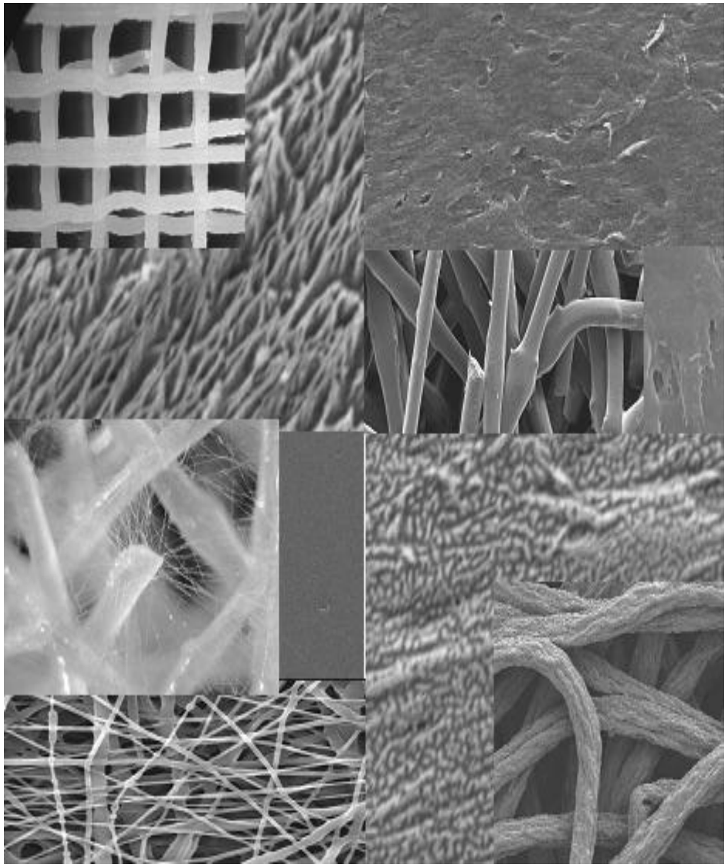
Surface Characterization of Materials

The surFACE



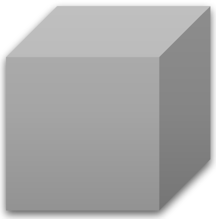
- The surface emits, absorbs and scatters, it is where **interactions with the outside world start**.

- The surface is also what one **sees, touches and smells**, it is where human feelings and emotions begin.

Surface vs Bulk

❖Number of atoms (1cm^3)

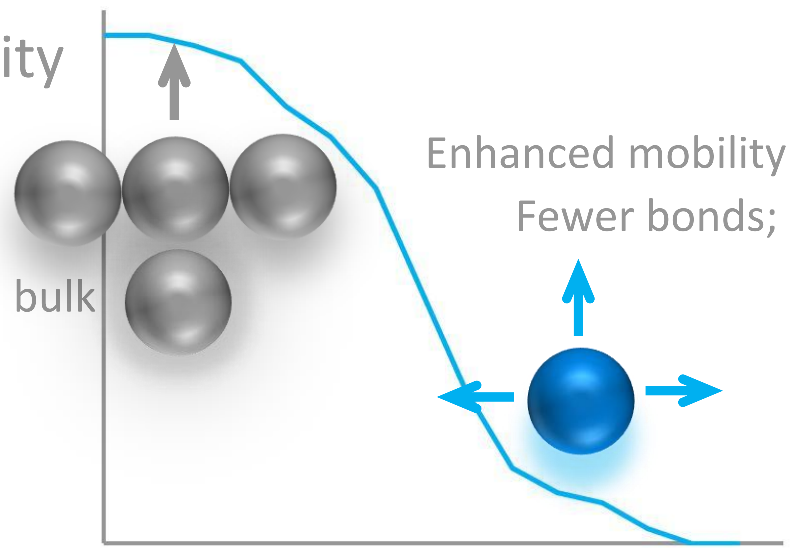
10^{15} atoms (10 ng)



10^{23} atoms

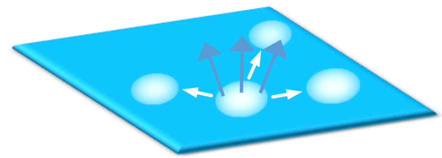
❖*Requires special characterization tools*

❖Atom mobility

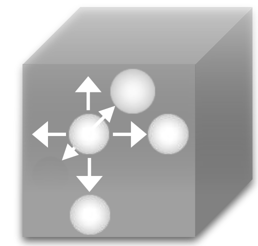


❖*Facilitates rate-limited processes* (phase transformations, crystallization, corrosion…)

Surface vs Bulk



Atoms/molecules with unsatisfied (dangling or strained) bonds

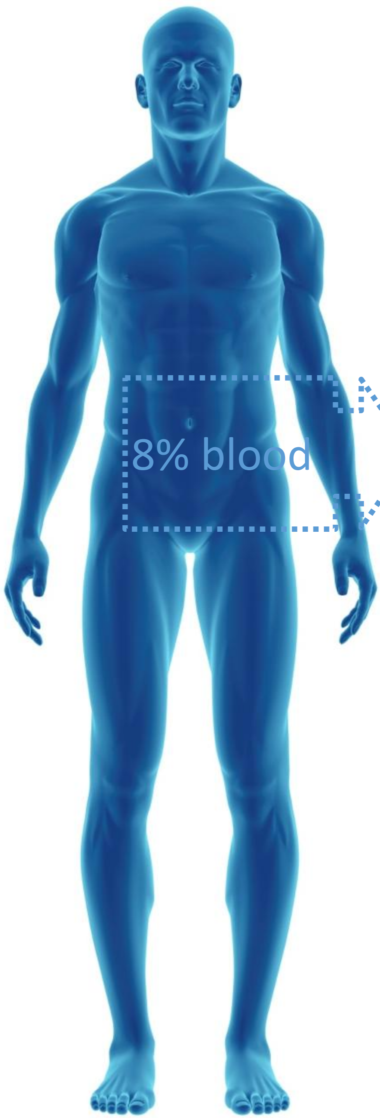


- Energy state

- *High reactivity and susceptibility to adsorbates*

*NOTE: The development of **MODERN SURFACE SCIENCE** did not begin until the early 1960s as the tools (UHV techniques) needed to detect the small numbers of surface atoms relative to the bulk atoms became available.*

Surface: where the bioreaction starts



8% blood

45% formed elements (cells)

- Erythtocytes 5million/mm^3
- Leukocytes 7000/mm^3
  - Neutrophils (65%)
  - Lymphocytes (21%)
  - Monocytes (5%)
  - Eosinophils (3%)
  - Basophils (1%)
- Platelets 300000/mm^3

55% plasma

- proteins
  - Albumins (55%)
  - Globulins (38%)
  - Fibrinogen (7%)
- other solutes
  - Gases, Food
  - Lipid, salts
- water

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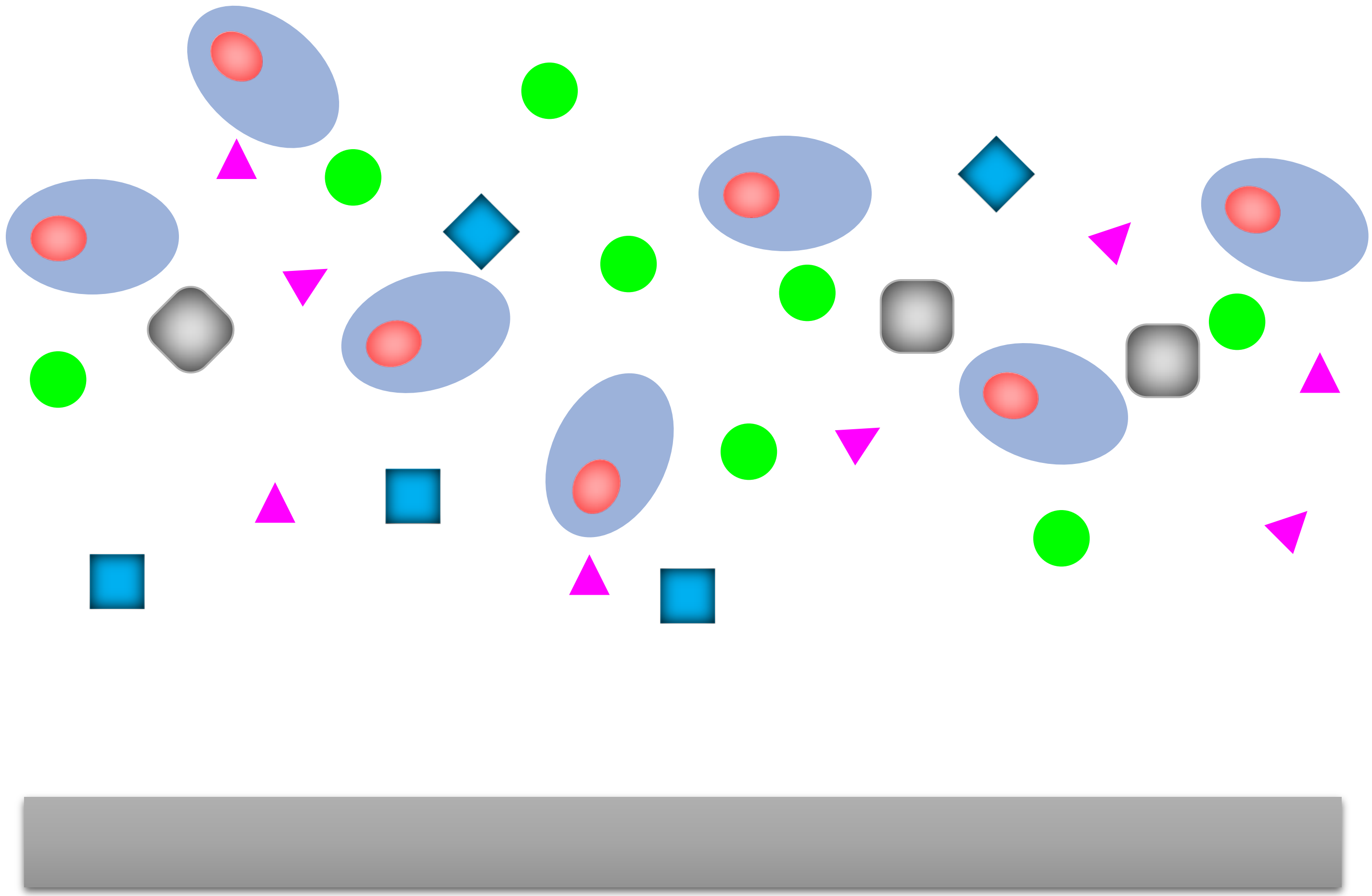
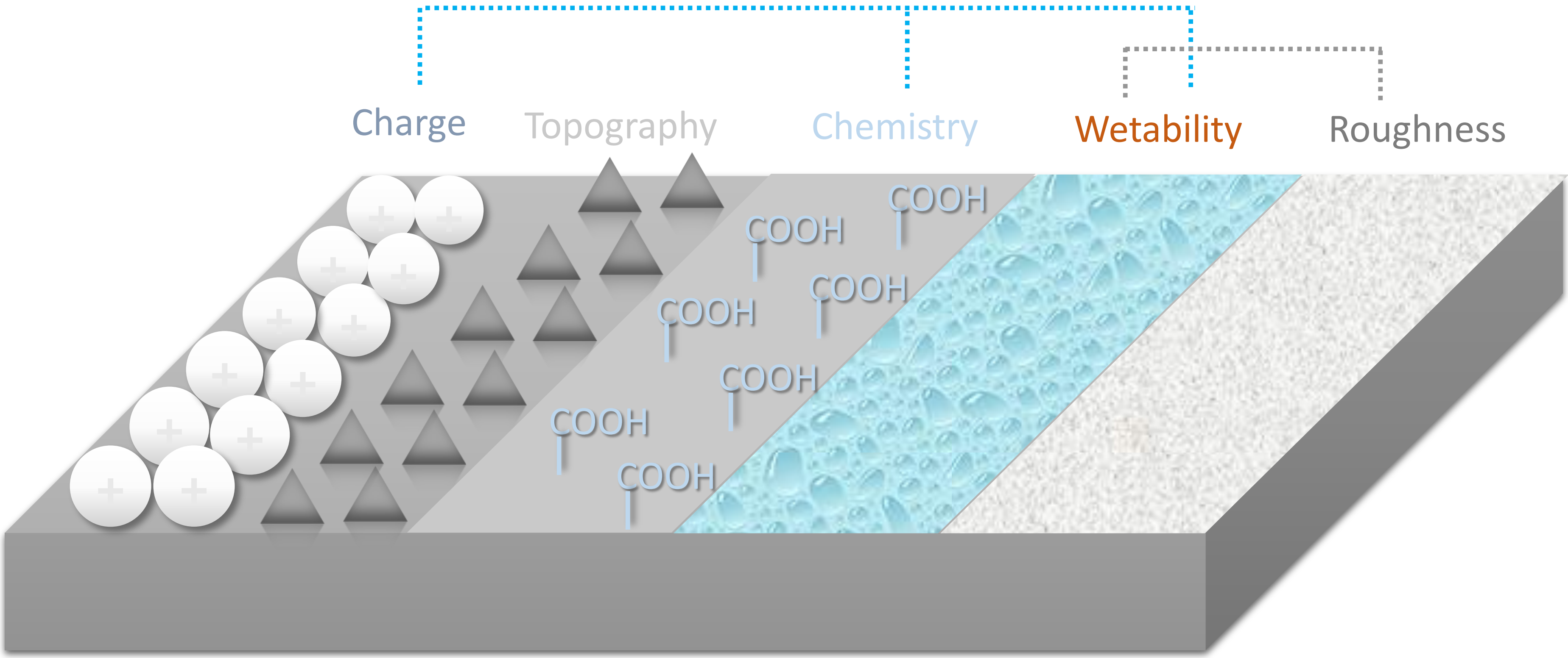
The surface and **cells society**

