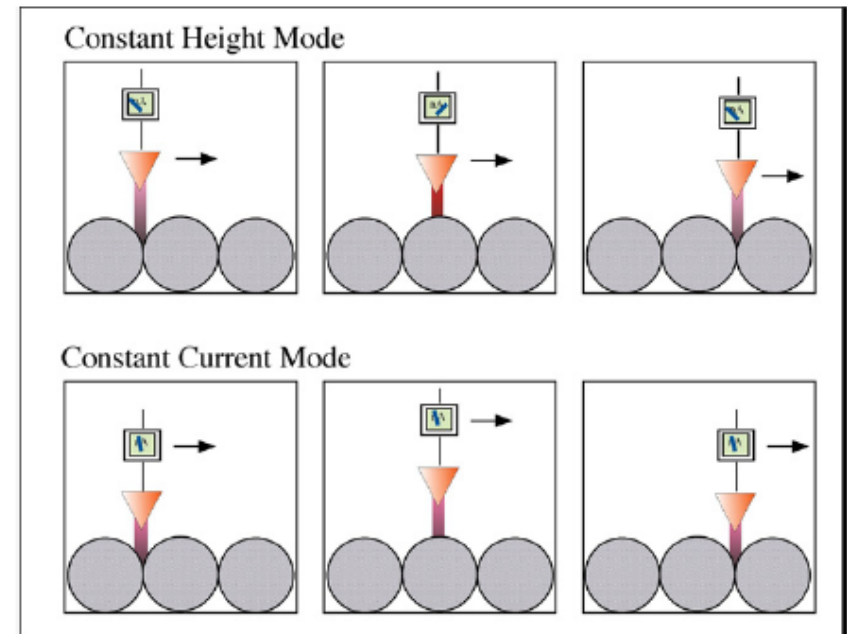

FIGURE I.1.5.14 Scanning tunneling microscopy can be performed in two modes. In constant height mode, the tip is scanned a constant distance from the surface (typically 5–10 Å) and the change in tunneling current is recorded. In constant current mode, the tip height is adjusted so that the tunneling current is always constant, and the tip distance from the surface is recorded as a function of distance traveled in the plane of the surface.

➤ Atomic Force Microscopy (AFM)

- The AFM uses a similar mechanism.
- Instead of recording tunneling current, the deflection of a tip mounted on a flexible cantilever arm due to van der Waals forces and electrostatic repulsion/attraction between an atom at the tip and an atom on the surface is measured.

