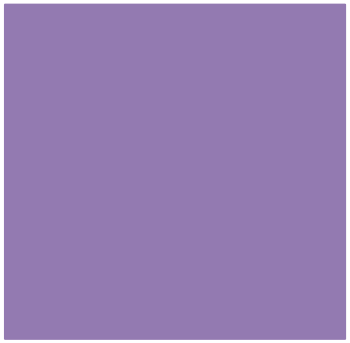
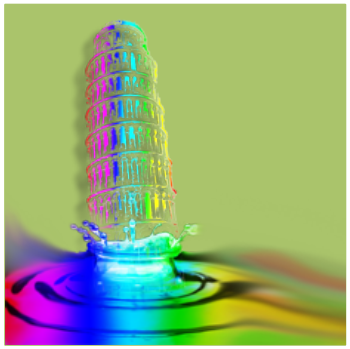




Biomaterials

G. Vozzi

A biomaterial is a material designed to interfere with biological materials to evaluate, treat, augment or replace any body tissue, organ or function (Chester 1991)

Other properties

- Biocompatible
- Bioadsorbable, bioerodible, bioresorbable

Biocompatibility

Ability of a material, device or system to perform its function, without a significant clinical response of the host, within a specific application.

Haemocompatibility

It is essentially described by the following phenomena:

1- platelet adhesion (evaluated, for example, by platelet counts)

2- activation of the coagulation system (evaluated for example by determining the Thrombin/AntiThrombin complex)

3- activation of the complement system (evaluated eg by measuring the ratio C3a / C5a)

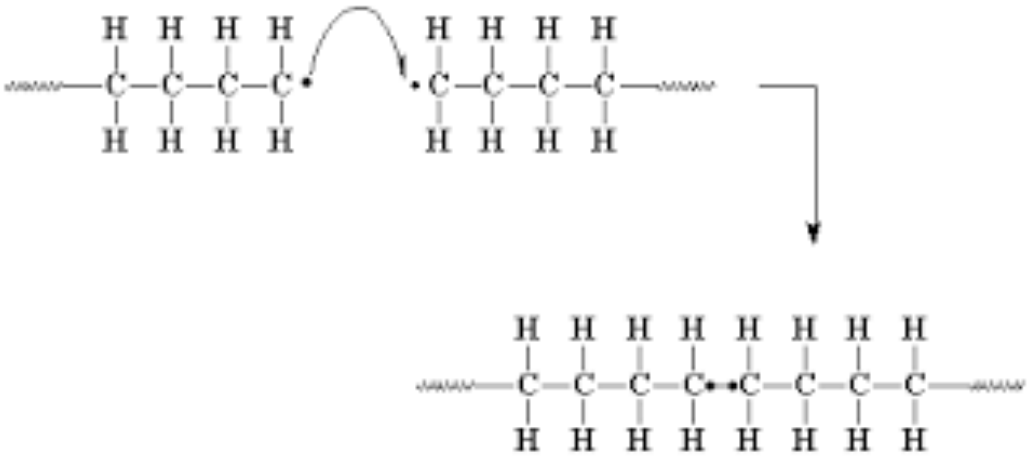
Classification

- Biopolymers: - synthetic
  - natural
- Metals
- Ceramics
- Composites

Synthetic Biomaterials

- Polymerisation process

poly-addition (chain reaction), when the monomer has double or triple bonds between carbon atoms



Synthetic Biomaterials

Polymerisation process

- poly-condensation (steps reaction) it is composed by three phase

- Initial phase

$$R^{\bullet} + M \rightarrow RM^{\bullet}$$

- Growing phase

$$RM_{n+1}M^{\bullet} + R^{\bullet} \rightarrow RM_{n+1}R$$

$$RM_{n+1}M^{\bullet} + RH \rightarrow RM_{n+1}H + R^{\bullet}$$

$$RM_{n+1}M^{\bullet} + RM_{m}M^{\bullet} \rightarrow RM_{n+1} + RM$$

$$RM^{\bullet} + M \rightarrow RMM^{\bullet}$$

- End phase

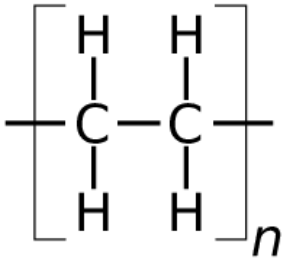
$$RMM^{\bullet} + nM \rightarrow RM_{n+1}M^{\bullet}$$

In polyaddition reaction there are no different reaction products from the initial molecules and the final polymers

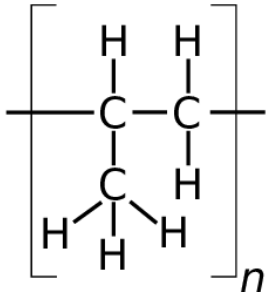
In polycondensation reaction there are other reaction products such as water, NaCl, methanol, HCl, etc.

Typical polymers obtained for polyaddition

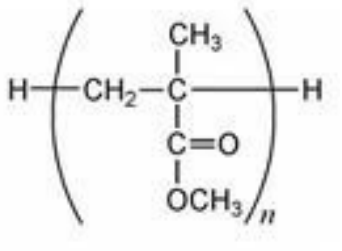
Polyethylene (PE)



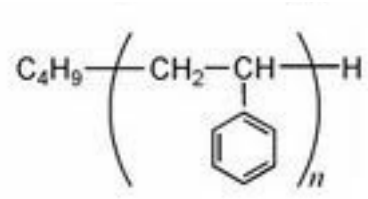
Polypropylene (PP)



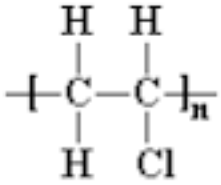
Poly(methyl methacrylate) (PMMA)



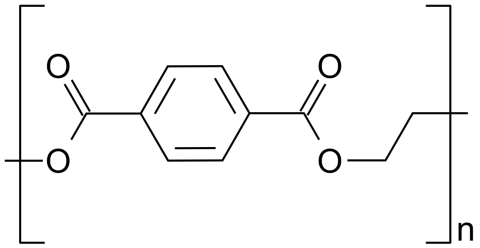
Polystyrene (PS)



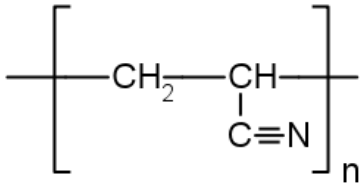
- Polyvinylclorure (PVC)



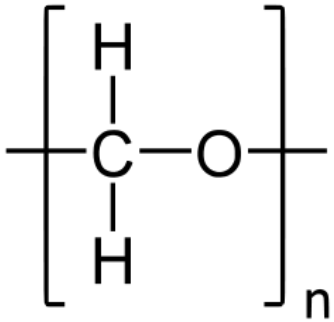
Polyethylenterephtalat (PTFE)



Polyacrylonitrile (PAN)

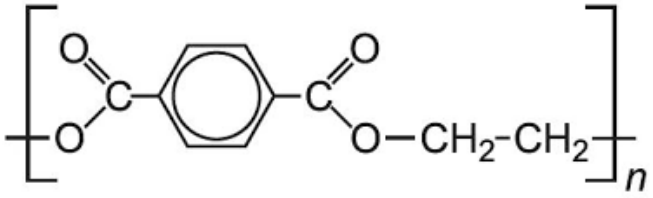


Polyoxymethylene (POM)

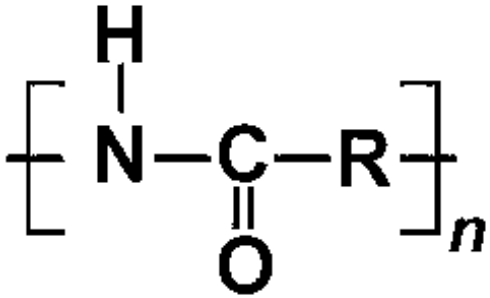


Typical polymers obtained for polycondensation

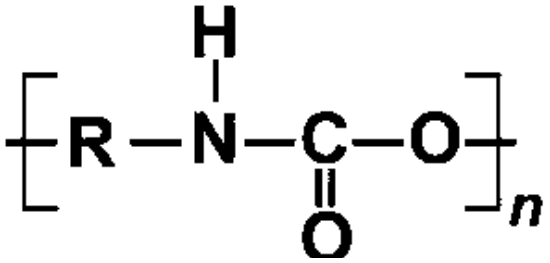
Polyesters



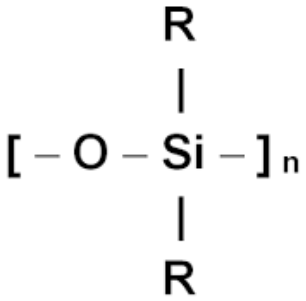
Polyamides



Polyurethanes



Polysiloxanes



Features of synthetic polymers

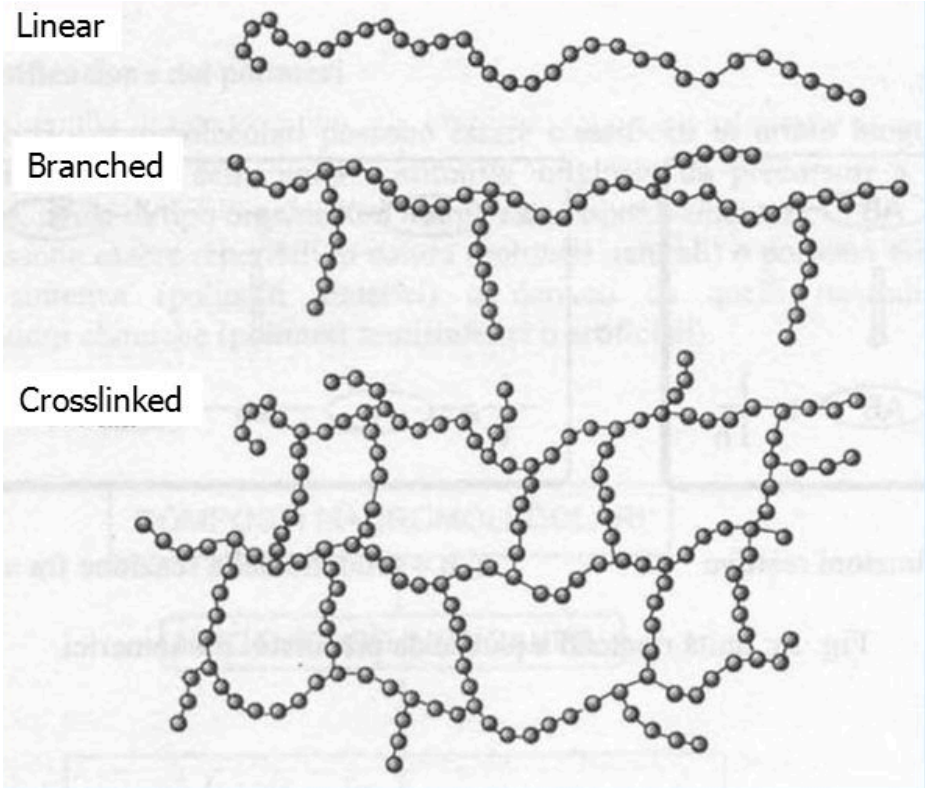
Molecular weight

TECHNIQUES TO DETERMINE MOLECULAR WEIGHT

Methods	Measured Parameter	M.Weight Measured	Upper Limit (g per mole)
Membrane osmometry	Osmotic pressure of polymer solvent	M_n	$5x10^4$
Light scattering (LS)	Intensity of light scattered by dilute polymer solutions	Mw, Mz	$1x10^8$
Gel permeation chromatography (GPC)	Elution volume of the polymer solution through a GPC column packed with porous microparticles	Mn , Mw	1×10^8
Viscometry	Flow time of polymer solution through a capillary	M v	1×10^8