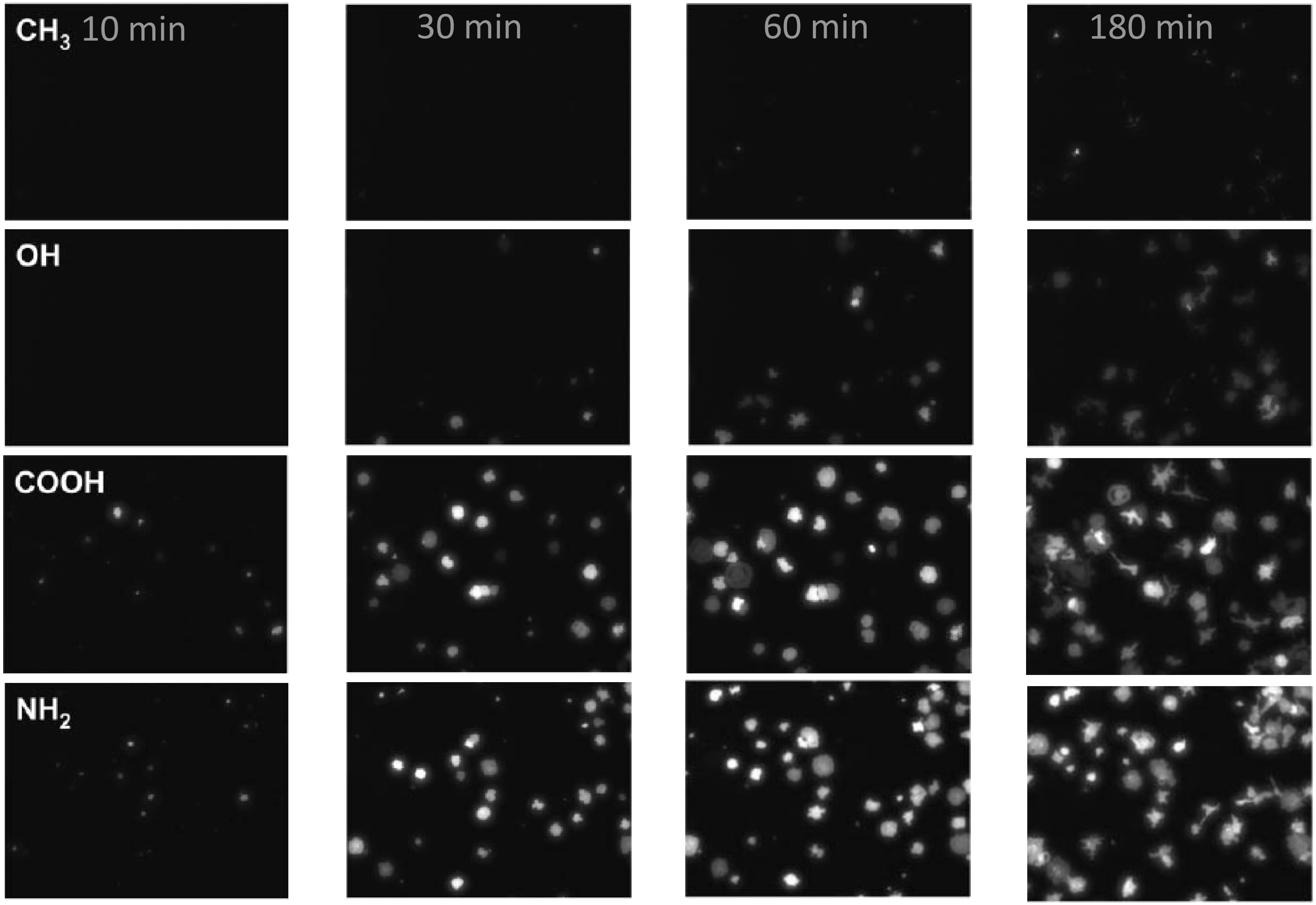


The surface dictates biological recognition!

Surface properties



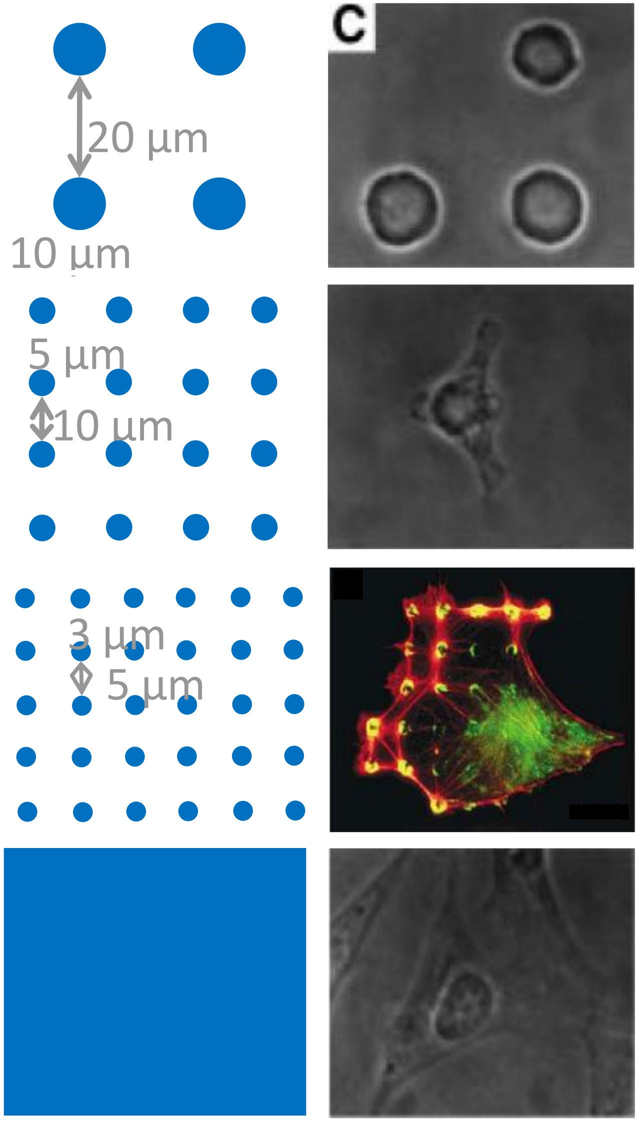
Cell sense of chemistry/wetability



Arima et al J Mat Chem (2007) 17:4079

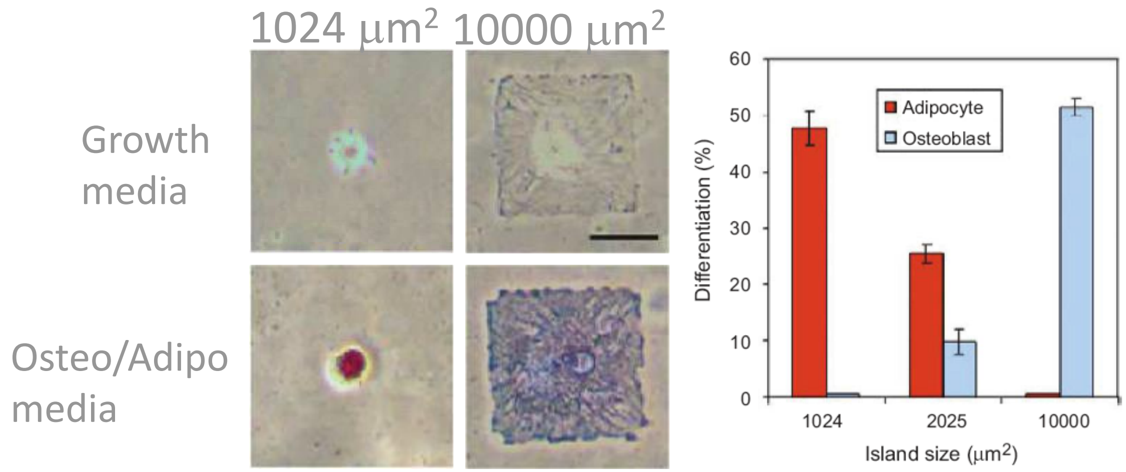
Cell sense of topography

Capillary endothelial cells



Chen C et al, Biotechnol Progr 1998, 14:356

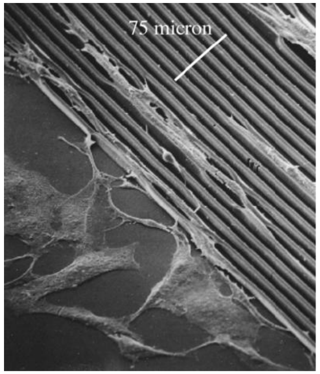
Mesenchymal stem cells differentiation



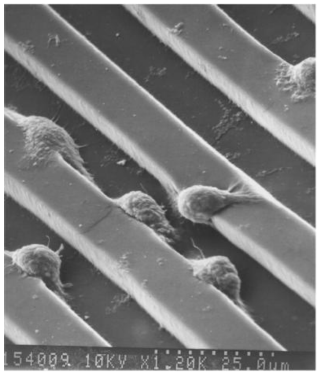
McBeath R et al, Dev Cell 2004, 6 (4): 483

Different cells behave differently

Epitenon fibroblasts



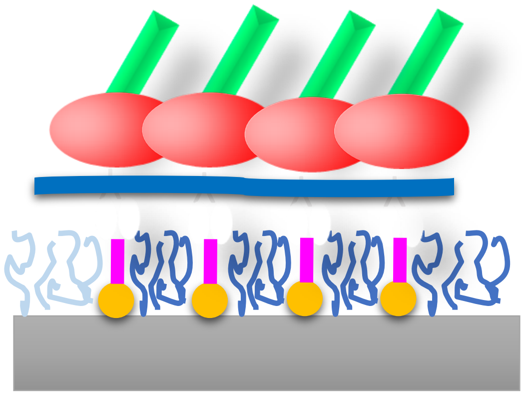
Human macrophages



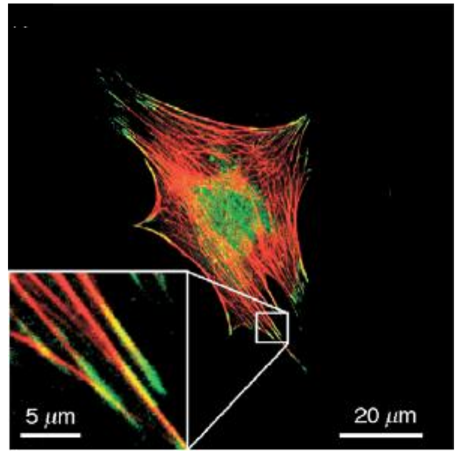
Curtis A, Eur Cells Materials 2004, 8:27.

Cell sense of size: the nano-scale

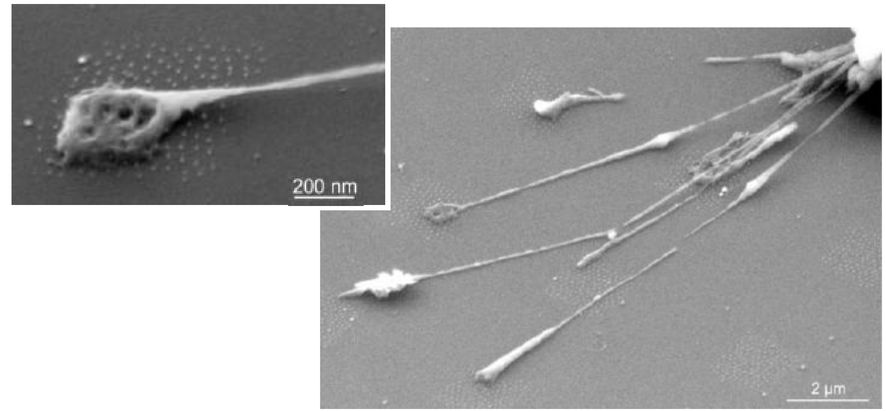
Efficient FA formation



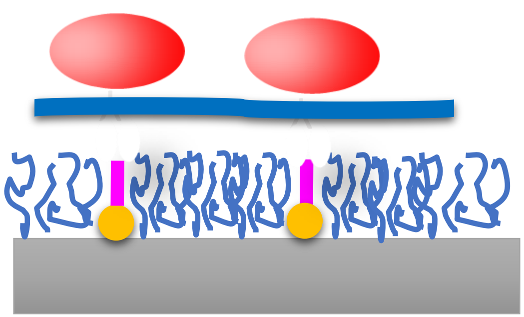
↔≤58nm



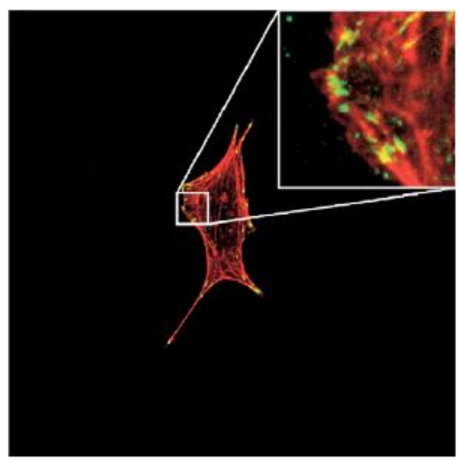
3T3-fibroblast adhering to micro-nanopatterns



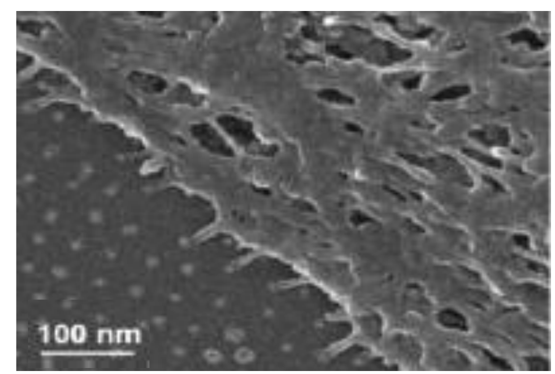
Limited cell attachment, spreading and actin stress fiber formation



↔≥73nm



3T3-fibroblast adhering to extended nanopatterns



Spatz JP and Geiger B, Methods in cell biology (2007) 83: 89

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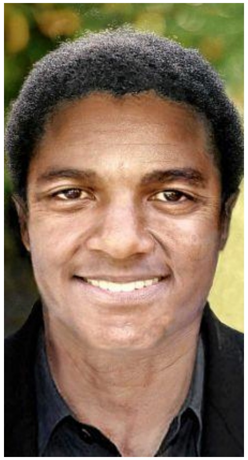
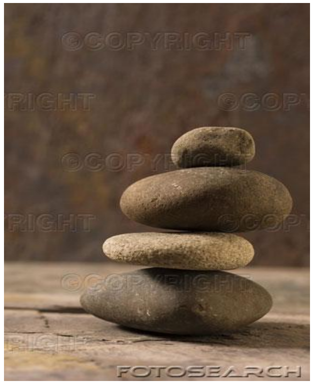
Tailoring the surface

❖**Modification:** The art of applying change to the original





Bulk modification

Surface modification

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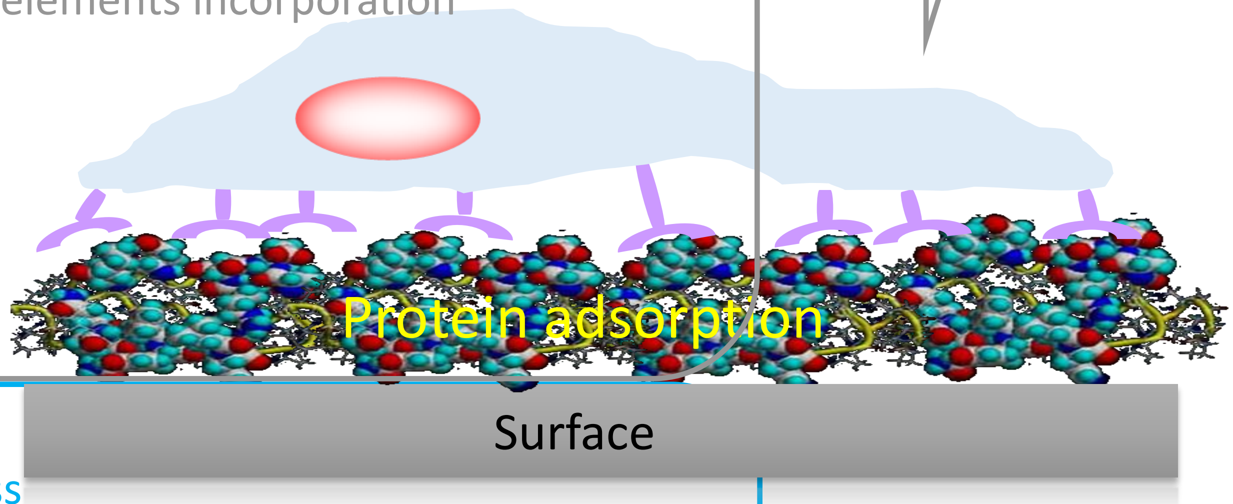
Surface Modification Approaches

❖Protein immobilization

❖Mimicking cell elements (membranes, receptors, etc.)