> AFM allows imaging of surfaces at sub-nanometer resolutions, and also provides detail on surface mechanics and molecular interactions.
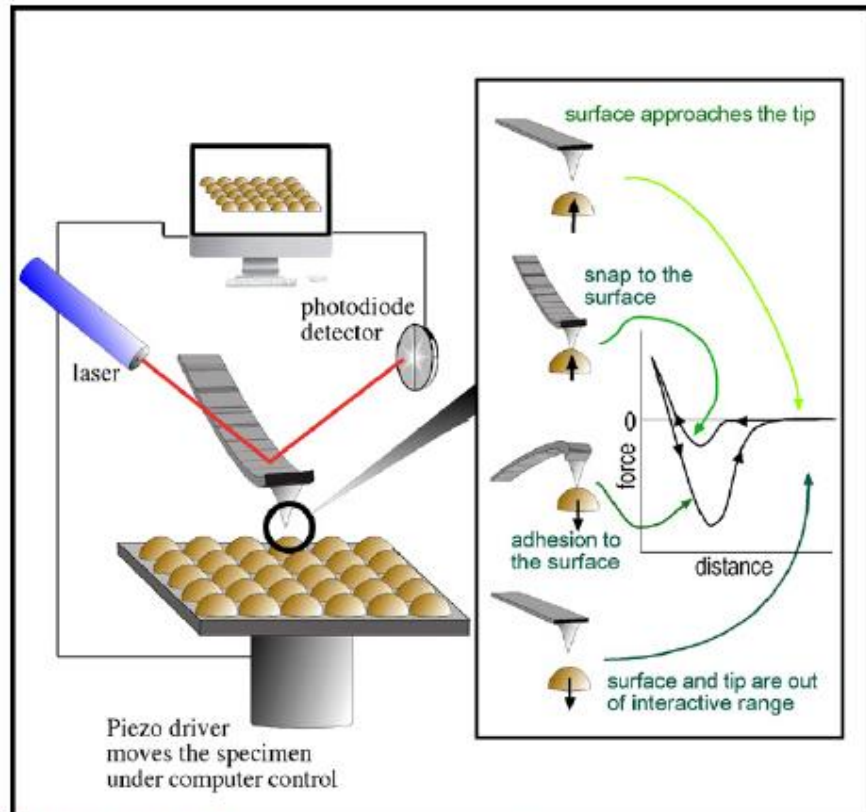
FIGURE I.1.5.15 Schematic diagram illustrating the principle of the atomic force microscope.
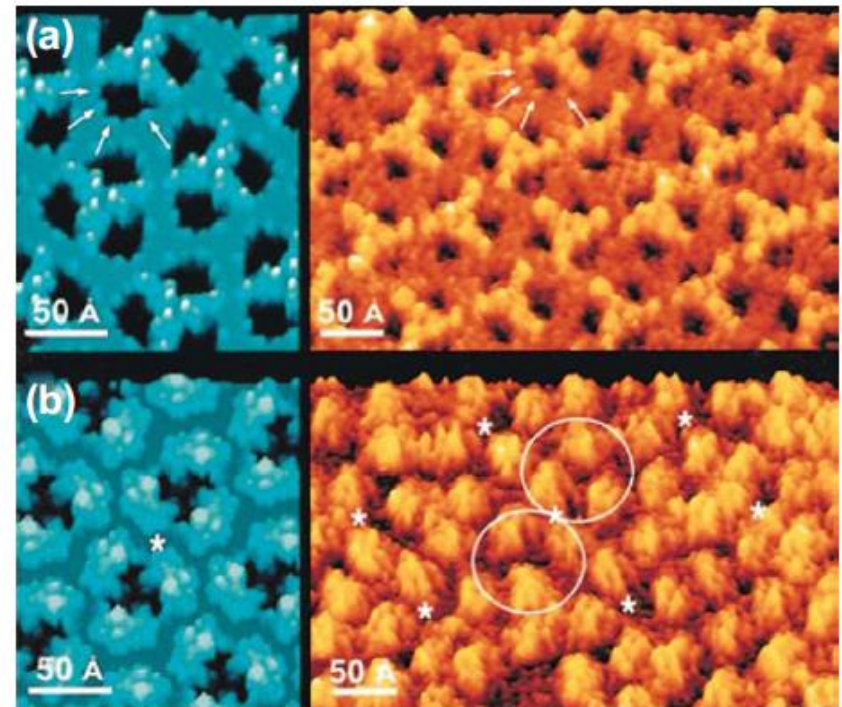
FIGURE I.1.5.16 An AFM image of porin proteins from the outer membrane of E. coli imaged with nanoscale resolution. Comparison of high-resolution AFM images of OmpF crystals (in brown-yellow) and the atomic model rendered at 3 Å (in blue). (From Müller, D. J. & Engel, A. (1999). Journal of Molecular Biology, 285, 1347, used with permission of the authors and the publisher.)

- The potential of the AFM to explore surface problems has been greatly expanded by ingenious variants of the technique. In fact, the term "atomic force microscopy" has been generalized to "scanning probe microscopy" (SPM).