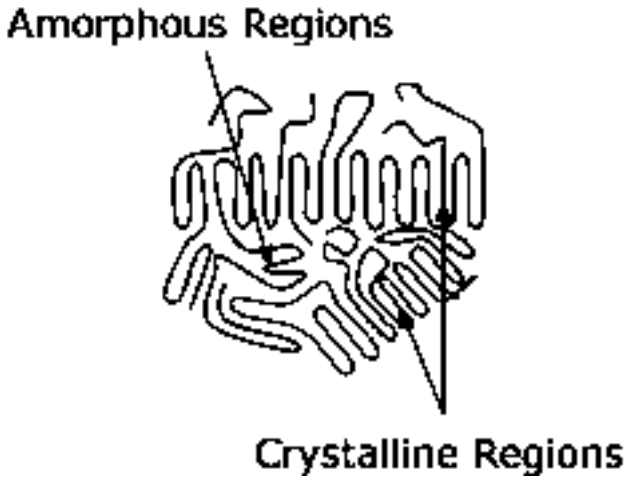
Features of synthetic polymers

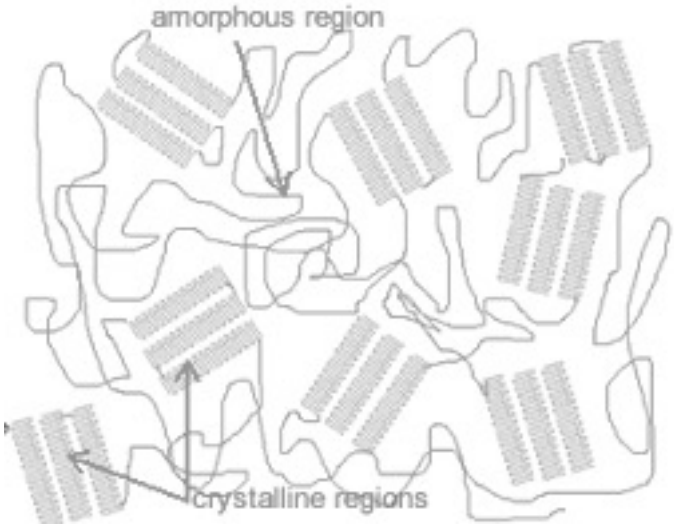
Chain structure



Features of synthetic polymers

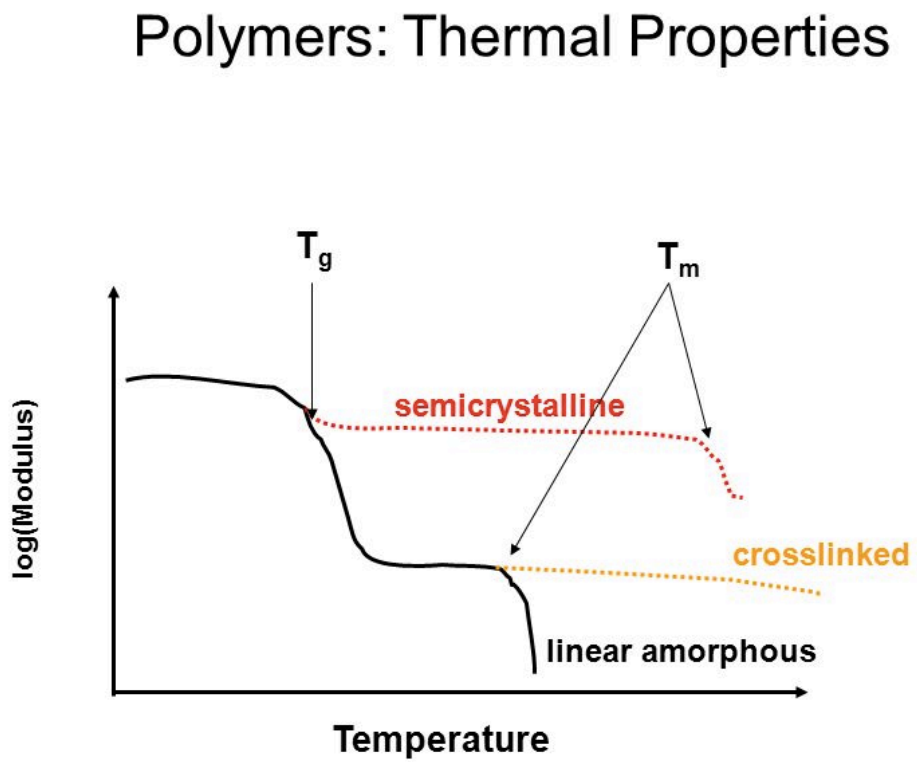
- Cristallinity



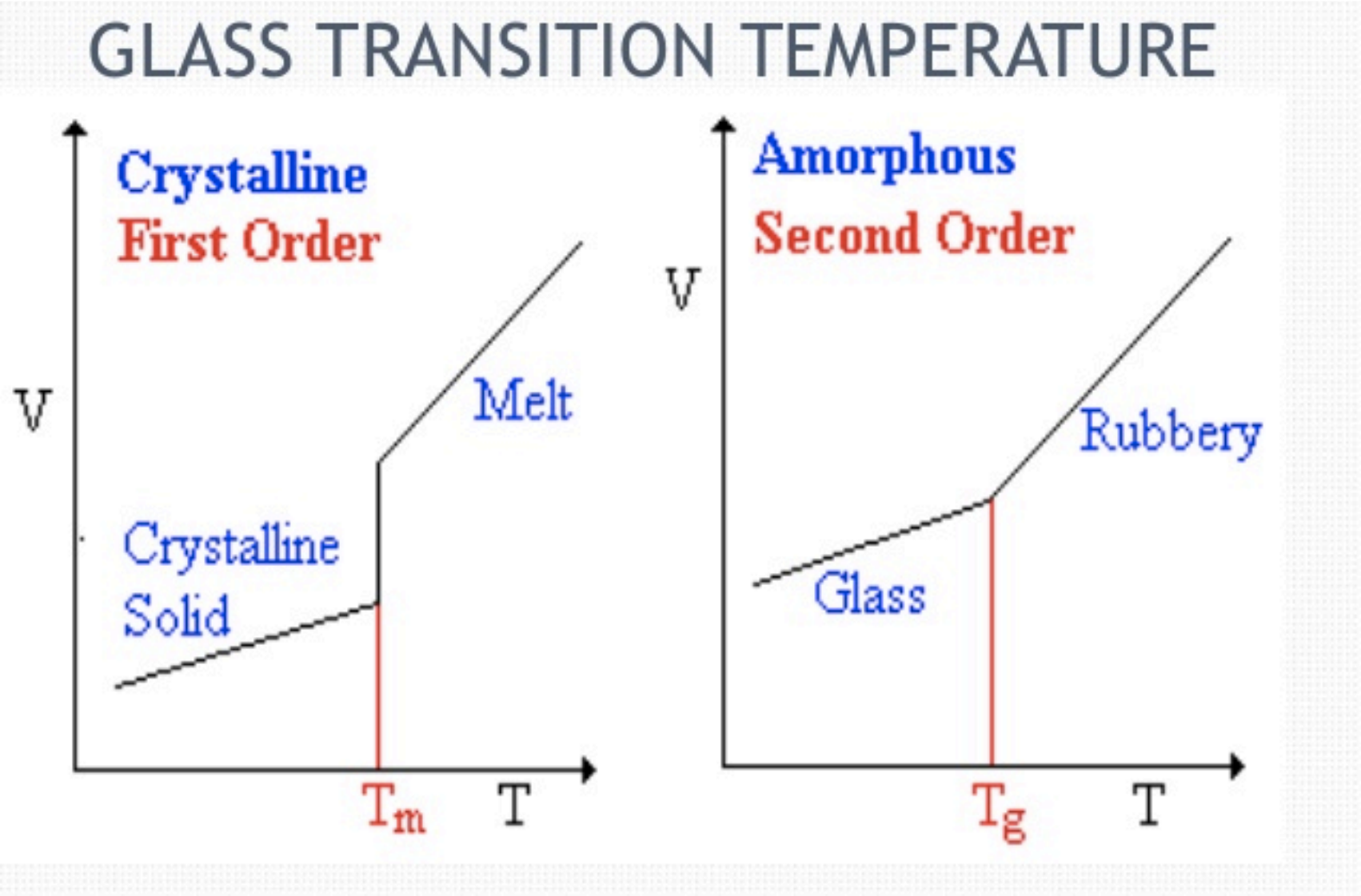


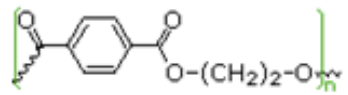
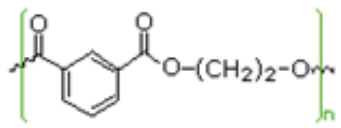
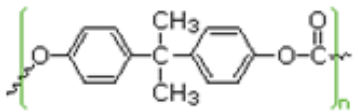
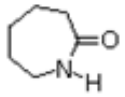
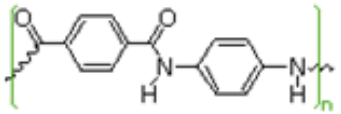
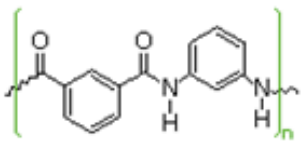
Features of synthetic polymers

Different thermal properties



Different thermal properties



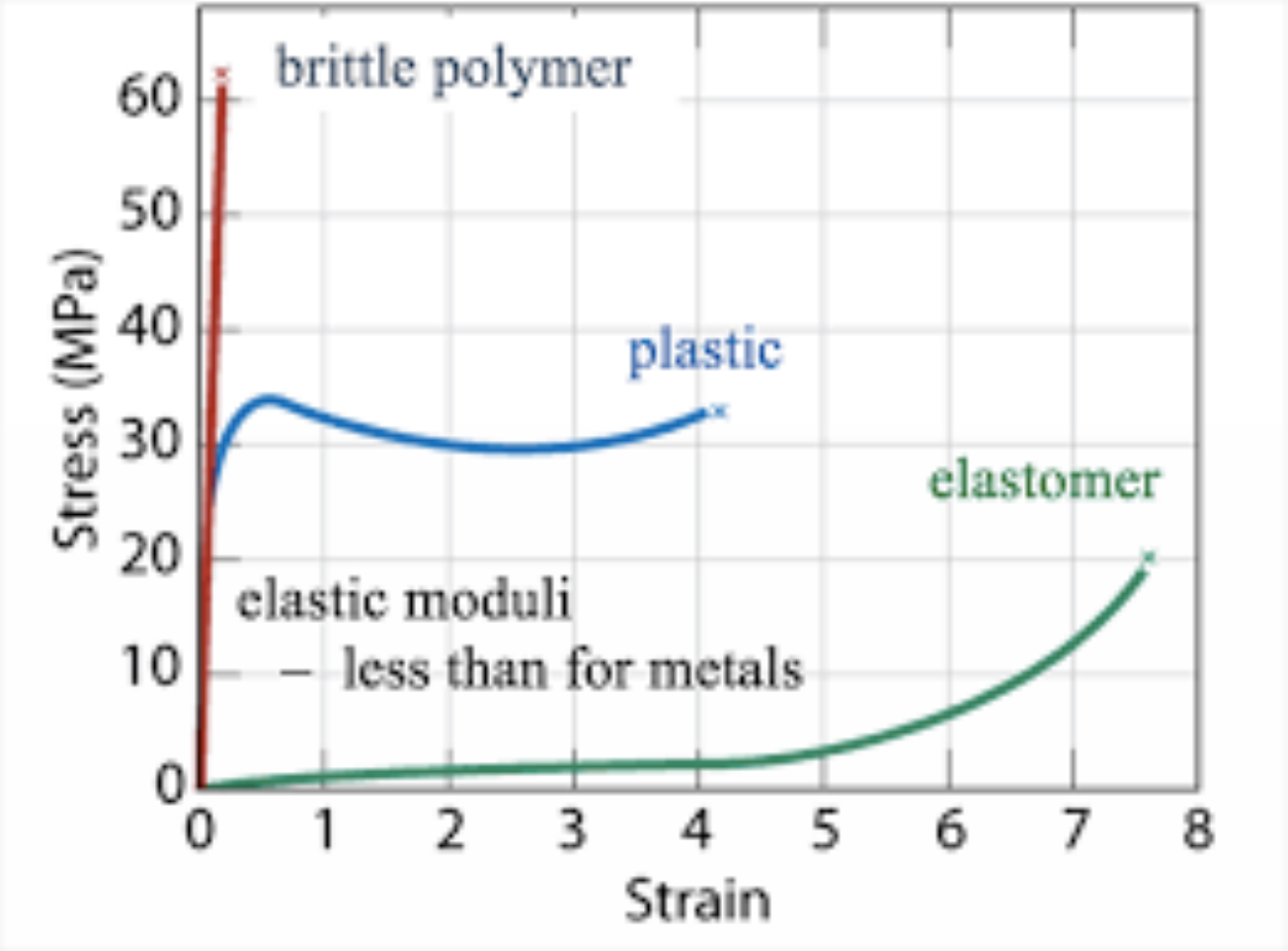
Formula	Type	Components	T_g ºC	T_m ºC
~[CO(CH$_2$)$_4$CO-OCH$_2$CH$_2$O]$_n$~	**polyester**	HO$_2$C-(CH$_2$)$_4$-CO$_2$H HO-CH$_2$CH$_2$-OH	< 0	50
	**polyester** Dacron, Mylar	para HO$_2$C-C$_6$H$_4$-CO$_2$H HO-CH$_2$CH$_2$-OH	70	265
	**polyester**	meta HO$_2$C-C$_6$H$_4$-CO$_2$H HO-CH$_2$CH$_2$-OH	50	240
	**polycarbonate** Lexan	(HO-C$_6$H$_4$-)$_2$C(CH$_3$)$_2$ (Bisphenol A) X$_2$C=O (X = OCH$_3$ or Cl)	150	267
~[CO(CH$_2$)$_4$CO-NH(CH$_2$)$_6$NH]$_n$~	**polyamide** Nylon 66	HO$_2$C-(CH$_2$)$_4$-CO$_2$H H$_2$N-(CH$_2$)$_6$-NH$_2$	45	265
~[CO(CH$_2$)$_5$NH]$_n$~	**polyamide** Nylon 6 Perlon		53	223
	**polyamide** Kevlar	para HO$_2$C-C$_6$H$_4$-CO$_2$H para H$_2$N-C$_6$H$_4$-NH$_2$	---	500
	**polyamide** Nomex	meta HO$_2$C-C$_6$H$_4$-CO$_2$H meta H$_2$N-C$_6$H$_4$-NH$_2$	273	390