❖Bioactive elements incorporation

BIO-APPROACHES



❖Roughness
❖Wettability
❖Surface mobility
❖Chemical composition
❖Crystallinity
❖Heterogeneity

ENGINEERING APPROACHES

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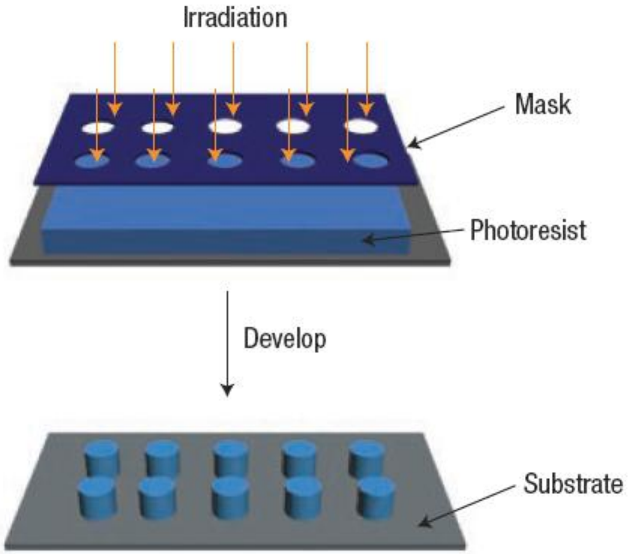
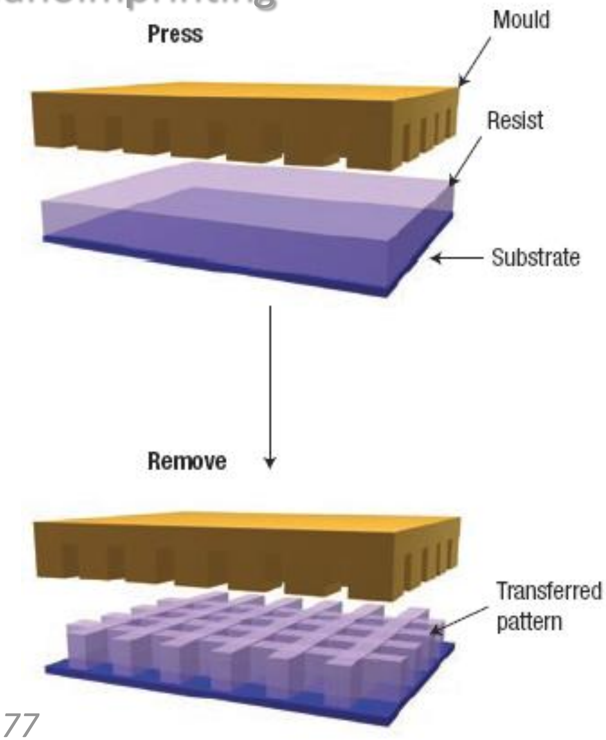
Surface topography: patterning

Photolithography



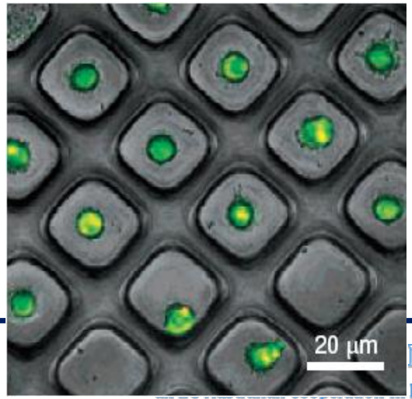
Nanoimprinting



Nie Z at al, Nature materials (2008) 7:277

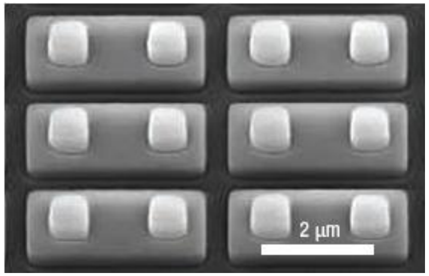
3T3 fibroblasts confined within PEG microwells with 30 × 30 μm individual dimensions

Revzin A et al, Langmuir (2003) 19:9855.



SEM image of multi-tier template.

Schmid GM et al. J. Vac. Sci. Technol. B (2006) 24:1283



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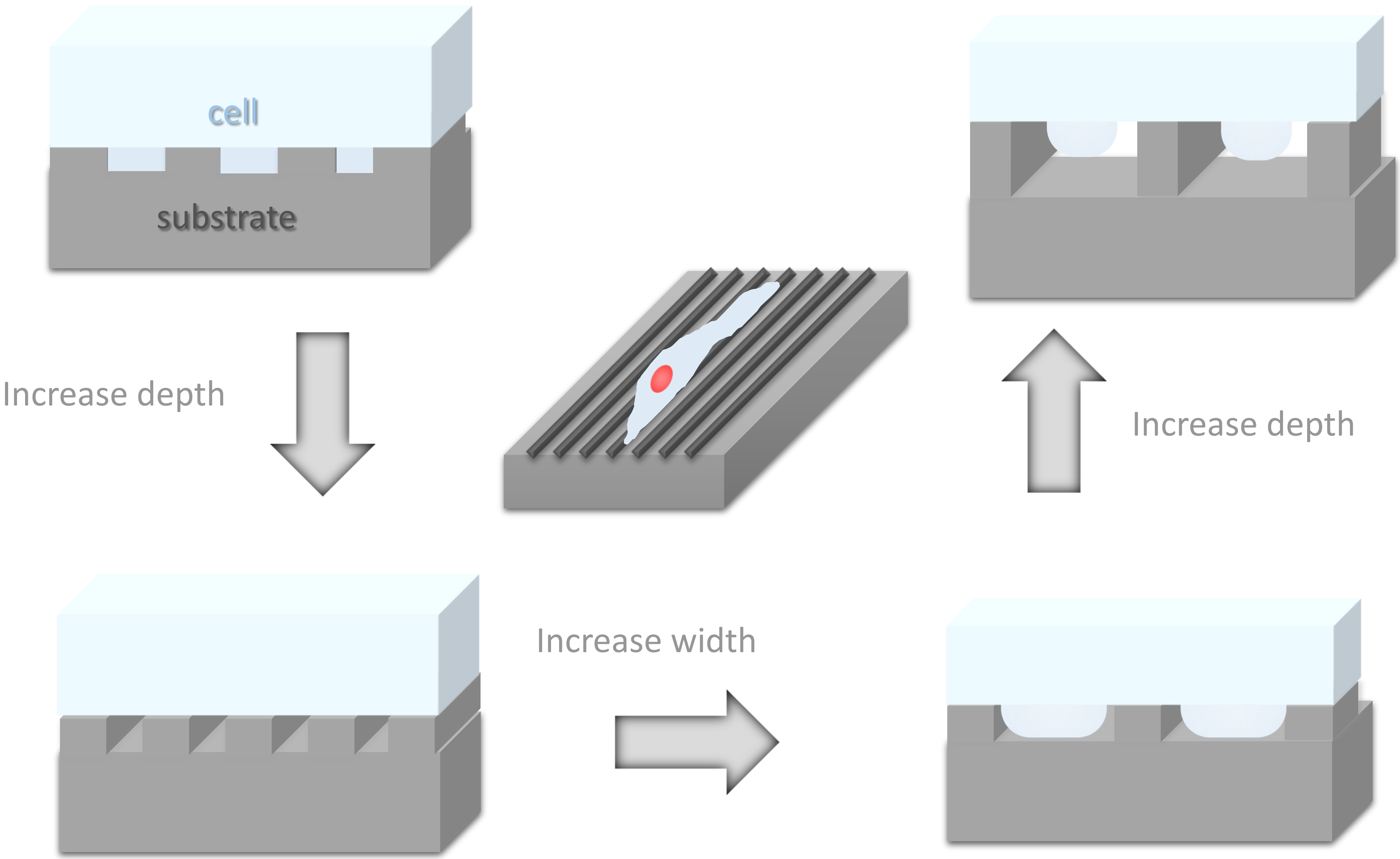
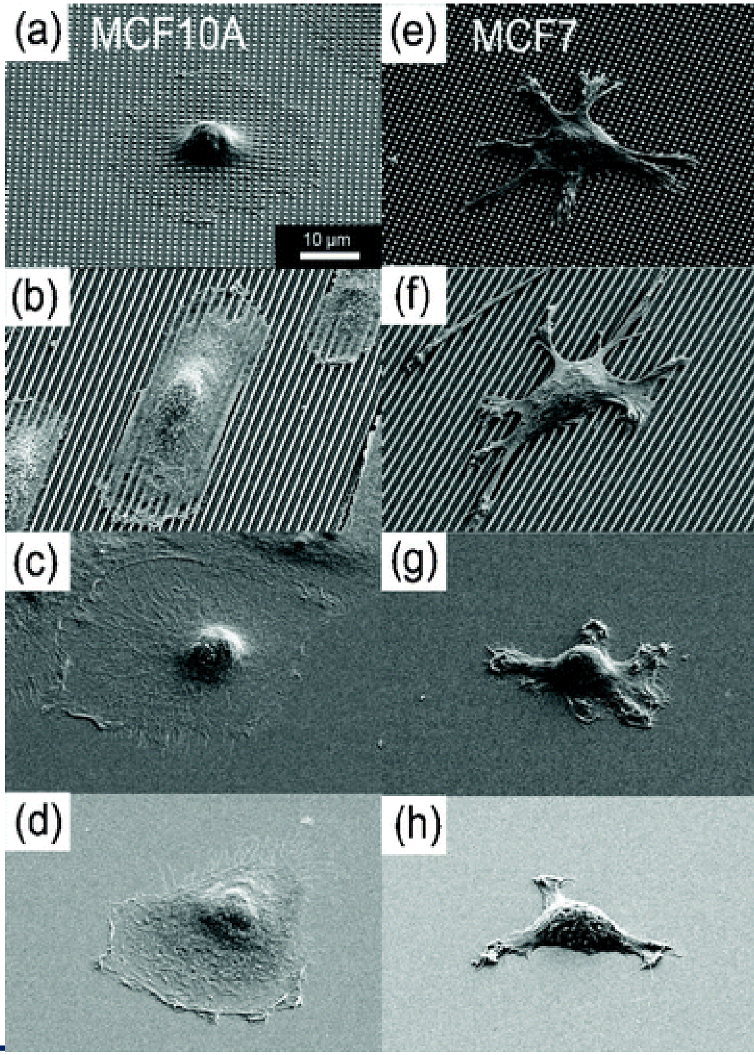
Surface topography: patterning



Yang et al, WIREs Nanomed Nanobiotechnol 2010 2:478

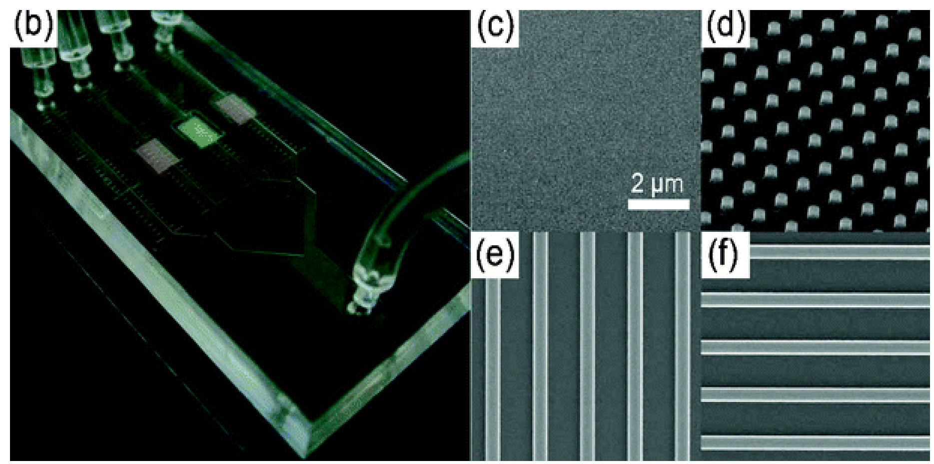
Surface topography: patterning

Label-free, microfluidic separation and enrichment of human breast cancer cells by adhesion difference



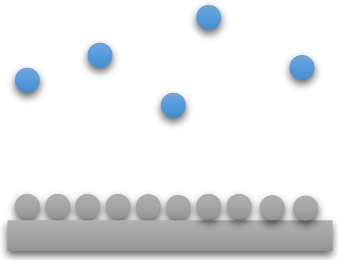
MCF10A: human breast epithelial cell line
MCF7: human breast cancer cell line