TABLE I.1.5.6	Scanning Probe Microscopy (SPM) Modes	
Name	**Acronym**	**Use**
Contact mode	CM-AFM	Topographic imaging of harder specimens
Tapping (intermittent force) mode	IF-AFM	Imaging softer specimens
Non-contact mode	NCM-AFM	Imaging soft structures
Force modulation (allows slope of force-distance curve to be measured	FM-AFM	Enhances image contrast based on surface mechanics
Scanning surface potential microscopy (Kelvin probe microscopy)	SSPM, KPM	Measures the spatial distribution of surface potential
Magnetic force microscopy	MFM	Maps the surface magnetic forces
Scanning thermal microcopy	SThM	Maps the thermal conductivity characteristics of a surface
Recognition force microscopy	RFM	Uses a biomolecule on a tip to probe for regions of specific biorecognition on a surface
Chemical force microscopy	CFM	A tip derivatized with a given chemistry is scanned on a surface to spatially measure differences of interaction strength
Lateral force microscopy	LFM	Maps frictional force on a surface
Electrochemical force microscopy	EFM	The tip is scanned under water and the electrochemical potential between tip and surface is spatially measured
Nearfield scanning optical microscopy	NSOM	A sharp optical fiber is scanned over a surface allowing optical microscopy or spectroscopy at 100 nm resolution
Electrostatic force microscopy	EFM	Surface electrostatic potential are mapped
Scanning capacitance microscopy	SCM	Surface capacitance is mapped
Conductive atomic force microscopy	CAFM	Surface conductivity is mapped with an AFM instrument
Nanolithographic AFM	NAFM	An AFM tip etches, oxidizes or reacts a space permitting pattern fabrication at 10 nm or better resolution
Dip-pen nanolithography	DPN	An AFM tip, inked with a thiol or other molecule, writes on a surface at the nanometer scale

- Since the AFM measures force, it can be used with both conductive and nonconductive specimens.
- Force must be applied to bend a cantilever, so AFM is subject to artifacts caused by damage to fragile structures on the surface
- Both AFM and STM can function well for specimens under water, in air or under vacuum
- For exploring biomolecules or mobile organic surfaces, the "pushing around" of structures by the tip is a significant concern.
- This surface artifact can be capitalized upon to write and fabricate surface structures at the nanometer scale
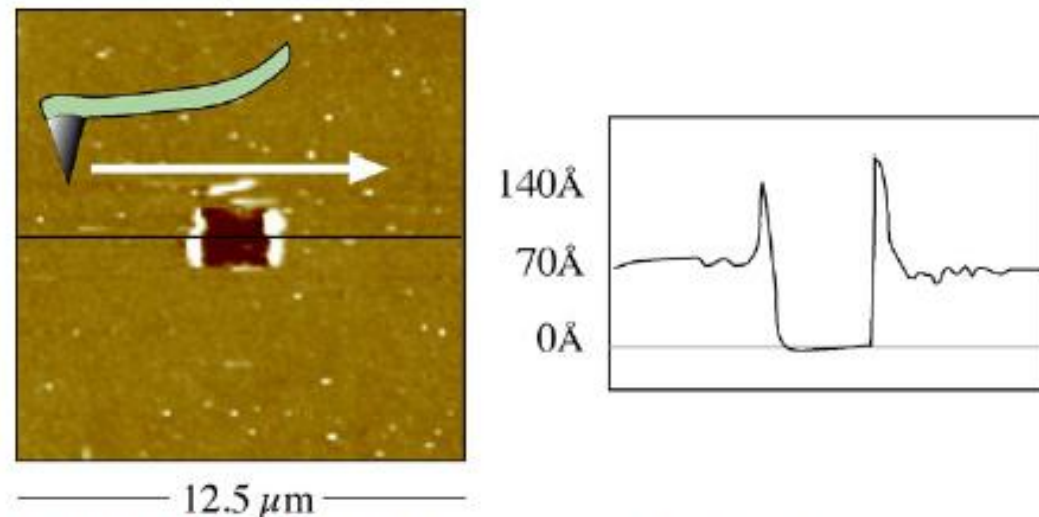
FIGURE I.1.5.17 An AFM tip, using relatively high force, was used to scratch a rectangular feature into a thin (70 Å) plasma-deposited film. The AFM also characterized the feature created.