Typical T_g and T_m Values for Polymers

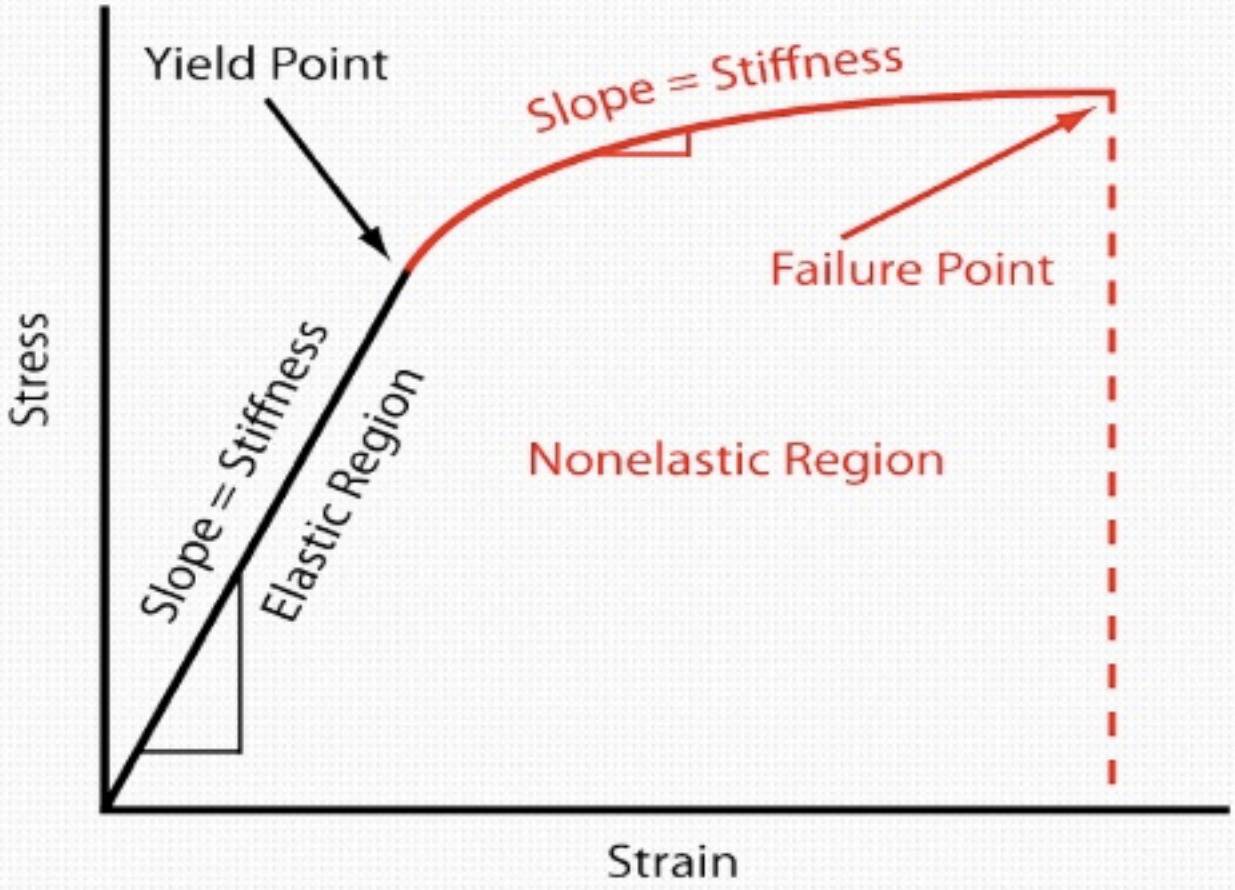
	Temperature (°C)	
	T_g	T_m
cis-Polybutadiene	−101	4
cis-Polyisoprene	−73	29
trans-Polyisoprene	−58	70
Linear polyethylene	−70 to −20	132
Polypropylene	−16	170
trans-1,4-Polybutadiene	−9	139
Nylon 6,6	47	235
Poly(methyl methacrylate)	49	155
Poly(vinyl chloride)	70	140
Polystyrene	94	227
Polycarbonate	152	267
Cellulose triacetate	111	300
Poly(tetrafluoroethylene)	135	327

Features of synthetic polymers

Different mechanical properties



MECHANICAL PROPERTIES



Principal classes of synthetic biomaterials

- Biodegradable linear aliphatic polyesters (e.g., polyglycolide, polylactide, polycaprolactone, polyhydroxybutyrate) and their copolymers
- Biodegradable copolymers between linear aliphatic polyesters in (1) and monomers other than linear aliphatic polyesters like, poly(glycolide-trimethylene carbonate) copolymer, poly(L-lactic acid-L-lysine) copolymer, polycarbonates,etc;
- Polyanhydrides;
- Poly(orthoesters);
- Poly(ester-ethers) like poly-p-dioxanone;
- Biodegradable polysaccharides like hyaluronic acid, chitin and chitosan;
- Polyamino acids like poly-L- glutamic acid and poly-L-lysine;
- Inorganic biodegradable polymers like polyphosphazene.

Degradation

Principal role in the degradation is due to free radicals, that induce change in the PH and in the binding forces between the different monomers

Natural biopolymers

- Collagen

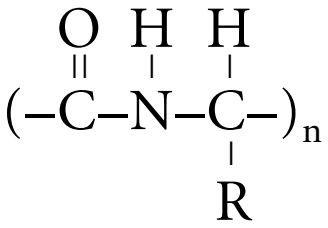
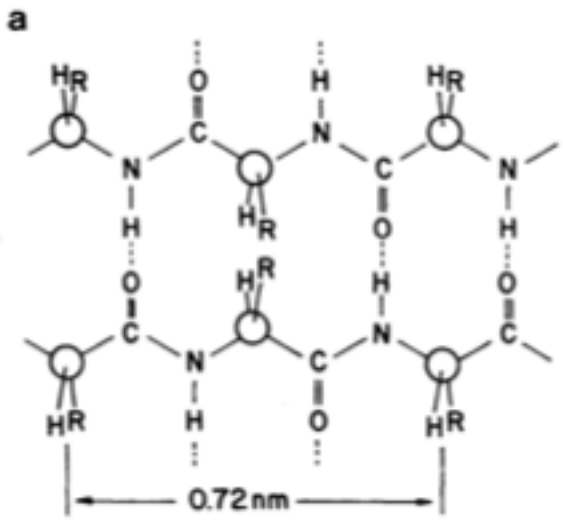


TABLE 42.1 Amino Acid Content of Collagen

Amino Acids	Content, residues/1000 residues*
Gly	334
Pro	122
Hyp	96
Acid polar (Asp, Glu, Asn)	124
Basic polar (Lys, Arg, His)	91
Other	233



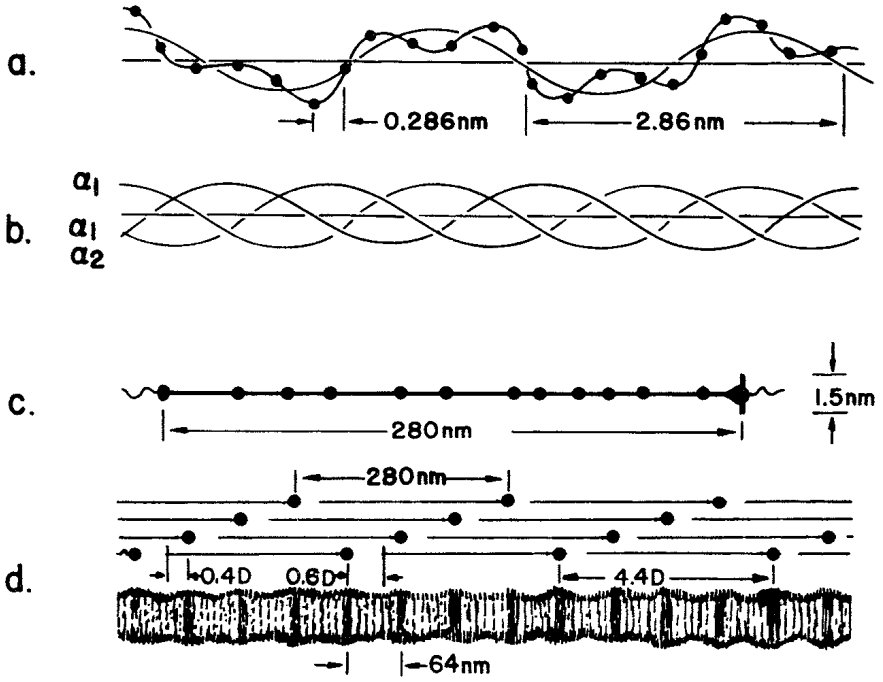
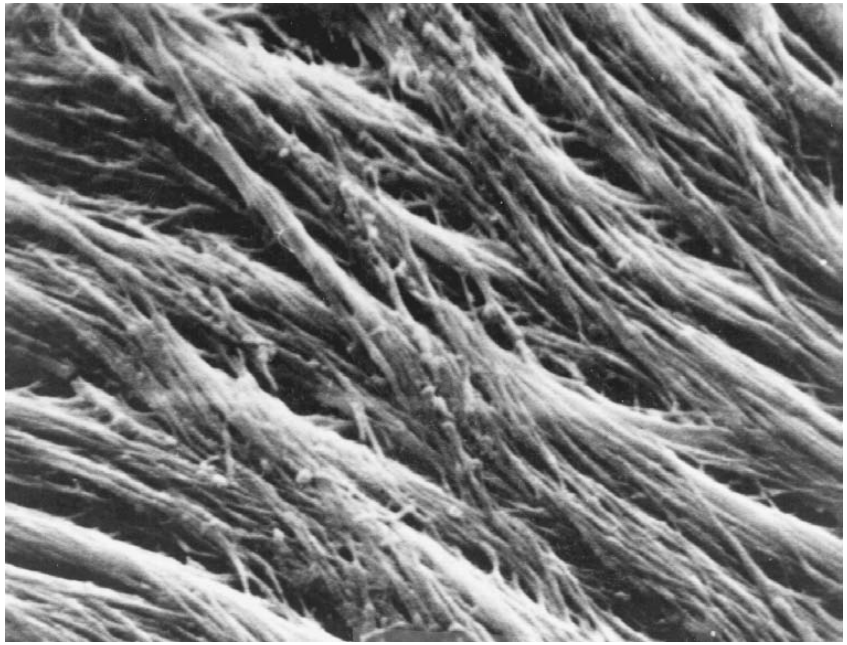
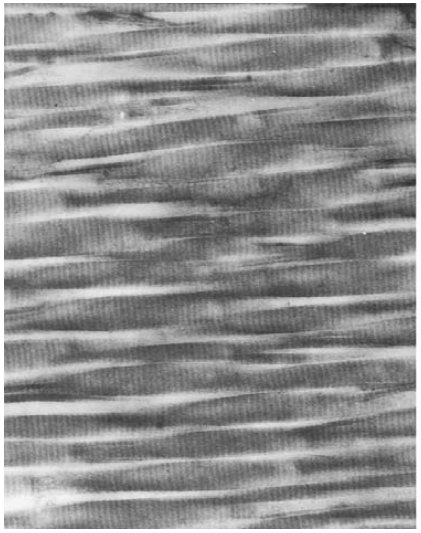


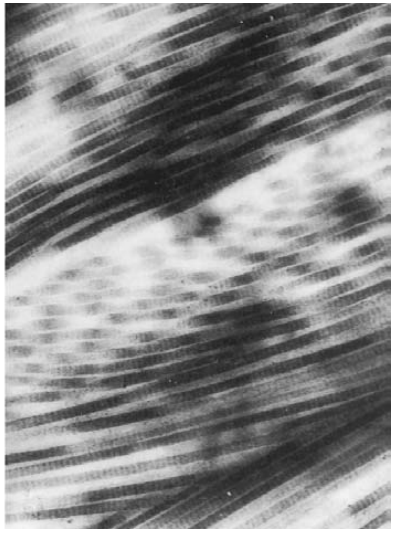
FIGURE 42.2 Diagram depicting the formation of collagen, which can be visualized as taking place in several steps: (*a*) single chain left-handed helix; (*b*) three single chains intertwined into a triple stranded helix; (*c*) a collagen (tropocollagen) molecule; (*d*) collagen molecules aligned in *D* staggered fashion in a fibril producing overlap and hole regions.