Cells cultured for 2 h on various surfaces used in the experiment

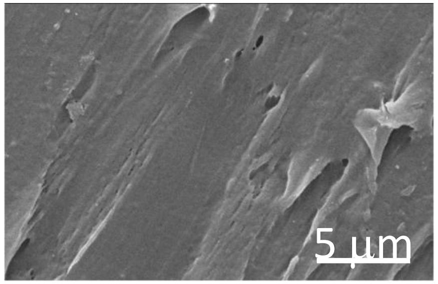


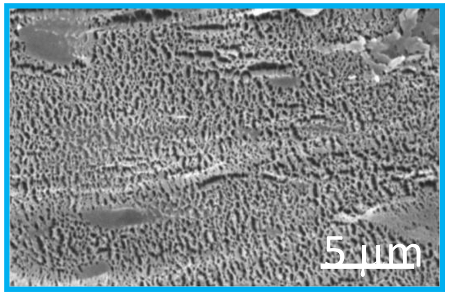
Kwon KW et al, Lab Chip (2007) 7:1461

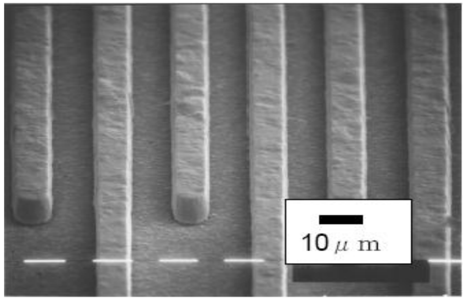
Surface roughness: etching

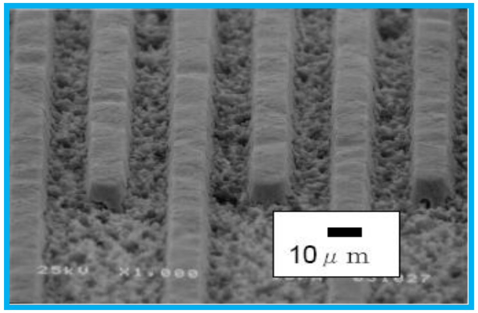


- ❖Plasma etching
- ❖Photo etching
- ❖Chemical etching



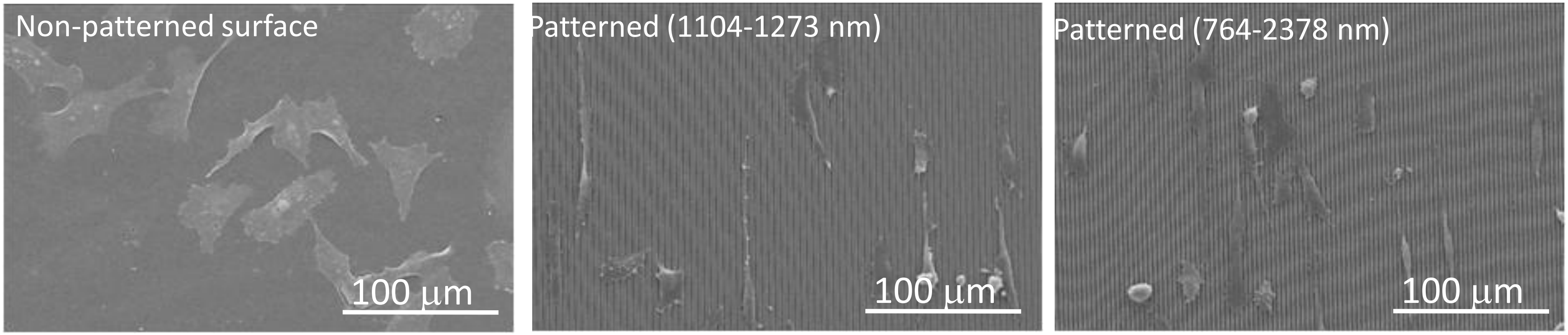






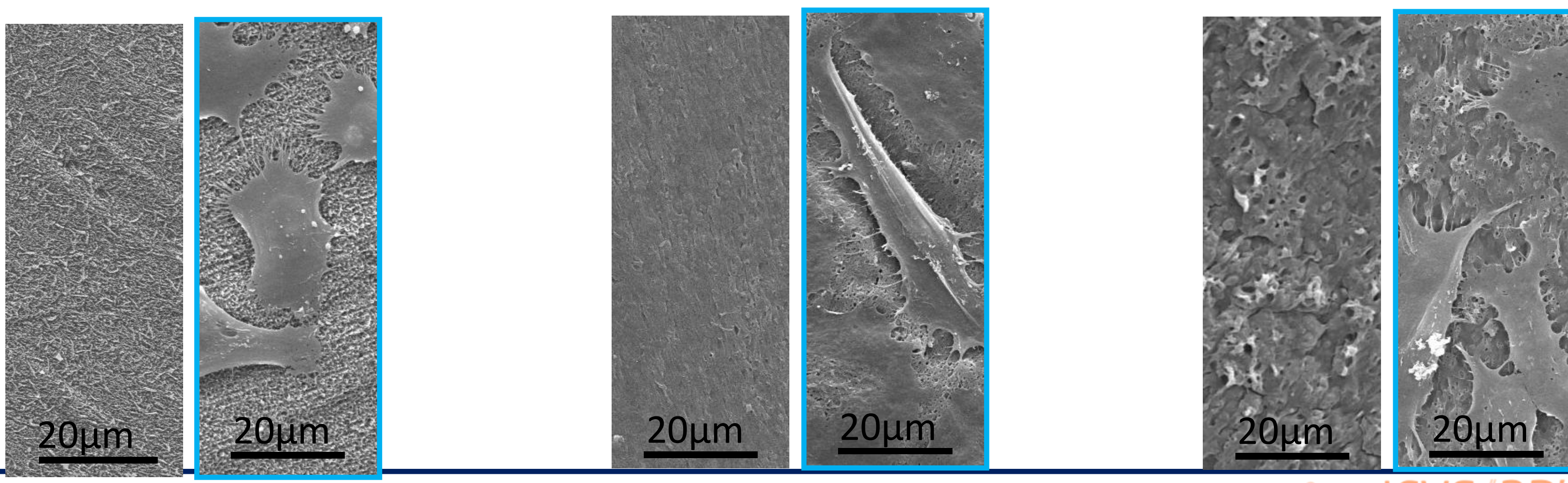
Surface roughness: cell response

❖**Anisotropic topographies** such as ridges and grooves: cells align along the anisotropic direction



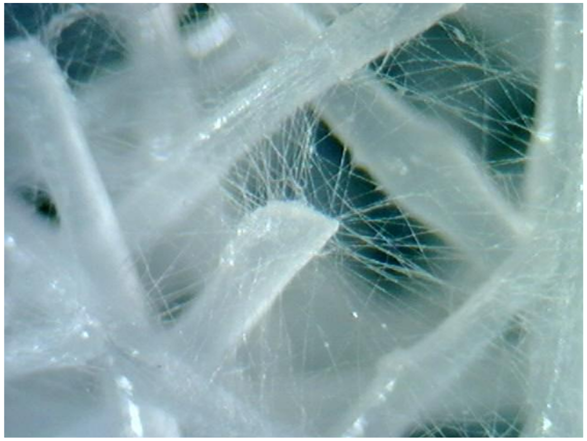
Baran ET et al, unpublished results

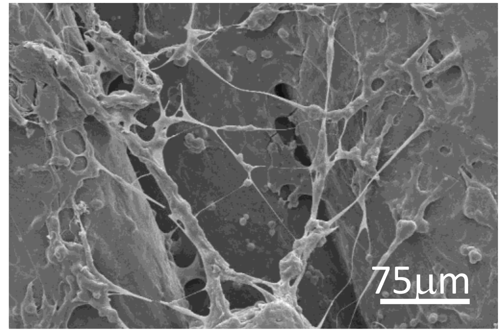
❖**Isotropic topographies** such as evenly or randomly distributed pits or protrusions, affect collective cell behaviors

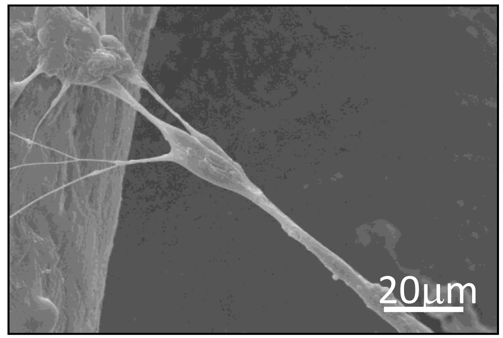


Surface morphology 3D: cell response

Nano/Macro Fibers

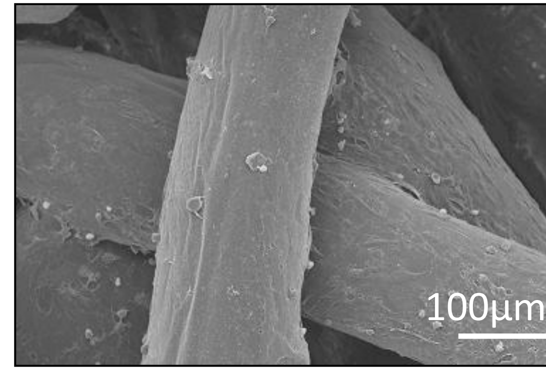






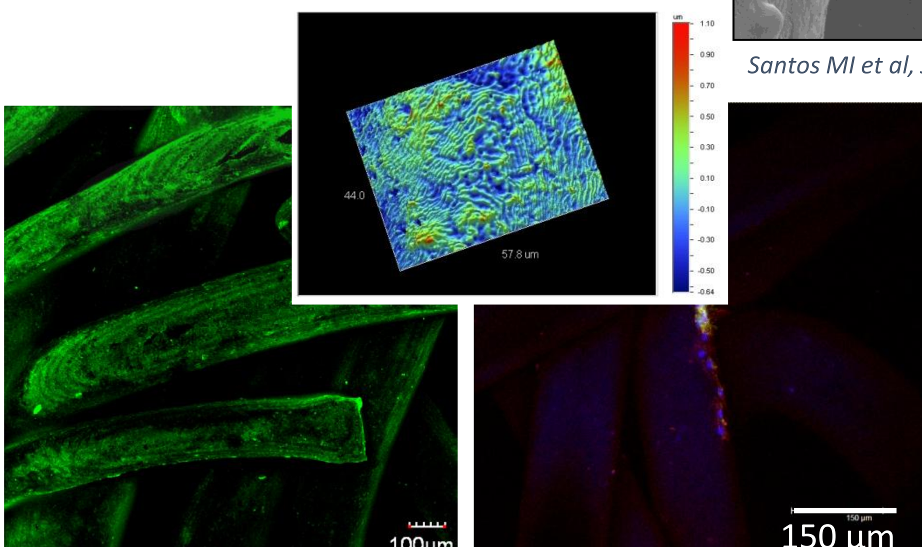
Santos MI et al, J Mat Chem (2009) 19:4091

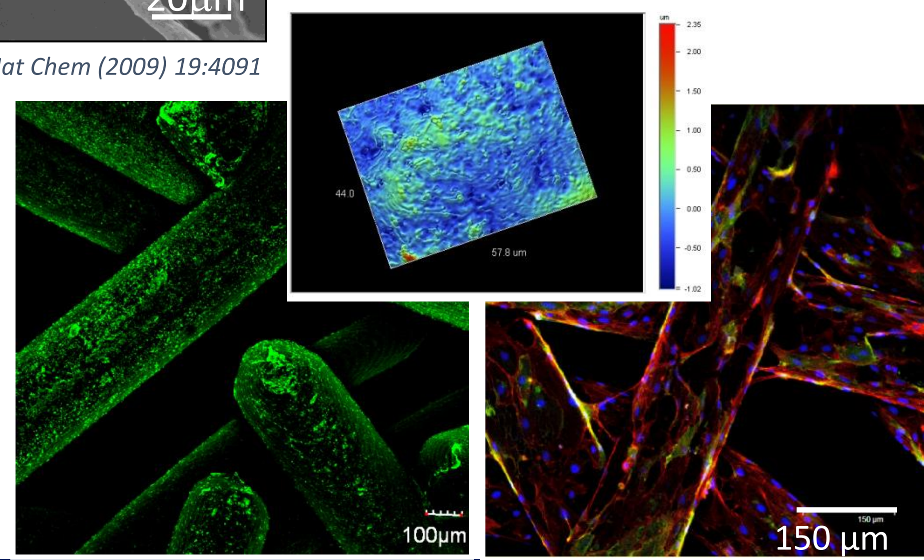
Human Osteoblast-like cell line (SaOs-2), 2 weeks



Tuzlakoglu K et al, J Mat Sci: Mat Med (2005) 16:1099

Plasma Etched Fibers





Surface chemistry: **coating**

Francisco Goya

The nude Maja

The clothed Maja

No chemical bond!!

Easy to apply

Long term instability e.g. delamination

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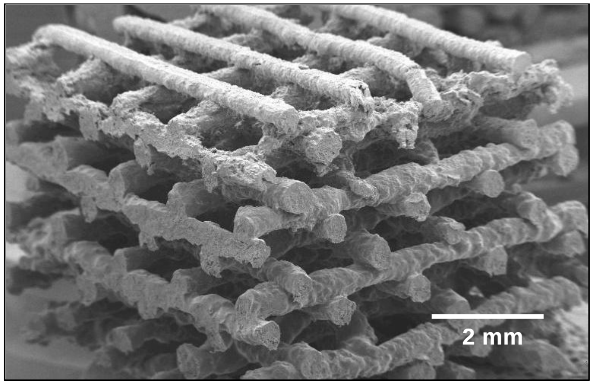
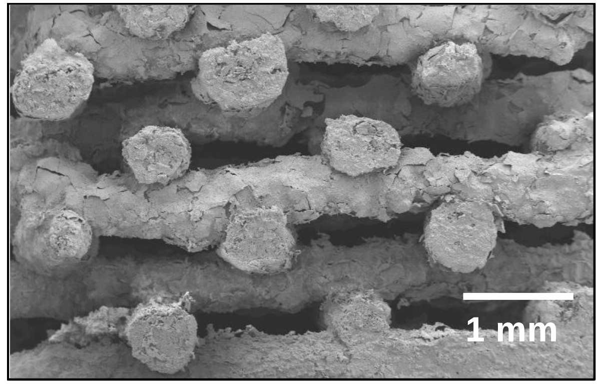
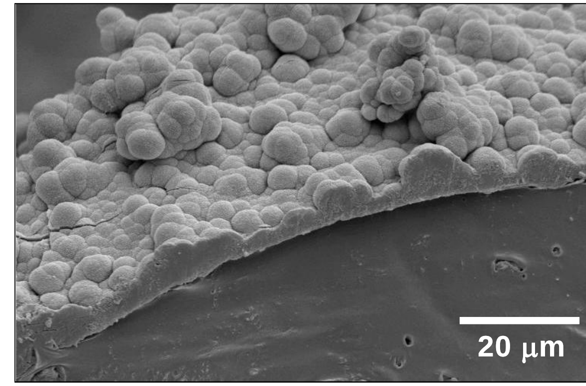
Surface chemistry: **coating**

SPCL scaffold



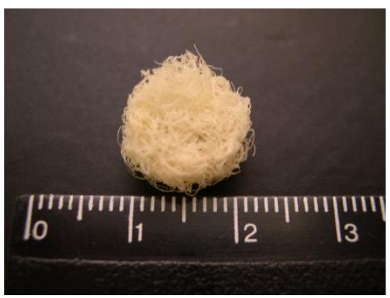
Surface modification and immersion in SBF