(a)



(b)



(c)

FIGURE 42.4 (*a*) Scanning electron micrograph of the surface of an adult rabbit bone matrix, showing how the collagen fibrils branch and interconnect in an intricate, woven pattern (×4800) [Tiffit, 1980]. (*b*) Transmission electron micrographs of (×24,000) parallel collagen fibrils in tendon [Fung, 1992]. (*c*) Transmission electron micrographs of (×24,000) mesh work of fibrils in skin [Fung, 1993].

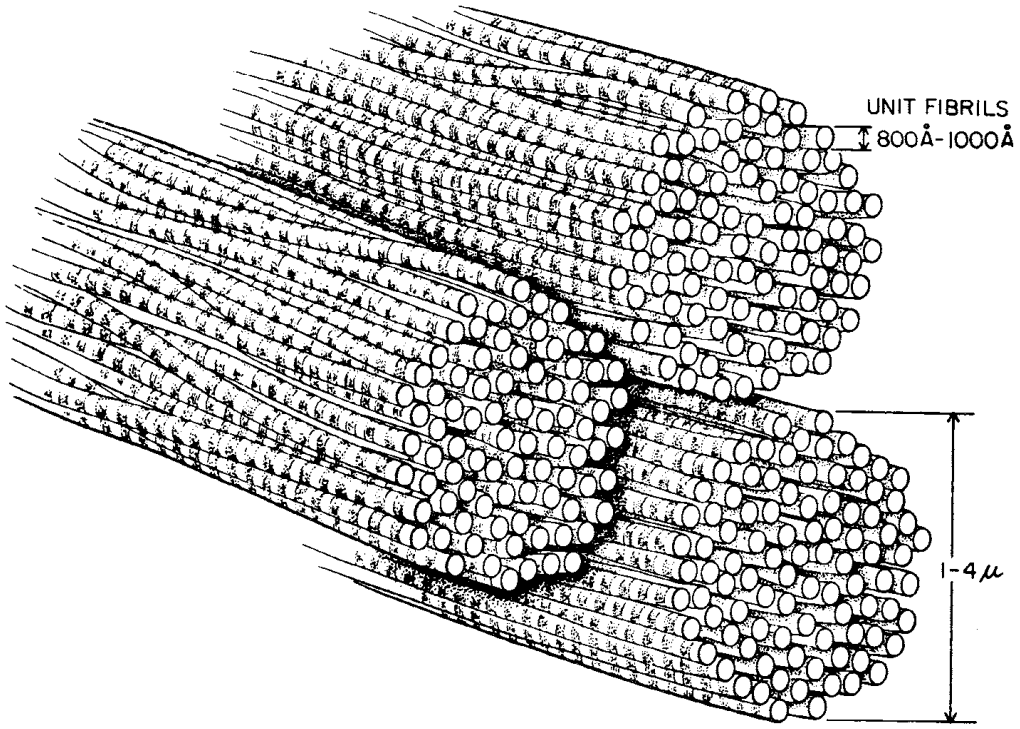
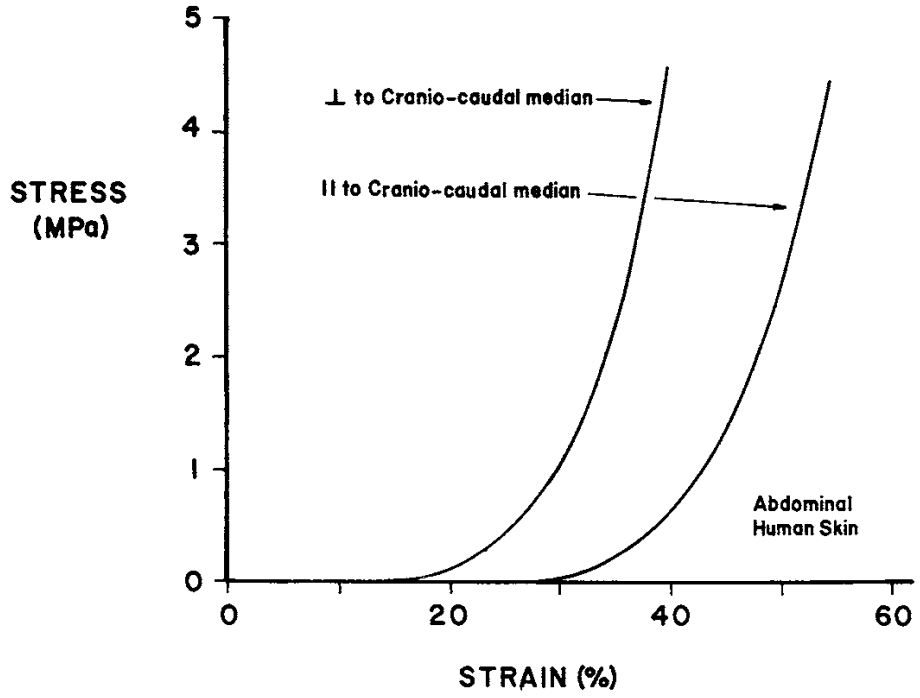


FIGURE 42.5 Diagram showing the collagen fibers of the connective tissue in general which are composed of unit collagen fibrils.


STRESS
(MPa)
5
4
3
2
1
0
⊥ to Cranio-caudal median
II to Cranio-caudal median
Abdominal
Human Skin
0
20
40
60
STRAIN (%)

Glysoaminoglycans

Glycosaminoglycans (GAGs), which consist of repeating disaccharide units in linear arrangement, usually include a uronic acid component (such as glucuronic acid) and a hexosamine component (such as n-acetyl-d-glucosamine). The predominant types of GAGs attached to naturally occurring core proteins of proteoglycans include chondroitin sulfate, dermatan sulfate, keratan sulfate, and heparan sulfate (Heinegard and Paulson, 1980; Naeme and Barry, 1993). The GAGs are attached to the core protein by specific carbohydrate sequences containing three or four monosaccharides.

The largest GAG is hyaluronic acid (hyaluronan),with unbranched units ranging from 500 to several thousand. Hyaluronic acid can be isolated from natural sources (e.g., rooster combs) or via microbial fermentation (Balazs, 1983). Because of its water-binding capacity, dilute solutions of hyaluronic acid form viscous solutions.

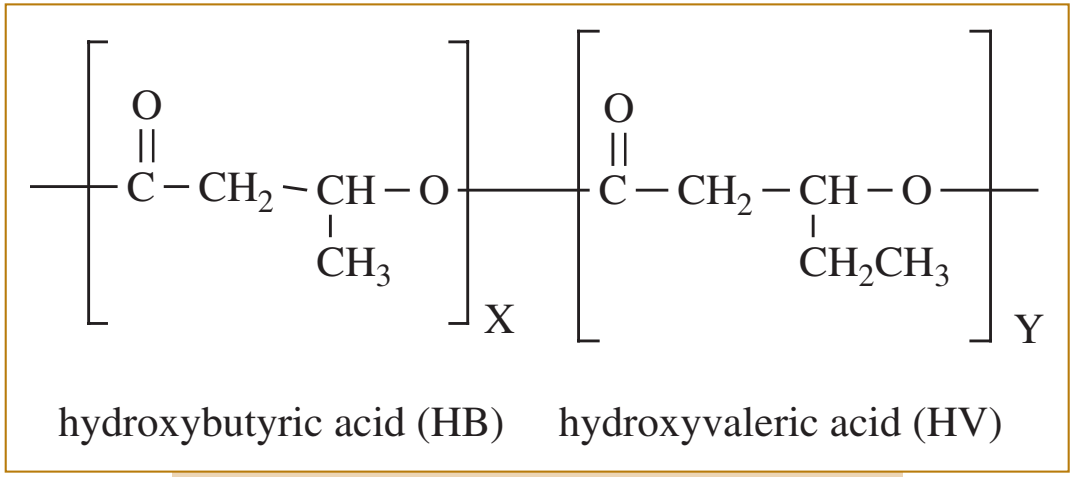
Hyaluronic acid can be cross-linked to form molecular weight complexes in the range 8 to 24 × 106 or to form an infinite molecular network (gels). In one method, hyaluronic acid is cross- linked using aldehydes and small proteins to form bonds between the C—OH groups of the polysaccharide and the amino or imino groups of the protein, thus yielding high- molecular-weight complexes (Balazs and Leshchiner, 1986). Other cross-linking techniques include the use of vinyl sulfone, which reacts to form an infinite network through sulfonyl-bis-ethyl cross-links (Balazs and Leshchiner, 1985).

Chitosan

Chitosan is a biosynthetic polysaccharide that is the deacylated derivative of chitin. Chitin is a naturally occurring polysaccharide that can be extracted from crustacean exoskeletons or generated via fungal fermentation processes.

Polyhydroxyalkanoates

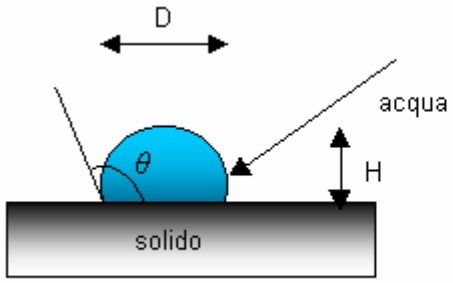
Polyhydroxyalkanoate (PHA) polyesters are degradable, biocompatible, thermoplastic materials made by several microorganisms (Miller and Williams, 1987; Gogolewski et al., 1993). They are intracellular storage polymers whose function is to provide a reserve of carbon and energy (Dawes and Senior, 1973). Depending on growth conditions, bacterial strain, and carbon source, the molecular weights of these polyesters can range from tens into the hundreds of thousands