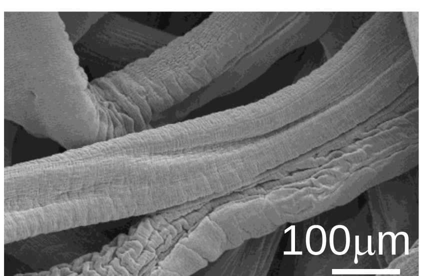


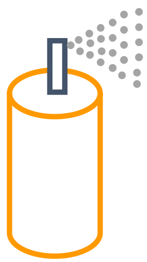


Oliveira AL, J Mat Sci:Mat Med (2007) 18:211

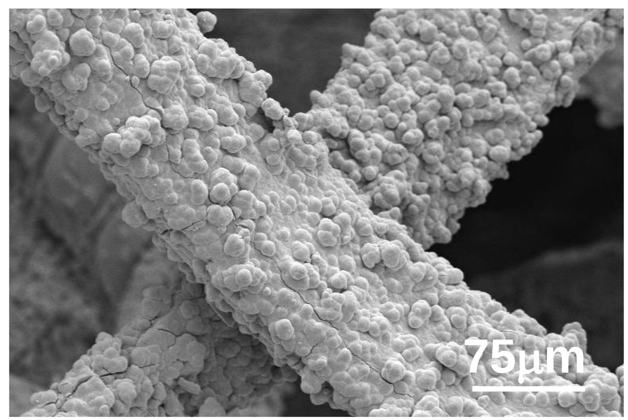
Chitosan scaffold

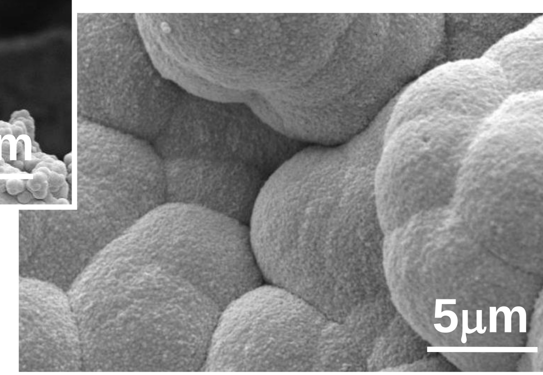






Bioglass® particles suspension, SBF



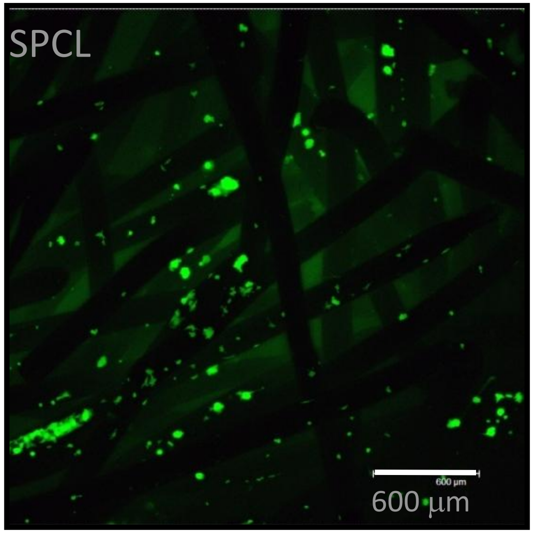


Tuzlakoglu K, J Mat Sci:Mat Med (2007) 18:1279

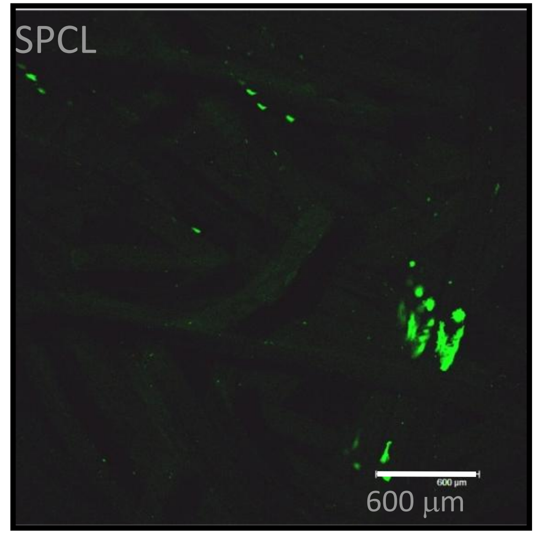
Surface chemistry: **coating**

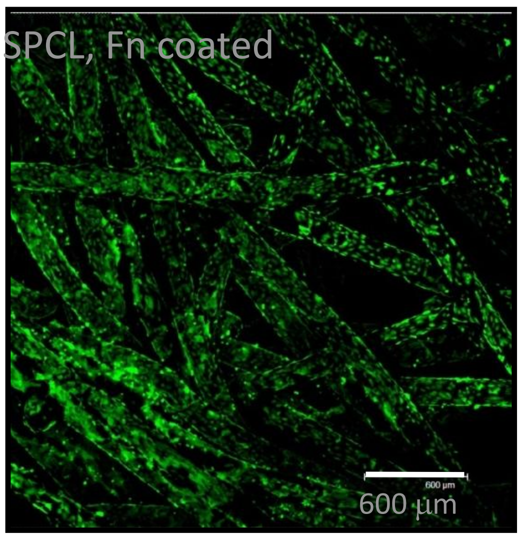
Calcein-AM viability assay of HUVEC cells

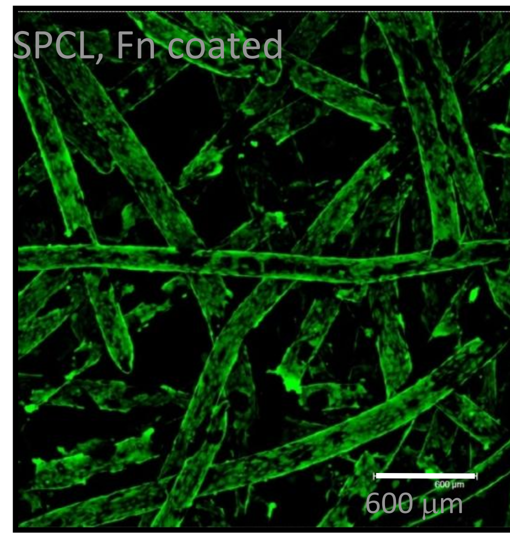
4 hrs



7 days







Santos MI et al, J Mat Chem (2009) 19:4091

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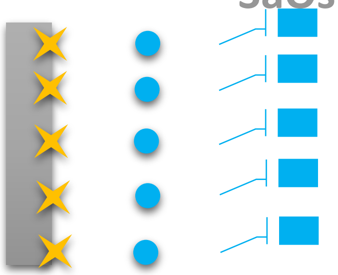
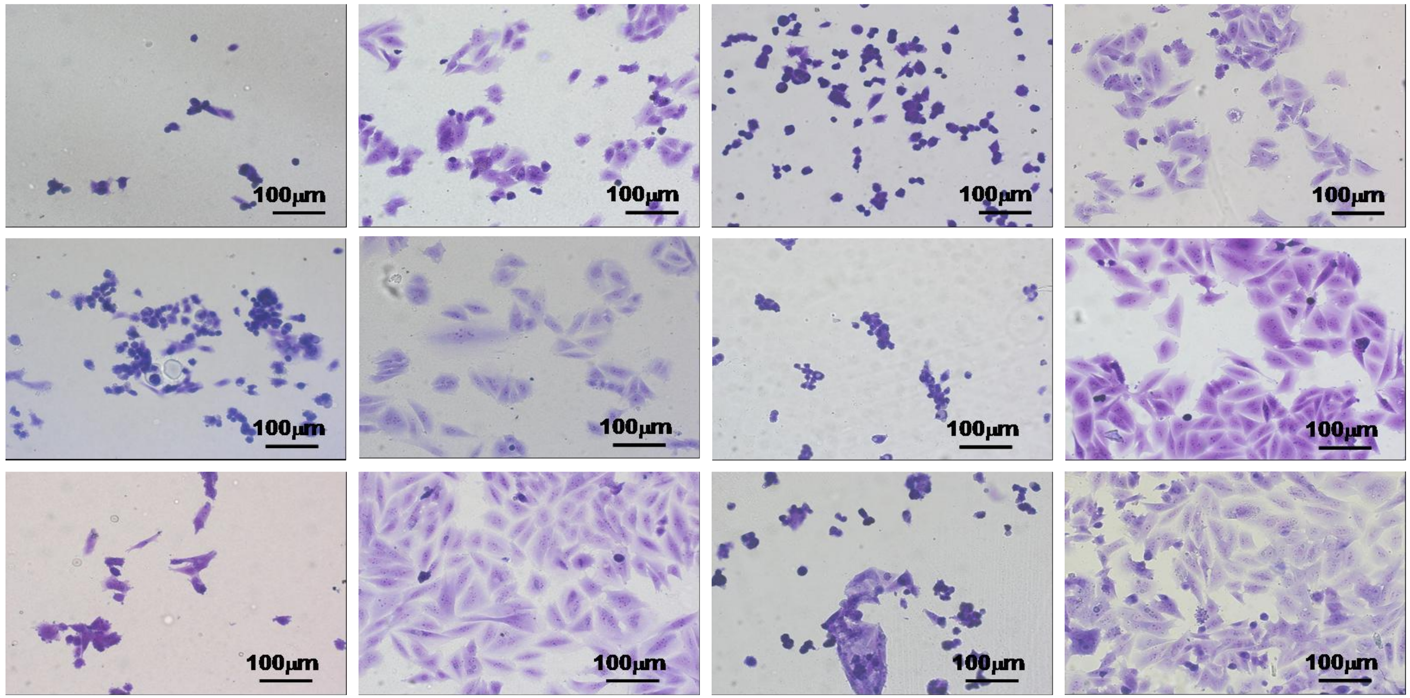
Surface chemistry: grafting

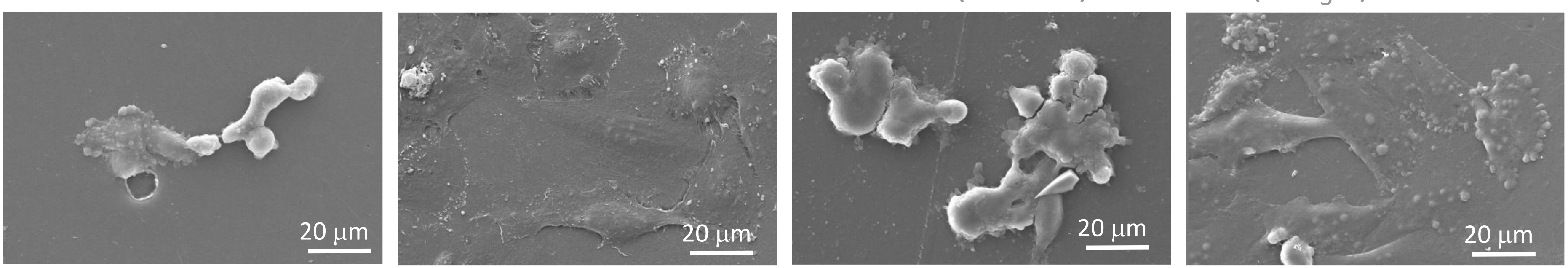
SaOs-2 on modified chitosan (1, 3 and 7 days of culture)



Covalent bond!!!



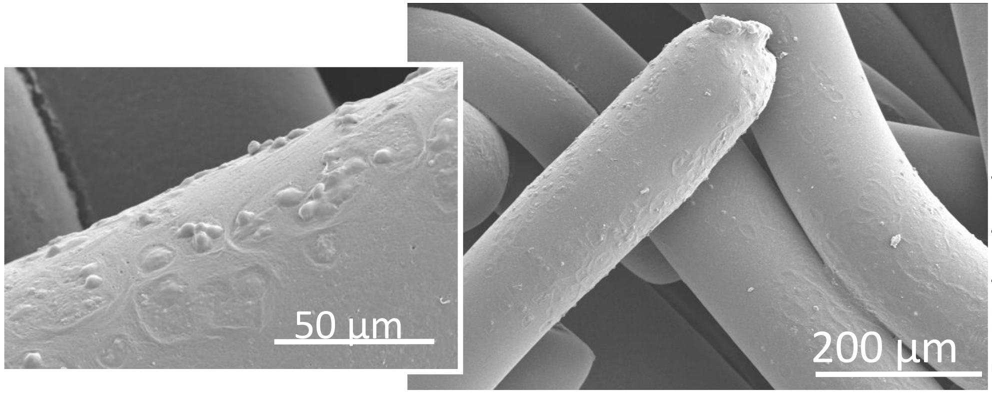
Chitosan Plasma AA (-COOH) VSA ($-SO_3H$)



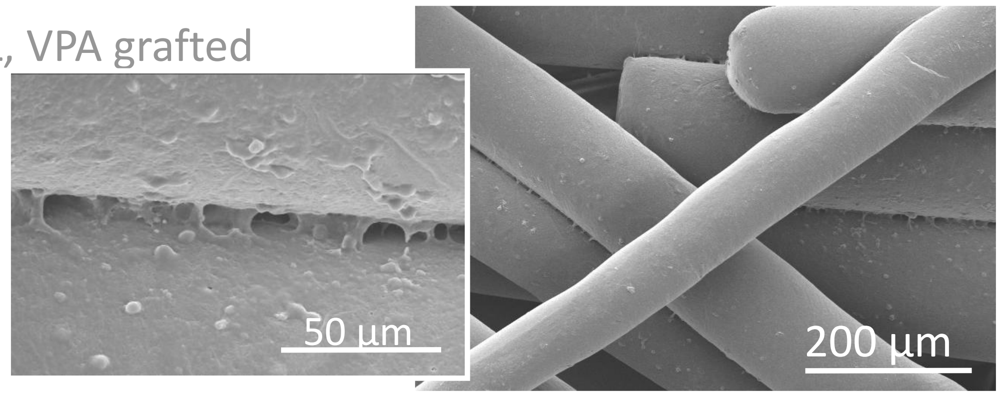
Lopez-Perez PM et al, J Mat Chem (2007) 17:4064