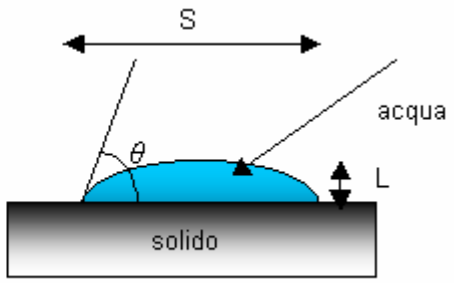


Biomaterial characterisation

Contact angle measurement

Water Drop method

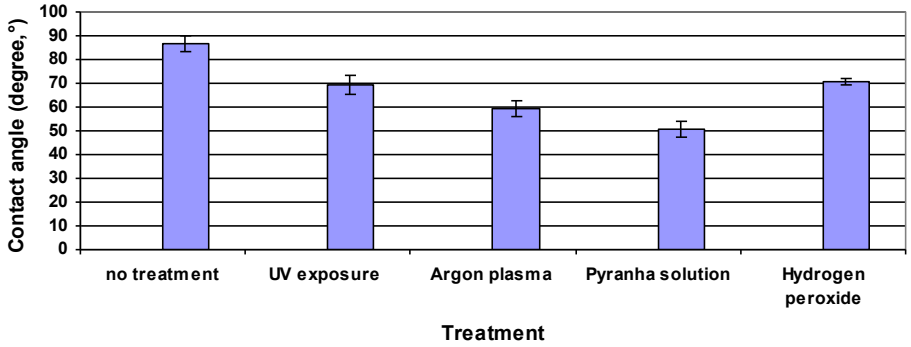




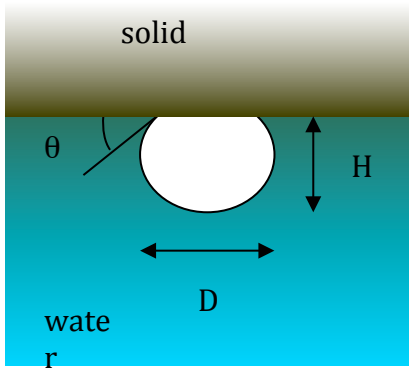
$$\theta = 180° - ar\cos\left|\left(\frac{2H}{D}\right) - 1\right|$$

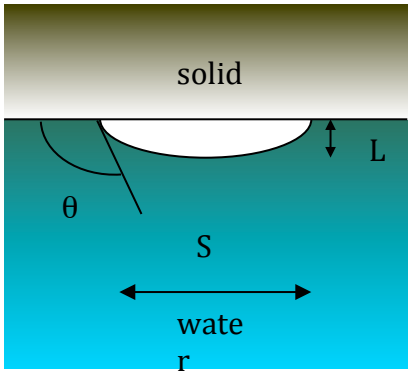
$$\theta = 2 \times arc\tan\left|\frac{2L}{S}\right|$$

Treatment inceases wettability (bubble-air on surface angle decreases)



Contact angle measurement





θ<90°
θ = arcos (2H/D –1)

θ>90°
θ = 180°- 2 arctan (2L/S)

- The measurement is based on the following principles:

1. the solid surface is rigid, unmoveable, and non-deformable. In practice, it means that elastic modulus of surface must be greater than 3.5 N/cm2;

2. the solid surface is almost smooth, so it is possible ignore the hysteris effects associated with the roughness of material;

3. the solid surface is uniform and homogenous;

4. the surface tension of the liquid is well known and constant and does not change during the experiment;

5. the solid surface does not interact with the liquid, even during the equilibrium between the three liquid-solid-aeroform phases;

6. the diffusion pressure of the liquid on the solid is zero. It means that the liquid vapours are not absorbed by the solid and so they do not alter it;

7. the solid surface is so rigid and unmoveable that the superficial groups cannot direct or equilibrate themselves after environmental changes.

The contact angle gives some information on the affinity between solid and liquid and air. The relationship between contact angle and interfacial tensing is:

$$\cos\theta = \frac{\gamma_{s/a} - \gamma_{s/l}}{\gamma_{l/a}}$$

- $\gamma_{s/a}$= interfacial solid-air tension
- $\gamma_{s/l}$= interfacial solid-liquid tension
- $\gamma_{l/a}$= interfacial liquid-air tension
- If the contact angle is small (the drop is very flat), $\cos\theta \Rightarrow 1$,and so $\gamma_s/a - \gamma_s/l = \gamma_l/a$.

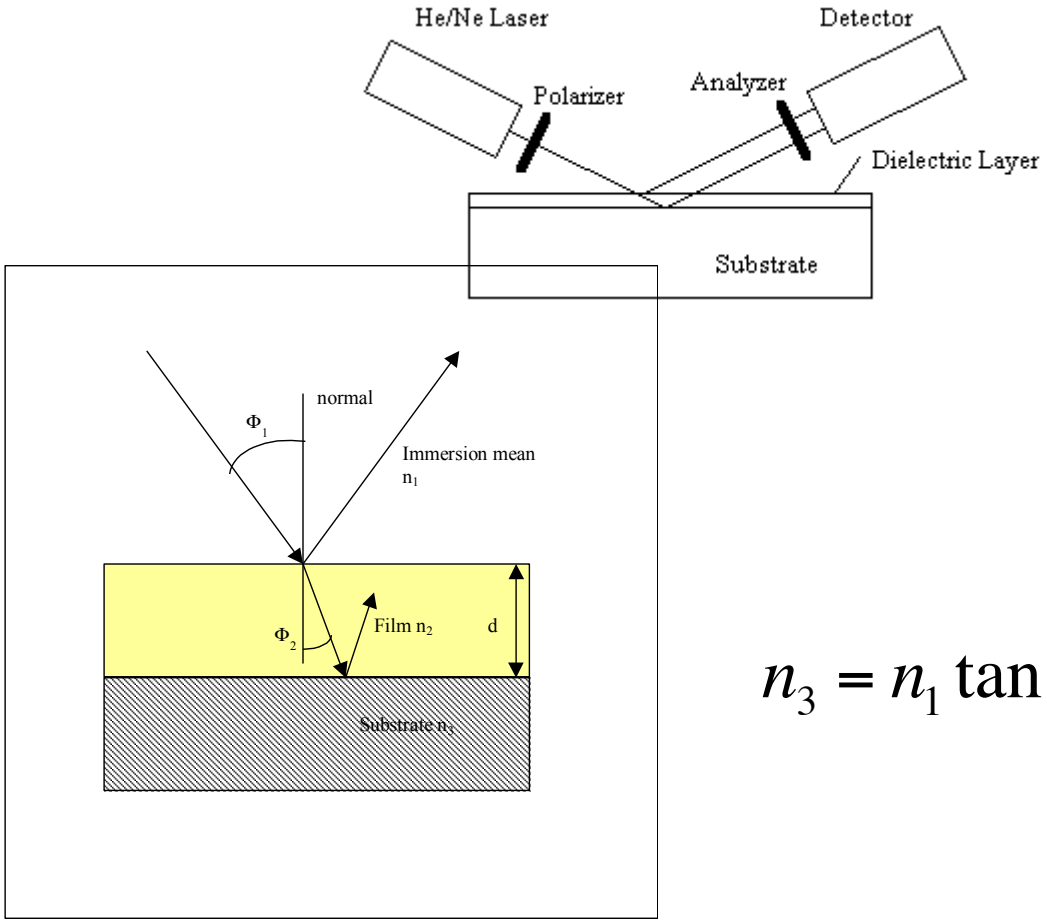
The three principal causes of hysteresis are:

1) the contamination of liquid or of the surface;
2) the presence of high roughness of material, that traps small quantities of air, altering the contact surface;
3) the rigidity of surface for which the positioning of bubble on the surface is difficult.

For these reasons, the measurements are not highly reproducible and there are variations in measured contact angle at different points on a surface.

Ellipsometry

Ellipsometry is a highly sensitive optical technique, useful for the thickness and optical density of a thin film



$$n_3 = n_1 \tan\varphi_1 \left[1 - \frac{4\rho \cdot \sin^2 \varphi_1}{(\rho + 1)^2} \right]^{\frac{1}{2}}$$

Ellipsometry is defined as the measurement of the Polarisation State of a wave vector. From the state of polarisation after reflection at an interface it is possible to obtain information on to the optical constants of the system that interacted with light ray modulating its polarisation.

The ellipsometric parameters measured are the complex ratio between the reflection coefficients relative to wave vectors polarised parallel and perpendicular to the plane of incidence. The electromagnetic theory of light allows the interpretation of the ellipsometric data obtained, giving equation that relate the complex amplitudes of reflections coefficients to the macroscopic properties that characterise the structure under examination.

Making measurements in different points of a surface, it is possible evaluate the uniformity of the layer. The lowest value of thickness that can be measured with this technique is almost an order of amplitude inferior than that measured with interferometric techniques, about 1 Angstrom.

The incident light can be separated into two components: one parallel and the other perpendicular to the plane of incidence. The reflection process introduces a phase difference Δ between these components and the variations in the ratio between their amplitudes, according to the law

$$\frac{|R_p|}{|R_S|}\tan\psi$$

where $tan\psi$ represents a measure of the absorptions of the two components. For this reason, the ratio between the reflection coefficient of polarised light in the plane of incidence and that in the plane of the surface is given by:

$$\rho = \frac{R_P}{R_S} = \tan\psi \cdot e^{j\Delta}$$

where ρ is the ratio between the reflection coefficients,

while ψ e Δ are functions of optical constants of surface, in other words of the wavelength of used light, the angle of incidence, thickness and refractive index of the film.

Kelvin Probe technique

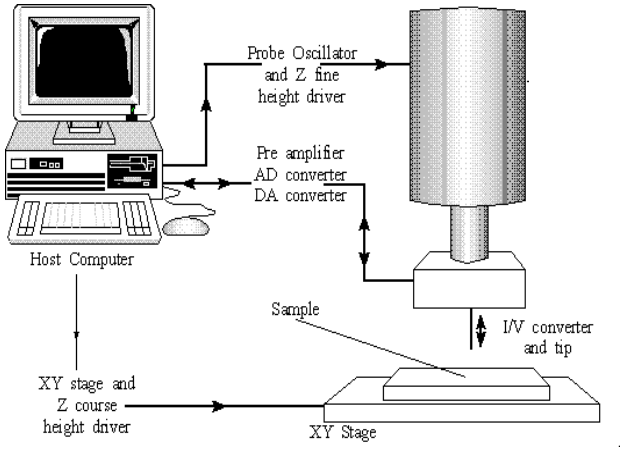
It is well none that cells, owing to the nature of the cytoplasmic lipid membrane, present a small negative external electrical charge. Interaction with positive surface are thus favoured. Cells are known to make a continuous contact with positively charged substrata, whereas in general, they present only discontinuous focal contacts with negatively charged substrata.

The measurement of the surface charge of a biopolymeric surface is hence an important in useful indicator of positive all-surface interaction. The measurement of the surface potential of polymer films was obtained with the Kelvin-probe technique. The Kelvin method was first postulated by the renowned Scottish scientist W. Thompson, later Lord Kelvin, in 1861. This method is based on the measurement of the potential difference between a fixed steel and vibrating plate, with and without a dielectric, positioning the two plates at a distance of a few millimetres. The difference between the two plates gives a measure of surface potential of the polymer.

Kelvin Probe technique

The Kelvin Method is also an indirect technique for the measuring work function of a surface. The work function is the least amount of energy required to remove an electron from the surface of a conducting material, to a point just outside the metal, with zero kinetic energy. As the electron has to move through the surface region, it's energy is influenced by the optical, electric and mechanical characteristics of the region. Hence, the work function is an extremely sensitive indicator of surface condition and is affected by absorbed or evaporated layers, surface reconstruction, surface charging, oxide layer imperfections, surface and bulk contamination

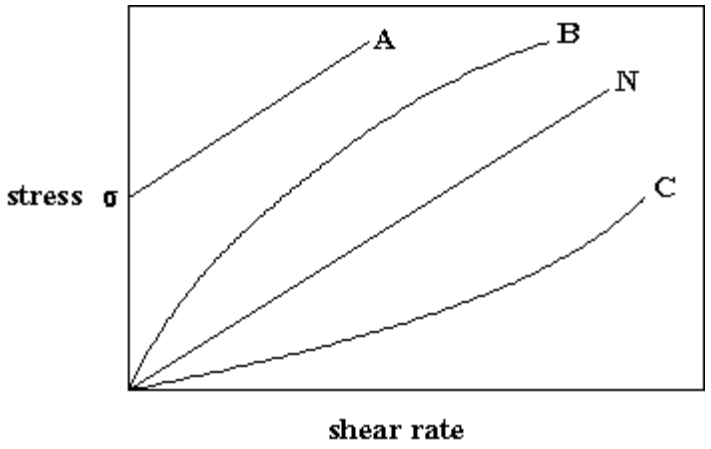
Kelvin Probe technique



$$C = \frac{Q}{V} = \frac{\varepsilon_0 A}{d}$$

$$\sigma = \frac{2\varepsilon_0 \varepsilon V}{d(\varepsilon - 1) + R}$$

Saybolt Viscometer

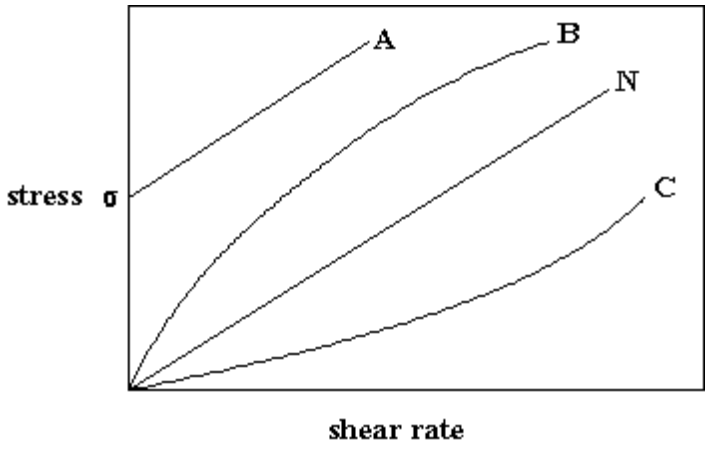


$$\sigma = K\left(\frac{dc}{dt}\right)^{n}$$

$$\eta = K^{'} c^{\partial} \overline{M_{w}^{3.4}}$$

The flow behaviour of polymer melts is often a very important parameter in industrial processes and is particularly relevant to injection moulding. The viscosity of the melt is the single most important characteristic to be considered when designing polymer systems for ease of injection moulding. In shear flow of a Newtonian liquid the shear stress is directly proportional to the shear strain rate and viscosity is independent of the shear rate, but several responses are possible for a polymer, as illustrated in figure.

Saybolt Viscometer



$$\sigma = K\left(\frac{dc}{dt}\right)^{n}$$

$$\eta = K^{'} c^{\partial} \overline{M}_{w}^{3.4}$$

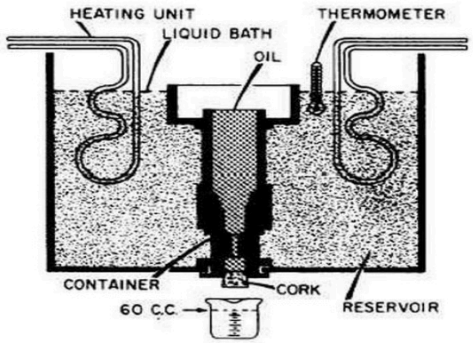
For two of the four types of viscosity behaviour illustrated stress is proportional to shear rate - these are the standard Newtonian (N) and Newtonian after a critical yield stress has been exceeded (A). The behaviour characterised by B and C is shear-rate thinning or pseudo plastic (B) and shear rate thickening or dilatent (C); several other types are possible

Saybolt Viscometer

the Saybolt viscometer**.** It consists of a cylindrical container for the polymer solution under examination with a receiving flask under it to catch and measure polymer solution discharged from the container. At the bottom of the container is an orifice of specified dimensions through which the polymer flows. The container is jacketed with a water bath to facilitate maintenance of a constant temperature. Two thermometers check temperatures, one in the polymer solution and one in the water bath. To adjust the temperature, an external source of heat is applied to the bath. Flow of polymer solution into the receiver is timed with a stop watch or equivalent device. The time of flow is taken to be proportional to the viscosity of the fluid.

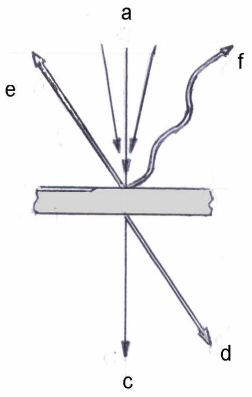
Viscosity is a linear function of temperature and it is possible express it as:

$\mu=aT+b.$



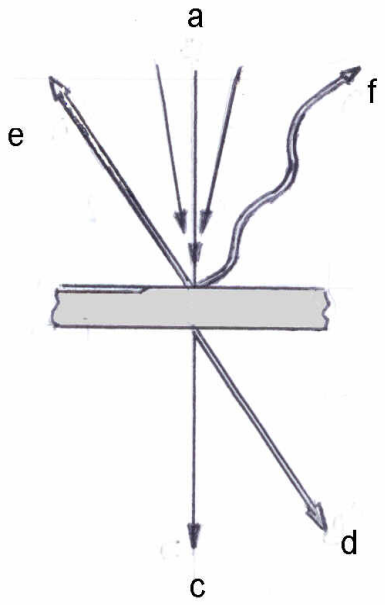
Scanning electrical Microscopy

Scanning electronic microscopy allows the use of a wide range of magnifications, between 15 until 500000, and has a high depth of field, in other terms the difference between maximum and minimum focusing positions, which enables surfaces with high topographic variations to be well focused. In SEM the different points of a sample are explored with a high power thin electronic beam, produced by an electronic gun, and focused with a magnetic lens system. Appropriate devices allow either motions of beam, that allow exploring a little square area, on motions of the sample relative to the beam, that allow varying not only the area under examination but also the inclination of the sample relative to the beam .



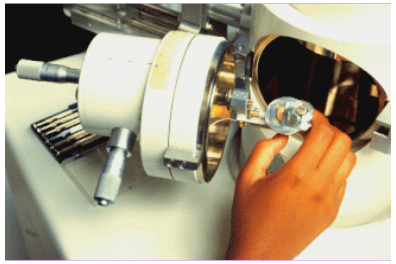
Scanning electrical Microscopy

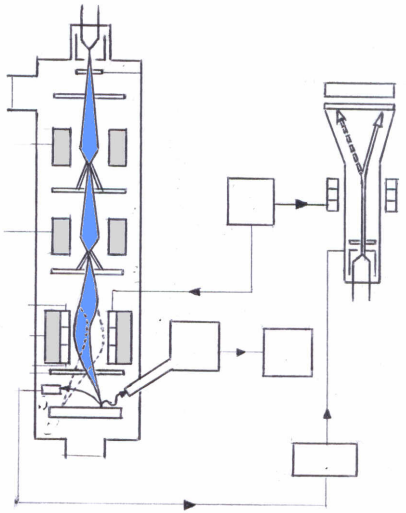
When a beam of electrons hits the surface of a material a part of these incident electrons, called primary electrons, preserve their energy and are reflected, (retrodiffusive electrons) (*e* in figure), while the other loose their energy by transferring it to the electrons of the solid, (*a, c* in figure). A fraction of electrons are then emitted with lower energy (*f, d* in figure).



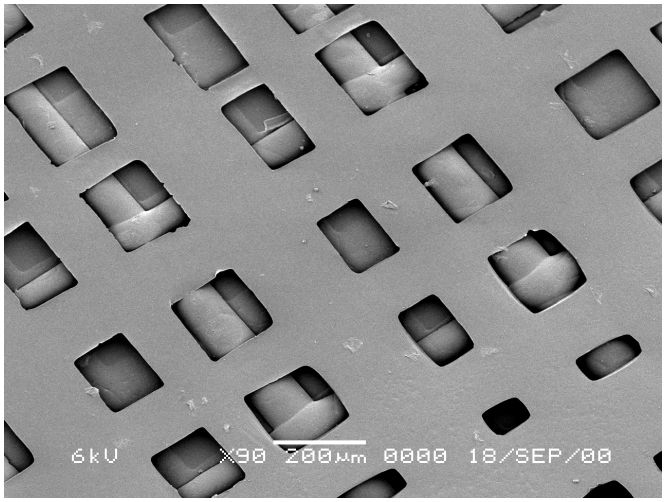
Scanning electrical Microscopy

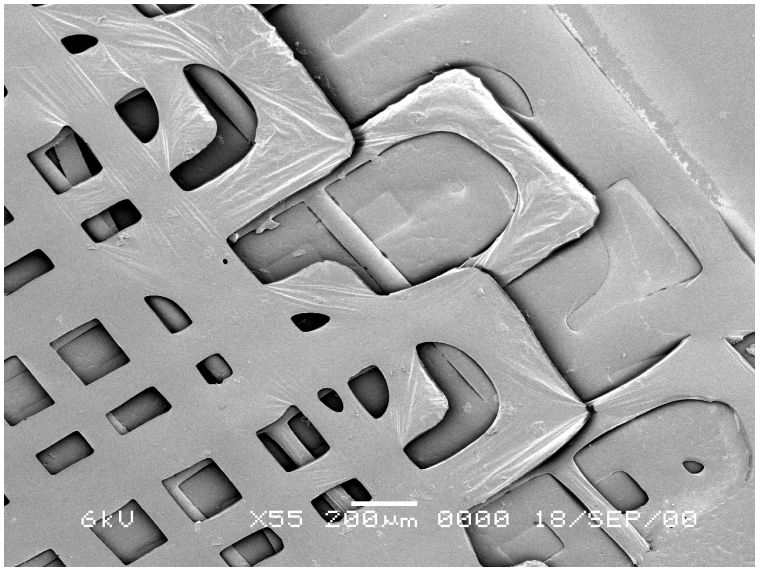
The incident electrons have high energy; they are able to ionise the interior energy levels of atoms of the material then go back to the fundamental state with photon emission. The X rays produced have energies that are characteristic of the atoms that emit them, and so they can be utilised to obtain information on the chemical composition of the sample. With an X-ray spectrum analyser, it is possibly have a spectrum that gives the relative peaks of the different elements. The intensity of characteristic line of one element is directly proportional to its concentration.





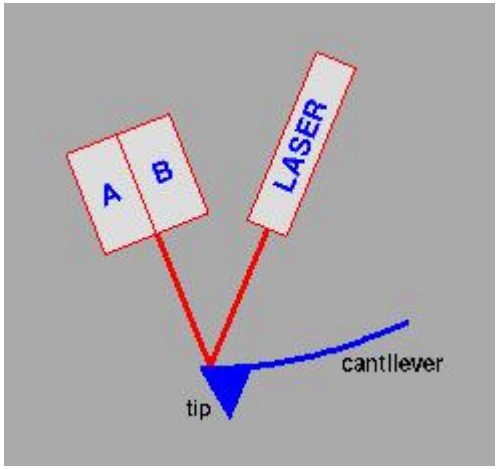
Scanning electrical Microscopy



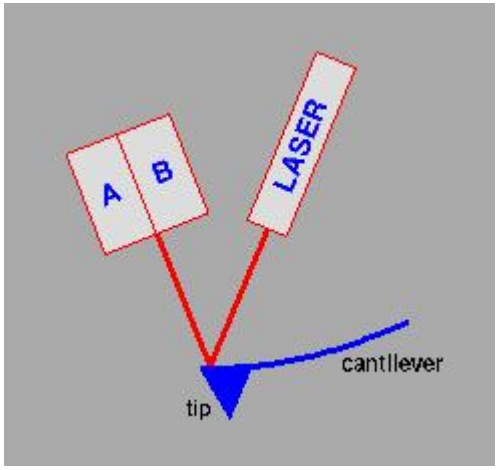


Atomic Force Microscopy

Binnig, Quate and Gerber invented the atomic force microscope (AFM) or scanning force microscope (SFM) in 1986. Like all other scanning probe microscopes, the AFM utilises a sharp probe moving over the surface of a sample in a raster scan. In the case of the AFM, the probe is a tip on the end of a cantilever, which bends in response to the force between the tip and the sample



Atomic Force Microscopy



The figure illustrates how this works; as the cantilever flexes, the light from the laser is reflected onto the split photo-diode. By measuring the difference signal (A-B), changes in the bending of the cantilever can be measured. Since the Cantilever obeys Hooke's Law for small displacements, the interaction force between the tip and the sample can be found. An extremely precise positioning device made from piezoelectric ceramics, most often in the form of a tube scanner performs the movement of the tip or sample. The scanner is capable of sub-angstrom resolution in x-, y- and z-directions. The z-axis is conventionally perpendicular to the sample.