Surface chemistry: **grafting (3D)**

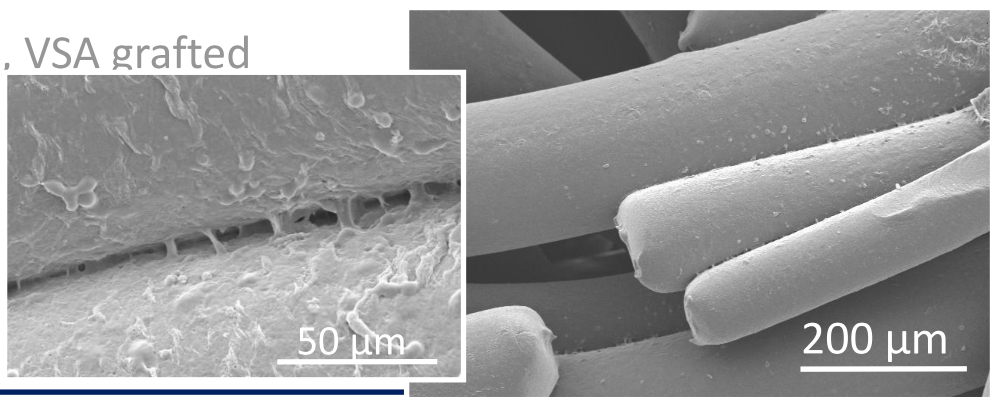
SPCL

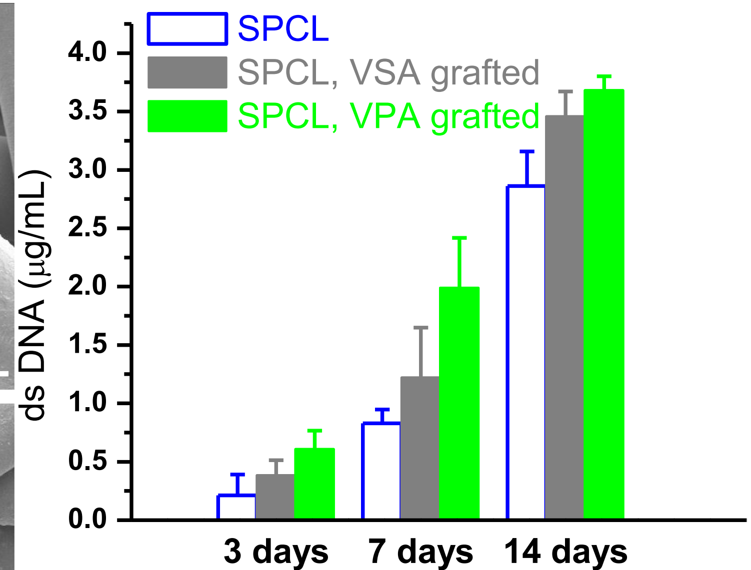


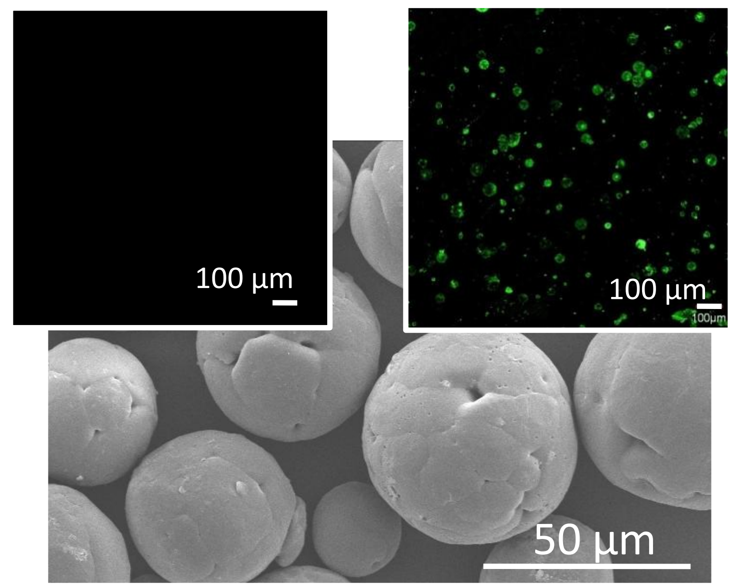
SPCL, VPA grafted



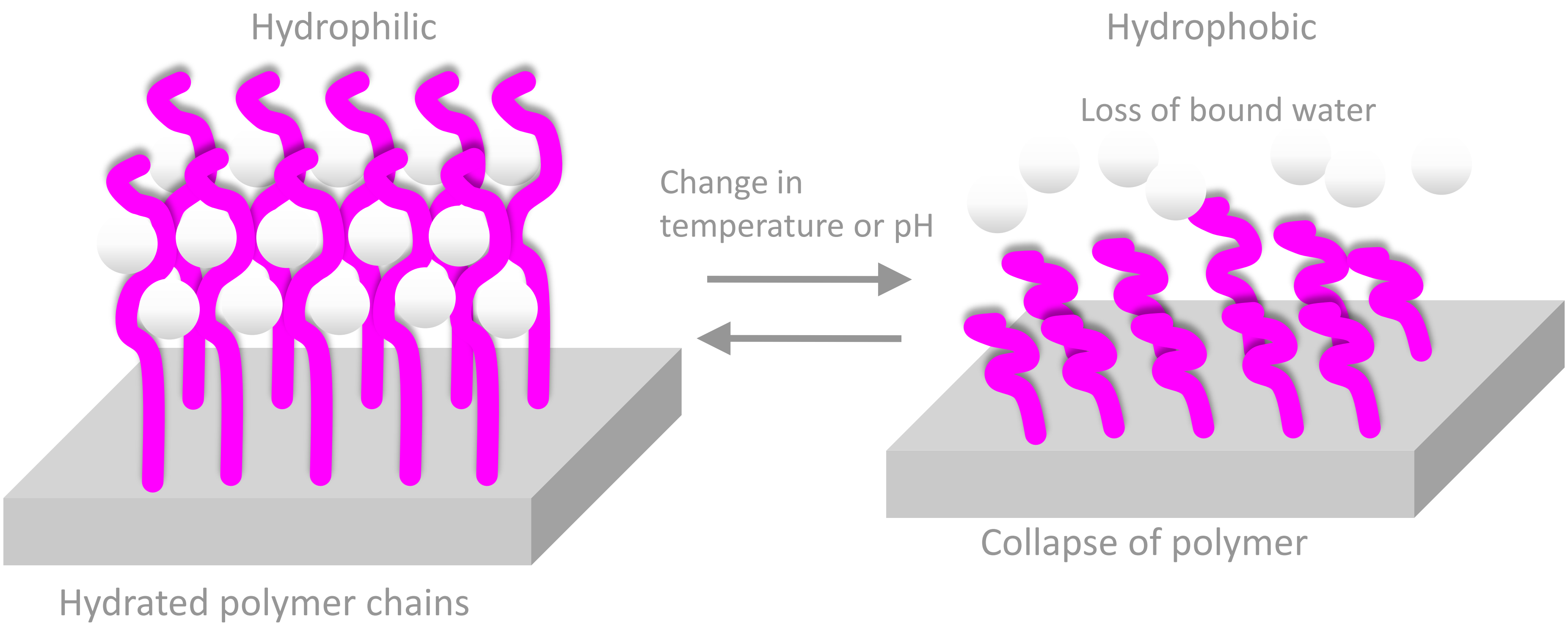
SPCL, VSA grafted





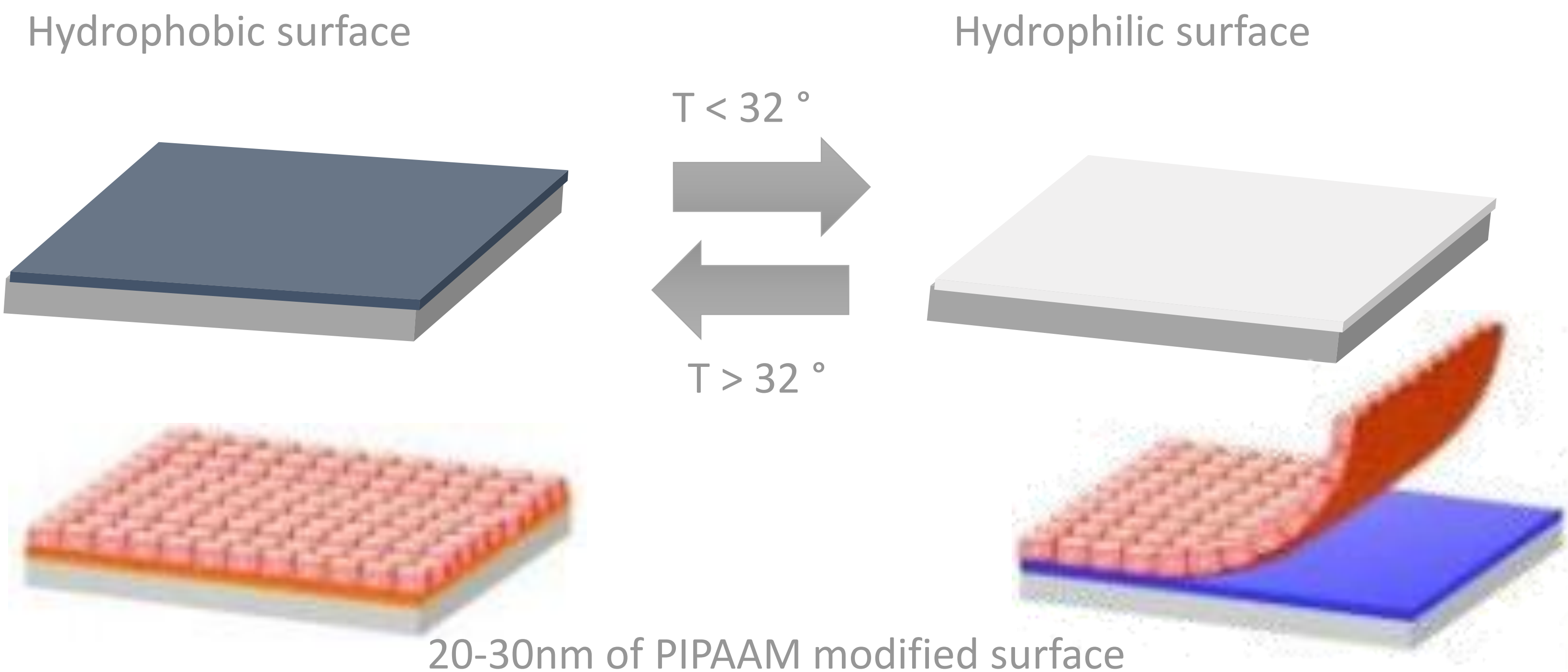


Wetability (applications)



Wetability (applications)

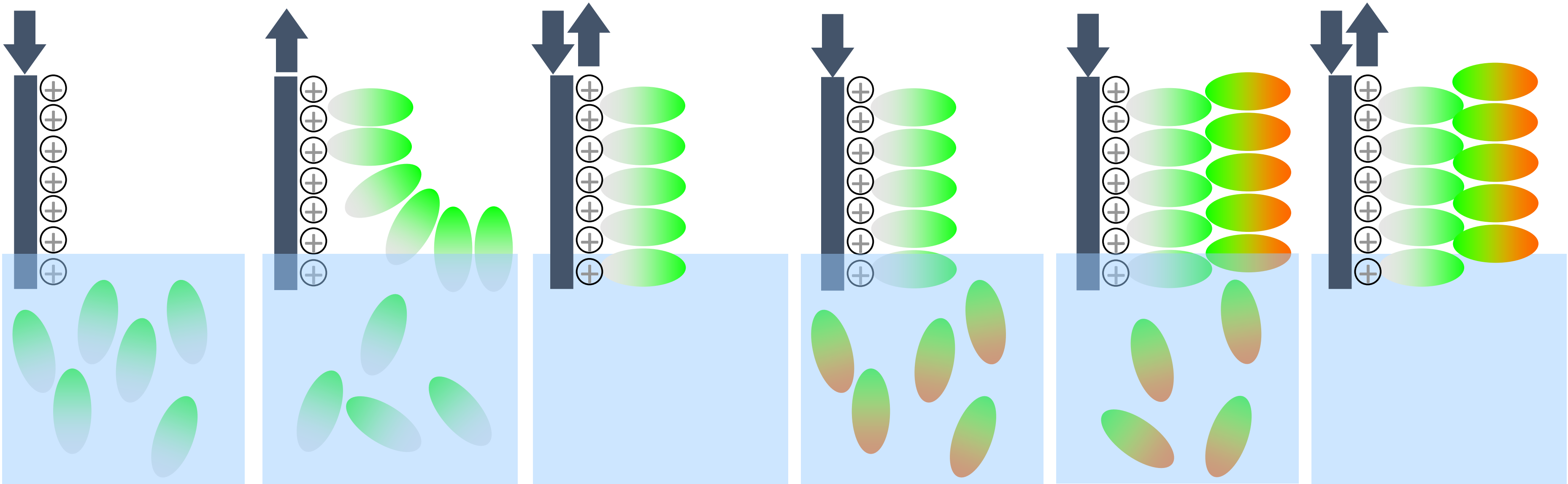
Manipulation of cell sheets through temperature-responsive surface



T. Okano

Surface chemistry: **layer by layer**

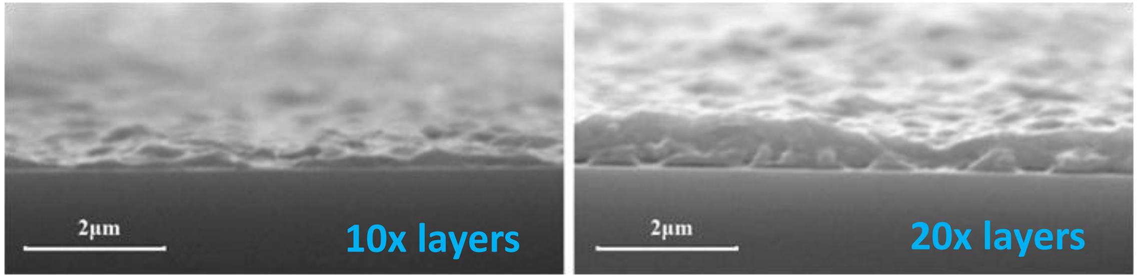
Rome Coliseum



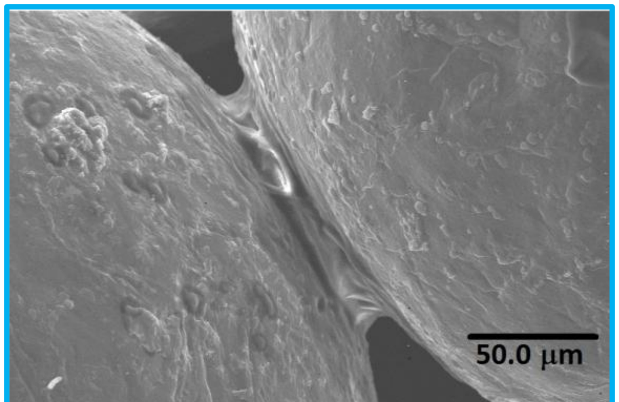
Polyanion solution Washing Polycation solution Washing

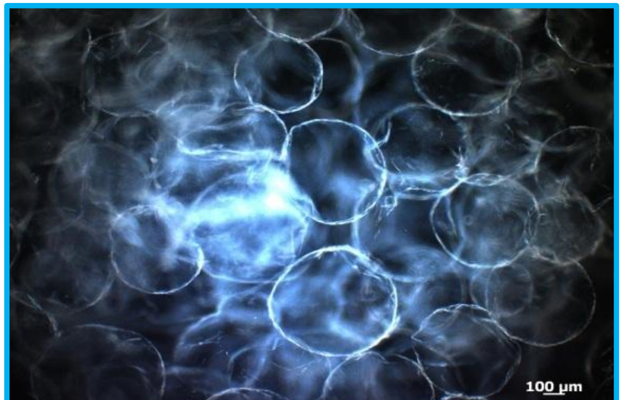
Surface chemistry: **layer by layer**

SEM: film tickness



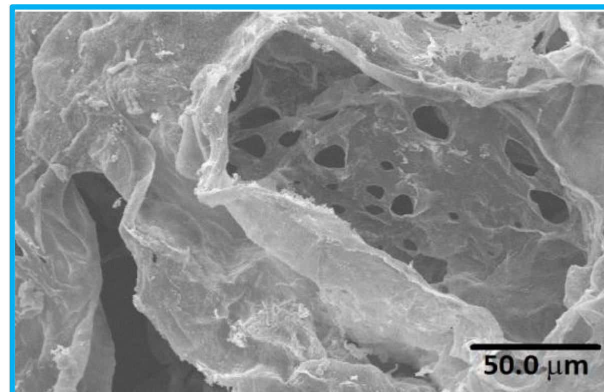
SEM of the particles coated using the LbL approach





Fluorescence imagining of the constructs after the particles leaching

SEM of the constructs after freeze drying



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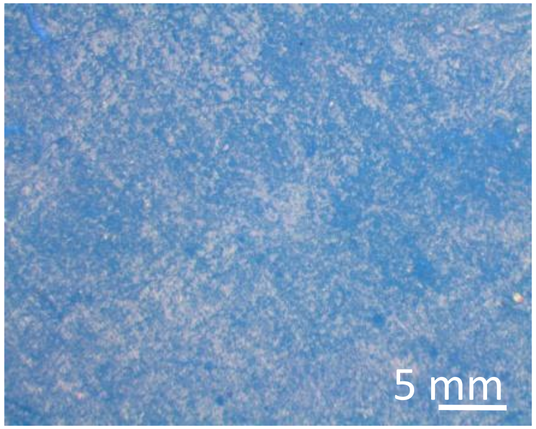
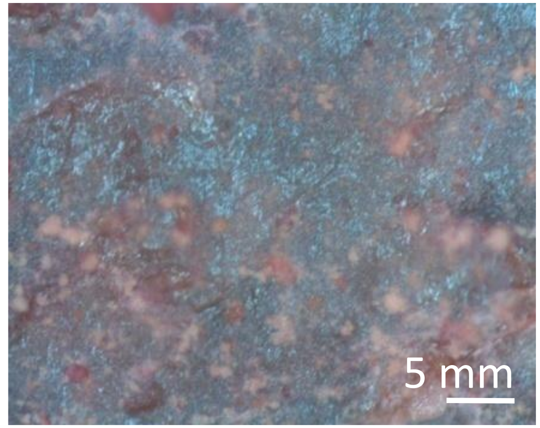
Surface chemistry: **blending**

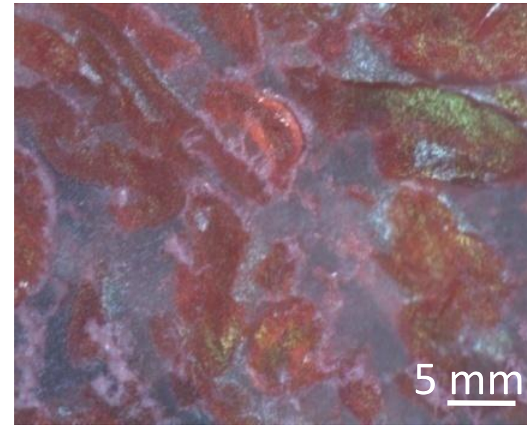
PBS



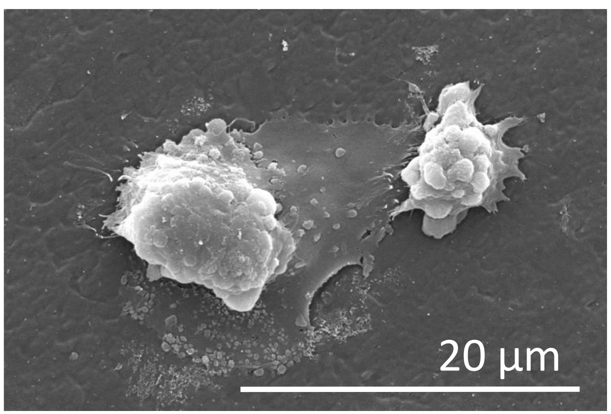
PBS/CHT

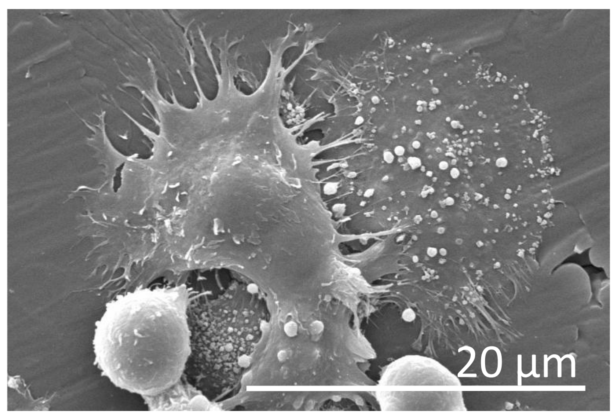


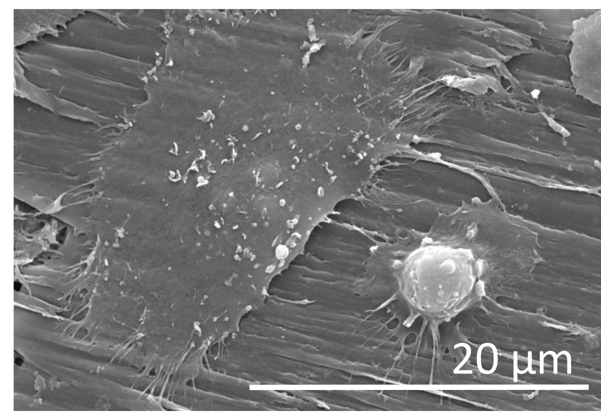
PBS/CHT bulk



SaOs-2, 1 day







Coutinho DF et al, Biomacromolecules (2008) 9:1139.

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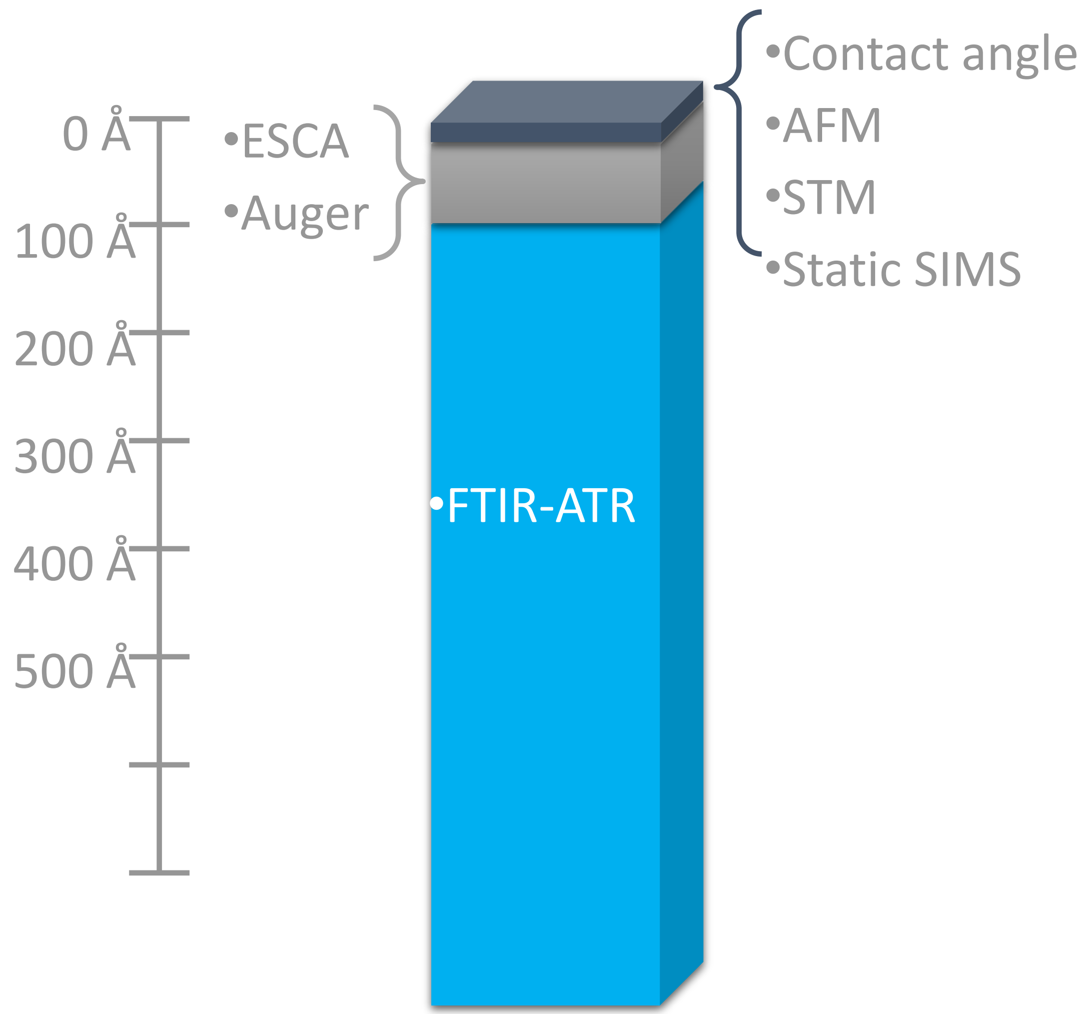
Surface characterisation

Surface characterization: methods

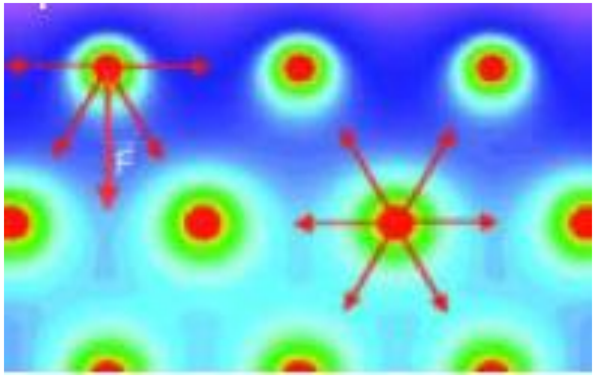
Method	*Abbr.*	*Analyzed depth*	*Resolution*	*Cost*
Contact angle measurements		3-20 Å	1 mm	Moderate
Scanning Tunneling Microscopy	STM	5 Å	1 Å	Expensive
Scanning Electron Microscopy	SEM	5 Å	40 Å	Expensive
X-Ray Photoelectron Spectroscopy	XPS/ ESCA	10-250 Å	10-150 µm	Very expensive
Auger Electron Spectroscopy	AES	50-100 Å	100 Å	Very expensive
Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance	FTIR-ATR	1-5 µm	10 µm	Expensive
Atomic Force Microscopy	AFM	Different	Very high	Expensive

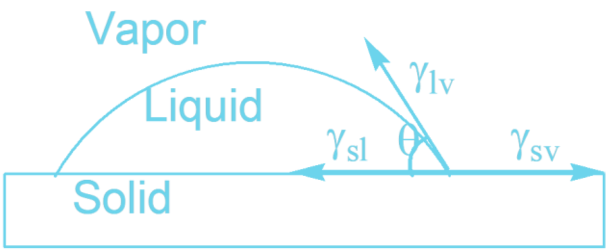
Surface characterisation

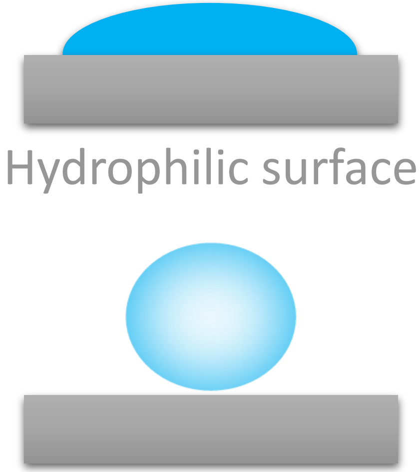


Contact angle and surface energy

The angle formed at a point on the line of contact of three phases by the tangents to the curves obtained by intersecting a plane perpendicular to the line of contact with each of three phases.







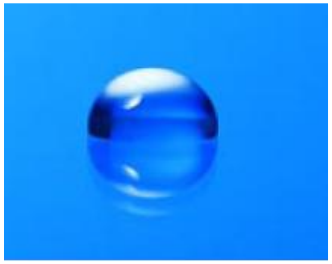
Advantages:

- ❖Inexpensive
- ❖Provides information, related to surface energy
- ❖Rapid

Drawbacks:

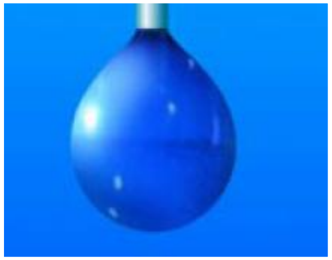
- ❖Noise (artifact)-prone
- ❖Only surface energetic information is obtained
- ❖Surface chemistry information is ambiguous

Contact angle and surface energy



Sessile drop method:

- ❖Wetting properties of a localized region on a solid surface;
- ❖Ideal for curved samples or where one side of the sample has different properties than the other.



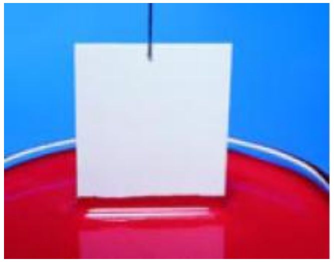
Pendant drop method:

- ❖Surface and interfacial tension even at high temperatures/pressures
- ❖Geometry of a drop is analyzed optically.

Bubble pressure method:

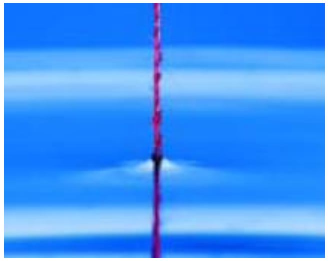
- ❖Surface tension at short surface ages.
- ❖Maximum pressure of each bubble is measured.

Du Noüy ring method



Dynamic Wilhelmy method

Single fiber method



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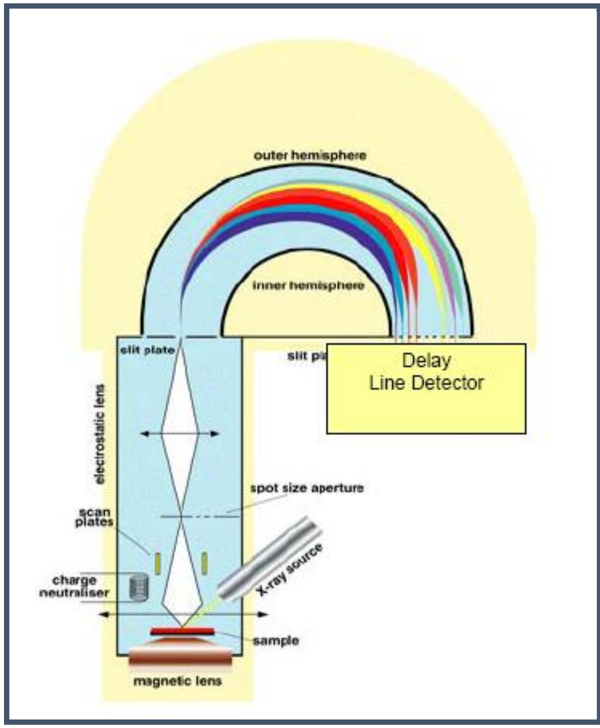
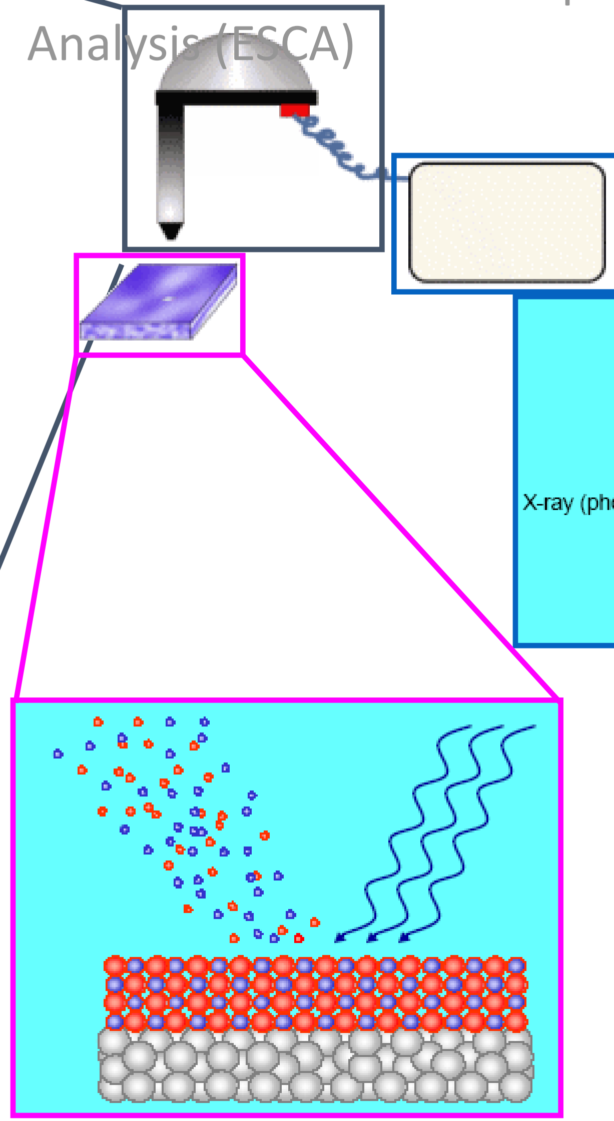
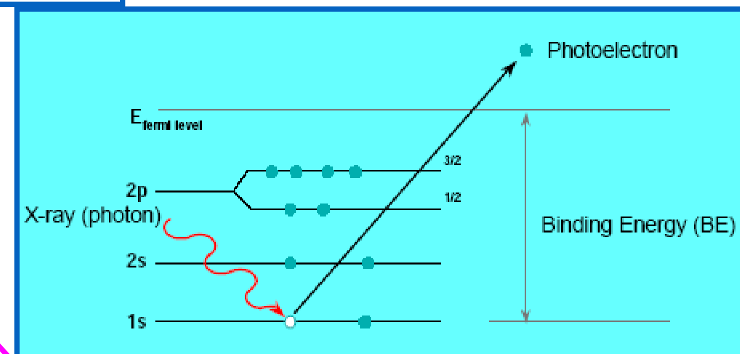
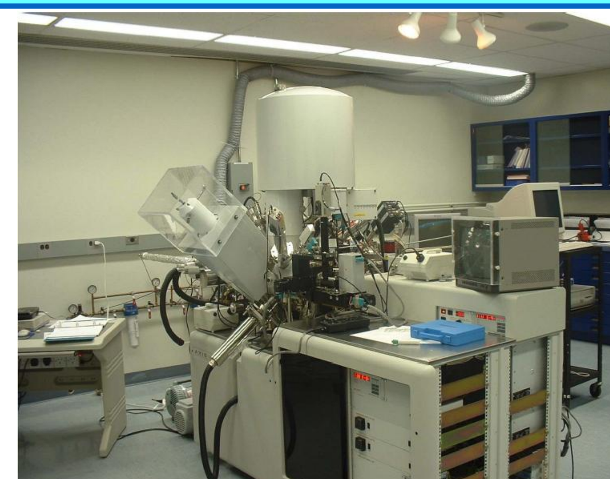
X-ray photoelectron spectroscopy (XPS)