Atomic Force Microscopy

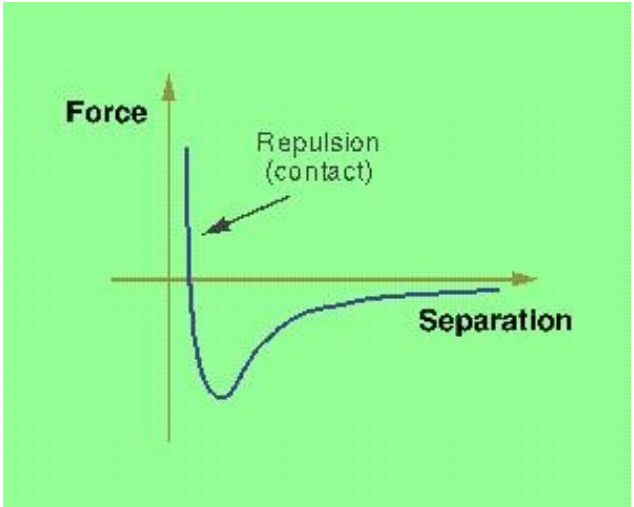
The AFM can be operated in two principal modes
with feedback control
without feedback control
If the electronic feedback is switched on, then the positioning piezo which is moving the sample (or tip) up and down can respond to any changes in force which are detected, and alter the tip-sample separation to restore the force to a pre-determined value. This mode of operation is known as *constant force*, and usually enables a fairly faithful topographical image to be obtained (hence the alternative name, *height mode*).
If the feedback electronics are switched off, then the microscope is said to be operating in *constant height* or *deflection* mode. This is particularly useful for imaging very flat samples at high resolution. Often it is best to have a small amount of feedback-loop gain, to avoid problems with thermal drift or the possibility of a rough sample damaging the tip and/or cantilever.

Atomic Force Microscopy

The way in which image contrast is obtained can be achieved in many ways. The three main classes of interaction are *contact mode*, *tapping mode* and *non-contact mode*.

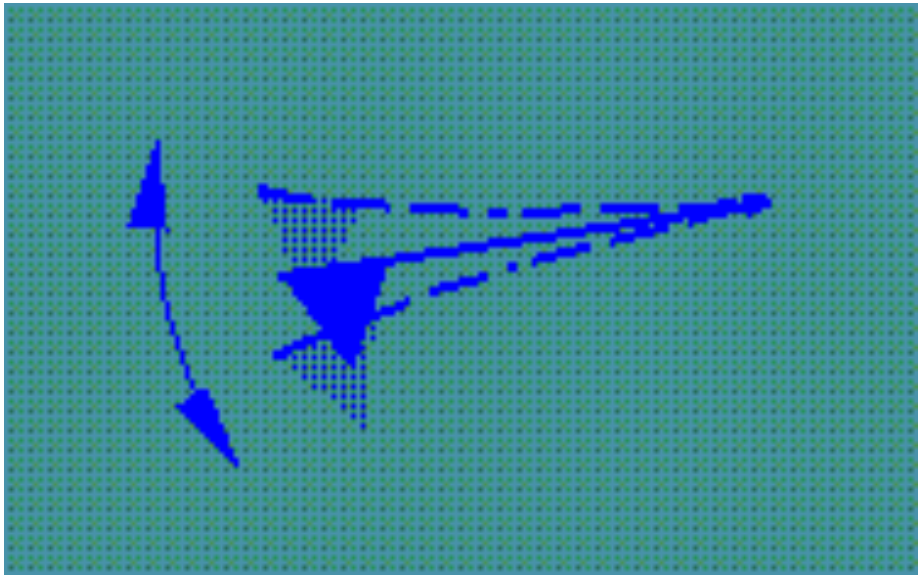
Contact mode is the most common method of operation of the AFM. As the name suggests, the tip and sample remain in close contact as the scanning proceeds. By "contact" we mean in the repulsive regime of the inter-molecular force curve.

The repulsive region of the curve lies above the x-axis. One of the drawbacks of remaining in contact with the sample is that there exist large lateral forces on the sample as the drip is "dragged" over the specimen.



Atomic Force Microscopy

Tapping mode is the next most common mode used in AFM. When operated in air or other gases, the cantilever is oscillated at its resonant frequency (often hundreds of kilohertz) and positioned above the surface so that it only taps the surface for a very small fraction of its oscillation period. This is still contact with the sample in the sense defined earlier, but the very short time over which this contact occurs means that lateral forces are dramatically reduced as the tip scans over the surface. When imaging poorly immobilised or soft samples, tapping mode may be a far better choice than contact mode for imaging.



Atomic Force Microscopy

Non-contact operation is another method, which may be employed when imaging by AFM. The cantilever must be oscillated above the surface of the sample at such a distance that we are no longer in the repulsive regime of the inter-molecular force curve. This is a very difficult mode to operate in ambient conditions with the AFM. The thin layer of water contamination, which exists on the surface on the sample, will invariably form a small capillary bridge between the tip and the sample and cause the tip to "jump-to-contact". Even under liquids and in vacuum, jump-to-contact is extremely likely, and imaging is most probably occurring using tapping mode.

Atomic Force Microscopy

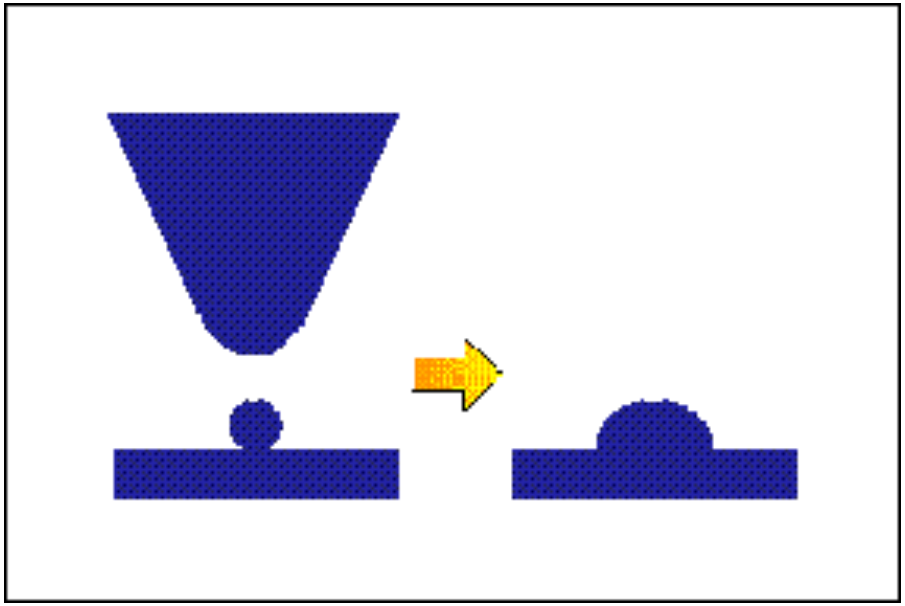
One of the most important factors influencing the resolution, which may be achieved with an AFM, is the sharpness of the scanning tip. The first tips used by the inventors of the AFM were made by gluing diamond onto pieces of aluminium foil. Commercially fabricated probes are now universally used. The best tips may have a radius of curvature of only around 5nm. The need for sharp tips is normally explained in terms of *tip convolution*. This term is often used (slightly incorrectly) to group together any influence, which the tip has on the image. The main influences are:

- broadening
- Compression
- interaction forces
- aspect ratio

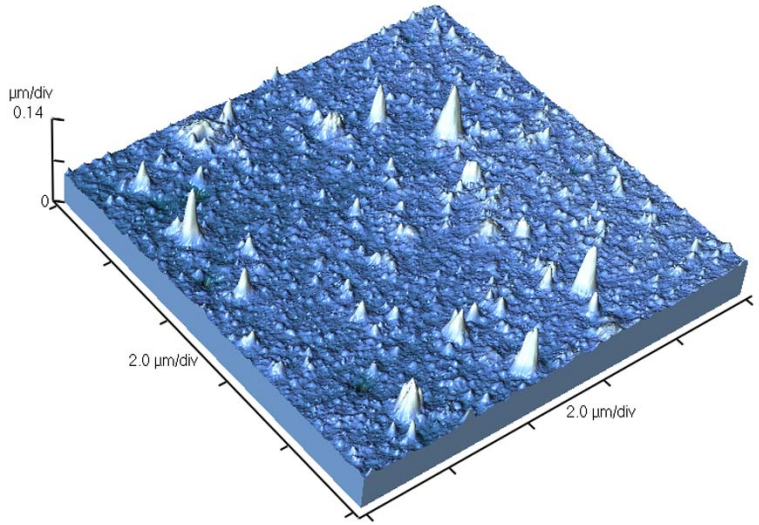
Tip broadening arises when the radius of curvature of the tip is comparable with, or greater than, the size of the feature trying to be imaged. The diagram illustrates this problem; as the tip scans over the specimen, the sides of the tip make contact before the apex, and the microscope begins to respond to the feature. This is what we may call tip convolution.

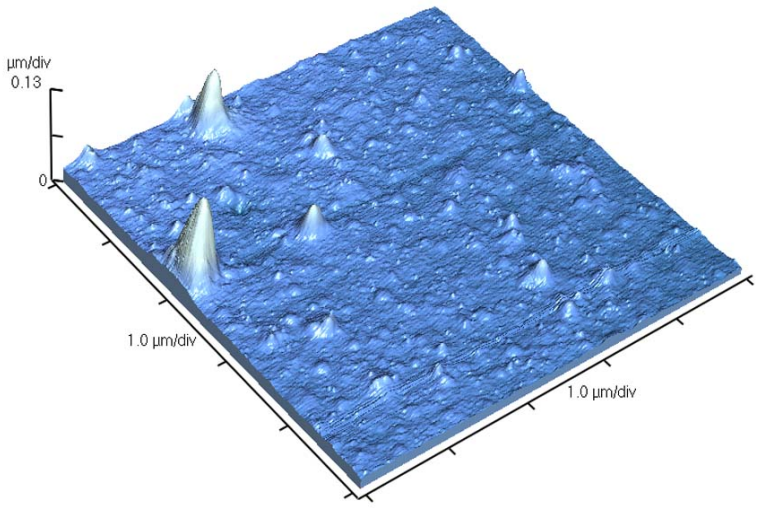
Atomic Force Microscopy

Compression occurs when the tip is over the feature trying to be imaged. It is difficult to determine in many cases how important this affect is, but studies on some soft biological polymers (such as DNA) have shown the apparent DNA width to be a function of imaging force.



Atomic Force Microscopy

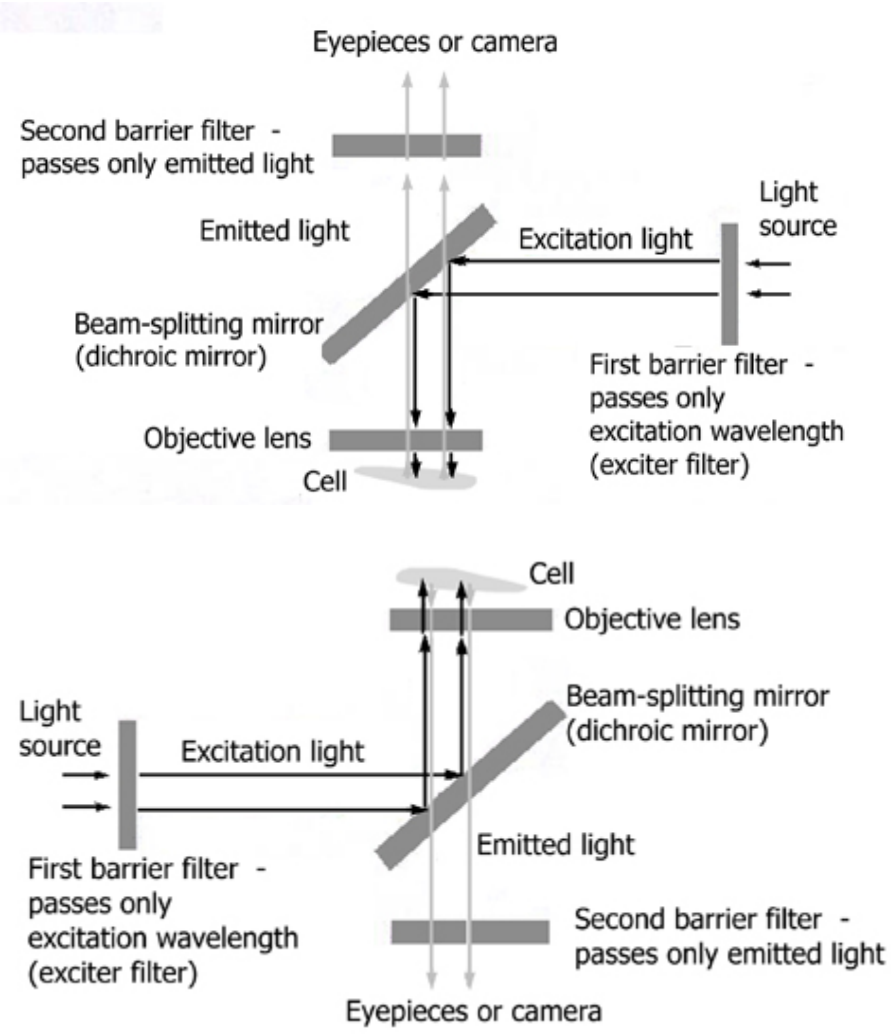




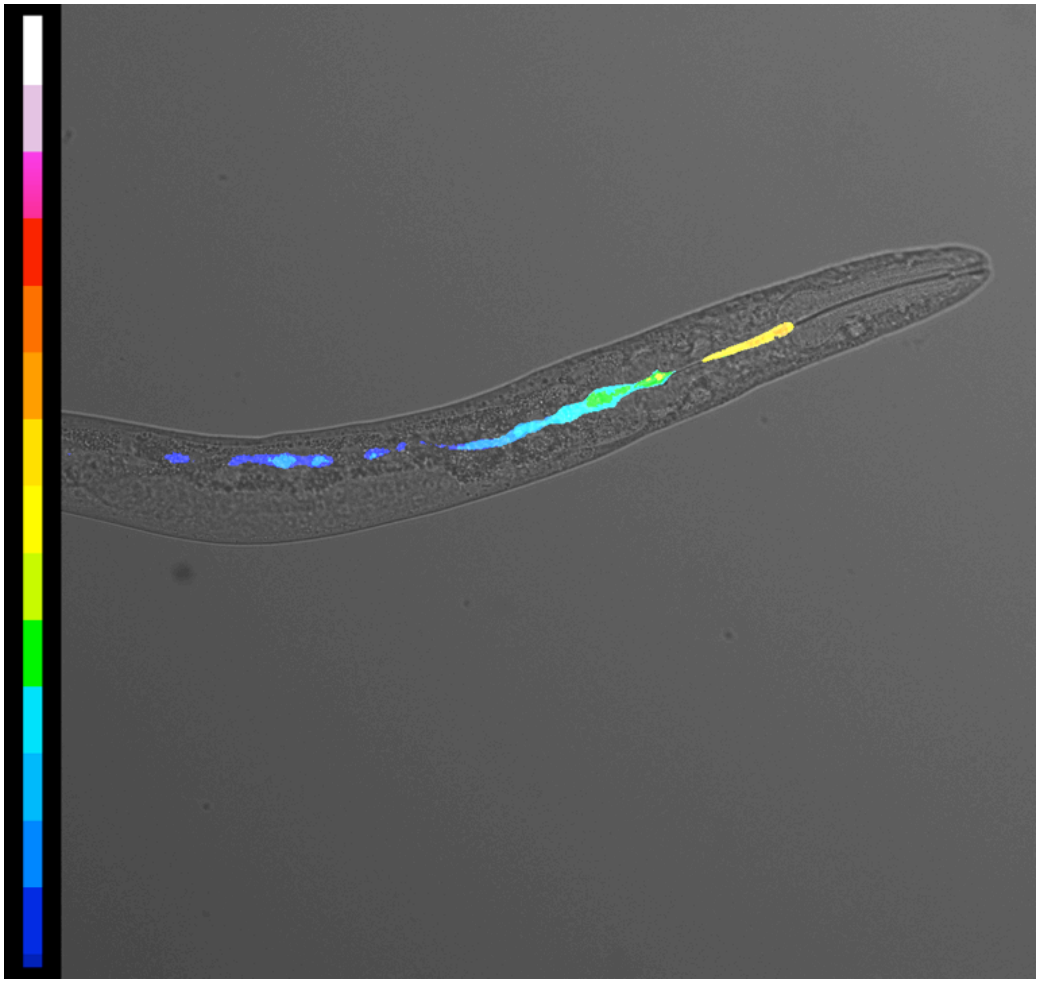
Fluorescence Microscopy

Fluorescence microscopy is used to detect structures, molecules or proteins within the cell. Fluorescent molecules absorb light at one wavelength and emit light at another, longer wavelength. When fluorescent molecules absorb a specific absorption wavelength for an electron in a given orbital, the electron rises to a higher energy level (the excited) state. Electrons in this state are unstable and will return to the ground state, releasing energy in the form of light and heat. This emission of energy is fluorescence. Because some energy is lost as heat, the emitted light contains less energy and therefore is a longer wavelength than the absorbed (or excitation) light. In fluorescence microscopy, a cell is stained with a dye and the dye is illuminated with filtered light at the absorbing wavelength; the light emitted from the dye is viewed through a filter that allows only the emitted wavelength to be seen. The dye glows brightly against a dark background because only the emitted wavelength is allowed to reach the eyepieces or camera port of the microscope.

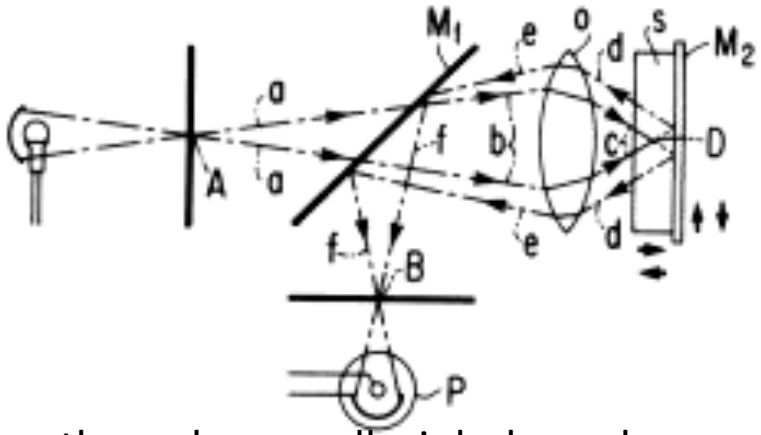
Fluorescence Microscopy



Fluorescence Microscopy

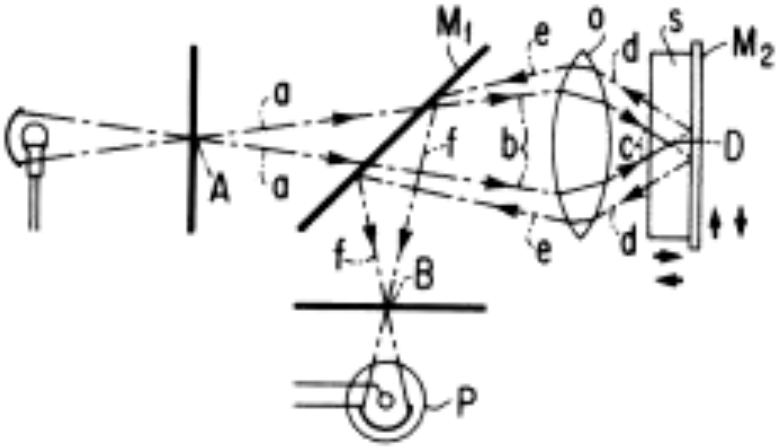


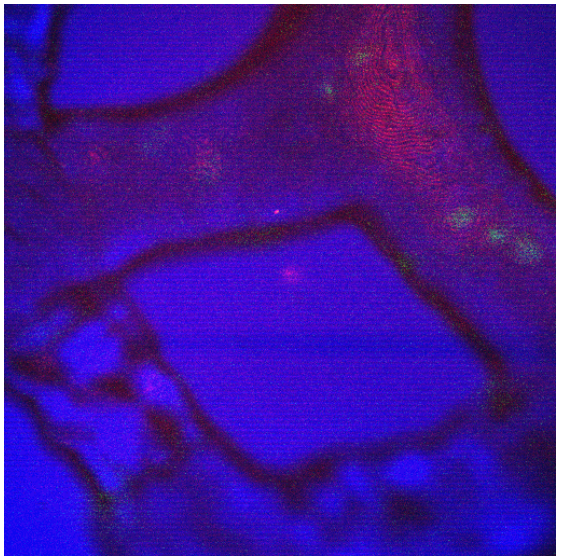
Confocal Microscopy

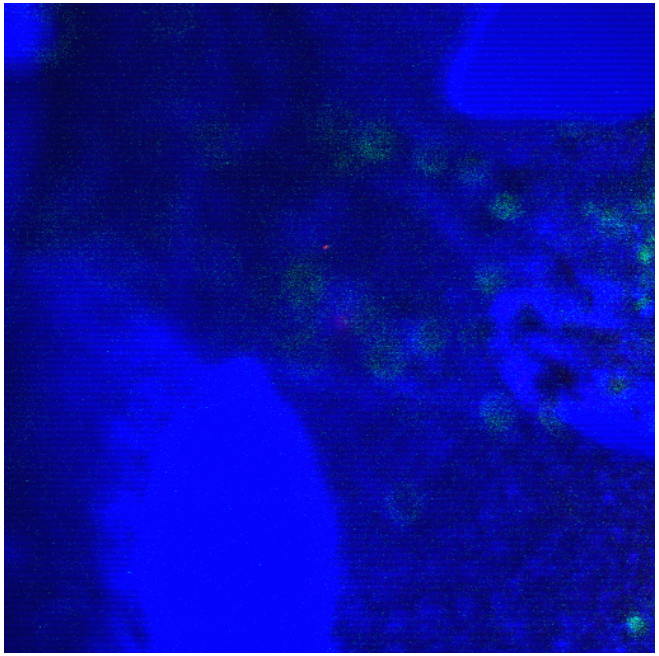


Light from a laser passes through a small pinhole and expands to fill the entrance pupil of a microscope objective lens. The objective lens focuses the light to a small spot on the specimen, at the focal plane of the objective lens. Light reflected back from the illuminated spot on the specimen is collected by the objective and is partially reflected by a beam splitter to be directed at a pinhole placed in front of the detector. This confocal pinhole is what gives the system its confocal property, by rejecting light that did not originate from the focal plane of the microscope objective. Light rays from below the focal plane come to a focus before reaching the detector pinhole, and then they expand out so that most of the rays are physically blocked from reaching the detector by the detector pinhole. In the same way, light reflected from above the focal plane focuses behind the detector pinhole, so that most of that light also hits the edges of the pinhole and is not detected. However, all the light from the focal plane is focused at the detector pinhole and so is detected at the detector. This ability to reject light from above or below the focal plane enables the confocal microscope to perform depth discrimination and optical tomography. A true 3D image can be processed by taking a series of confocal images at successive planes into the specimen and assembling them in computer memory.

Confocal Microscopy



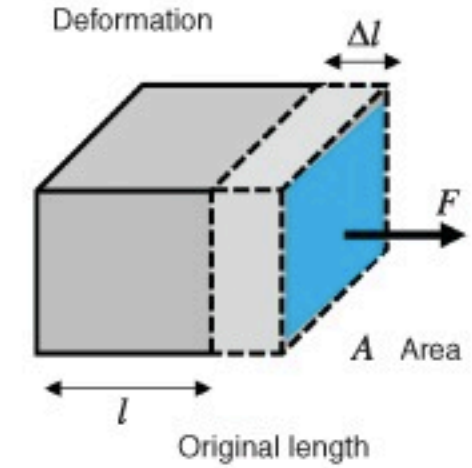




Mechanical properties

(a)

Tension/compression

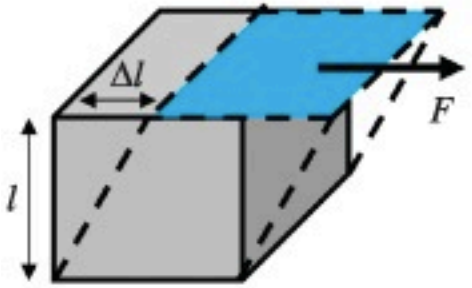


Normal strain $\varepsilon = \Delta l / l$

Normal tensile / compressive force

Normal stress $\sigma = F/A$

Shear



Tangential shear force

Shear stress $\tau = F/A$

Shear strain $\gamma = \Delta l / l$

Δt Time interval of deformation

$\dot{\gamma} = \Delta\gamma / \Delta t$ Shear strain rate / rate of deformation

(b)

Elastic solid

$\sigma = E\varepsilon$

E Elastic modulus

$\tau = G\gamma$

G Shear modulus

Viscous liquid

$\tau = \mu\dot{\gamma}$

Viscosity μ

(c)