Known also as Electron Spectroscopy for Chemical Analysis (ESCA)

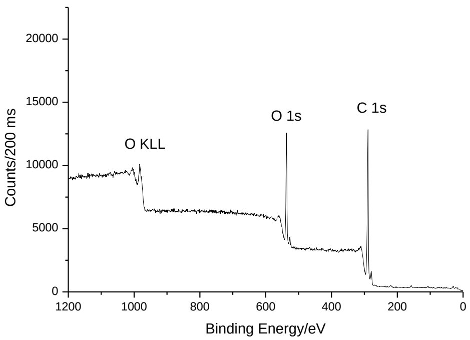


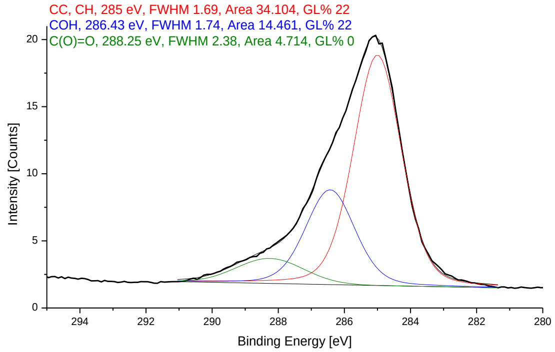


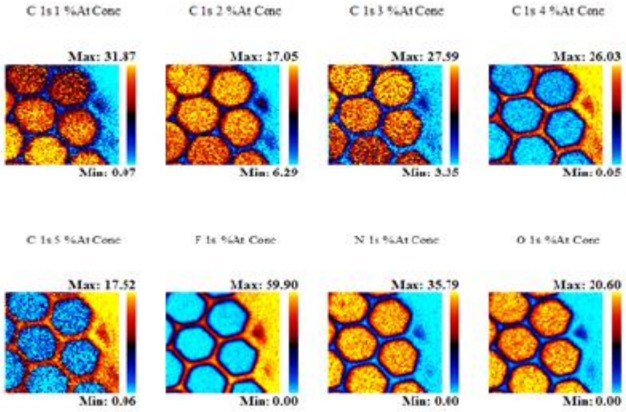




X-ray photoelectron spectroscopy (XPS)







Advantages:

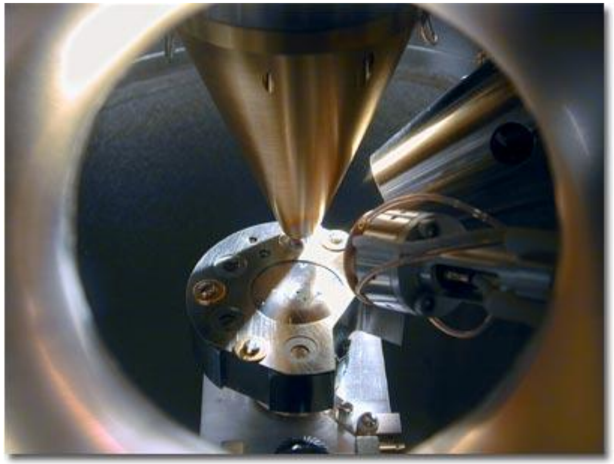
- ❖ Convenient and quick;
- ❖ Characterization of functional groups on surfaces and determination of valence states and/or bonding environment of atoms near the surface;
- ❖Relatively non-destructive ("soft" x-rays, 1.2 - 1.4 keV.

Disadvantages:

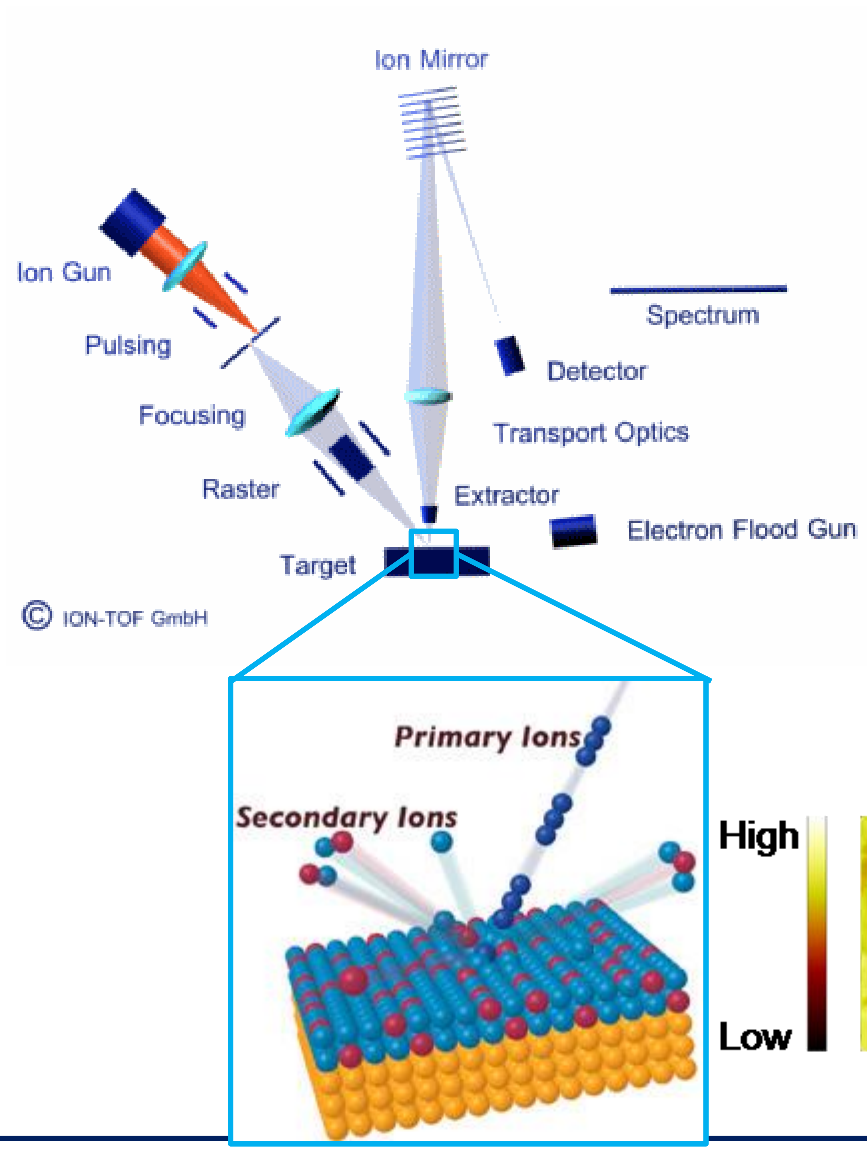
- ❖ Sensitivity 0.1 - 1.0 atomic %;
- ❖ Poor spatial resolution (>10's of microns);
- ❖ Depth profiling possible but slow.
- ❖Uses UHV

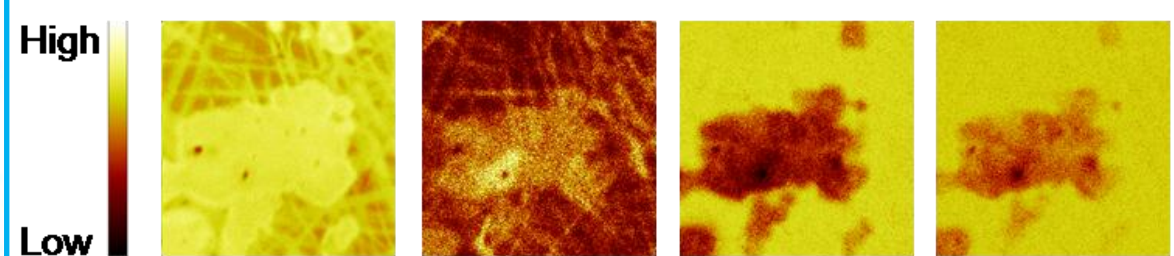
Time of Flight Secondary Ion Mass Spectrometry

- SIMS was developed by NASA (1960) to investigate the composition of Moon rocks. The method proved so successful, that the apparatus was commercially produced.
- Sensitivity: the outer 5 nm of a sample.
- Possible spatial or depth profiles of elemental or molecular concentrations.
- Generation of element specific images of the sample that display the varying concentrations over the area of the sample.



Time of Flight Secondary Ion Mass Spectrometry





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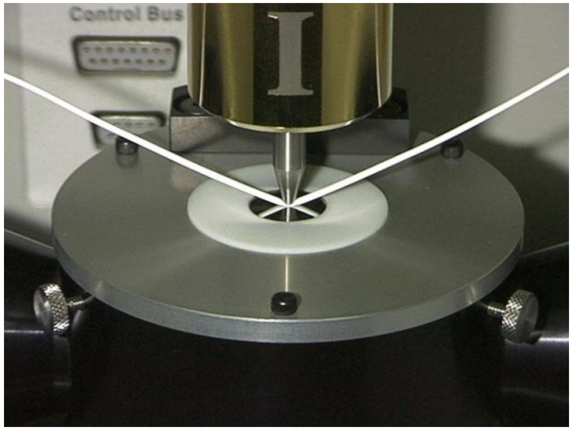
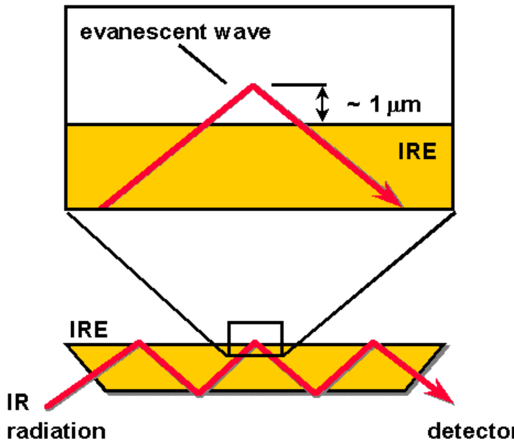
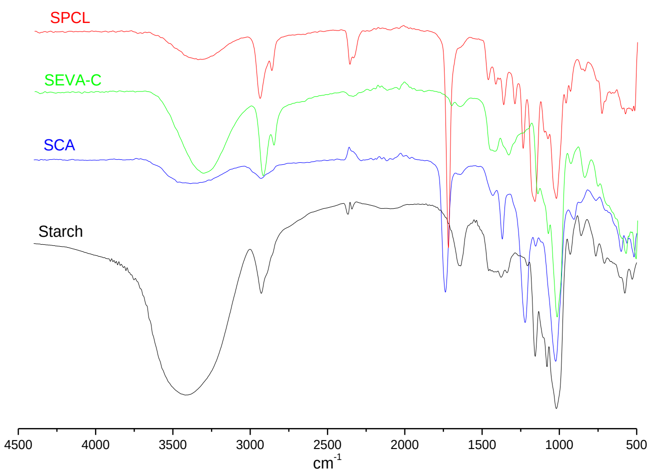
Surface characterization: chemistry (FTIR)





❖IR radiation penetrates a short distance (~1-5 µm) from the surface of the internal reflection element (IRE) into the polymer

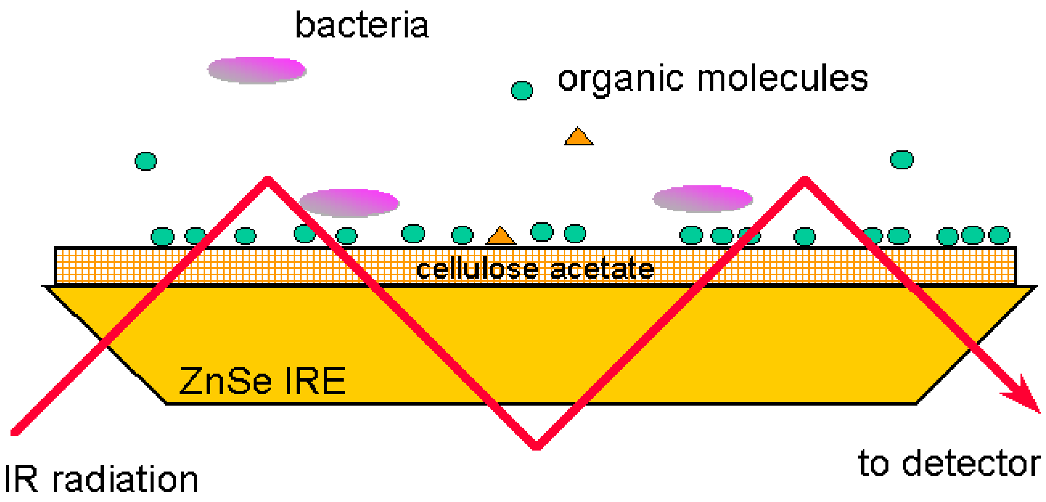
❖This is the unique physical phenomenon that enables one to obtain infrared spectra of samples placed in contact with the IRE.



❖Presence or absence of specific functional groups

❖Identification of unique features on the material after chemical surface modification - shifts in the frequency of absorption bands and changes in relative band intensities indicate changes in the chemical structure or changes in the environment around the material

Surface characterization: chemistry (FTIR)



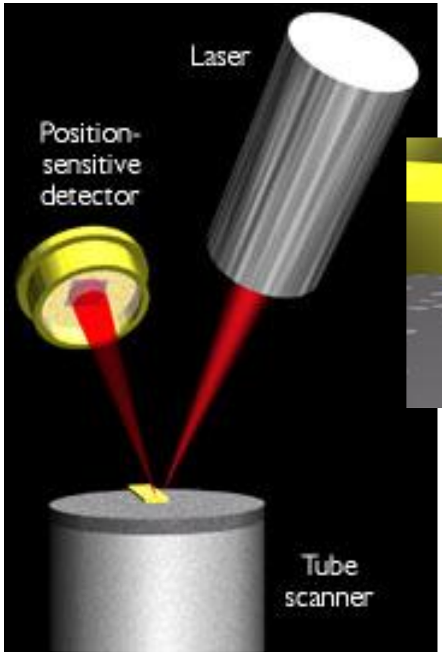
Material is cast onto IRE
The film (~200 nm) is made thin enough to allow light pass through to the adjoining aqueous phase.

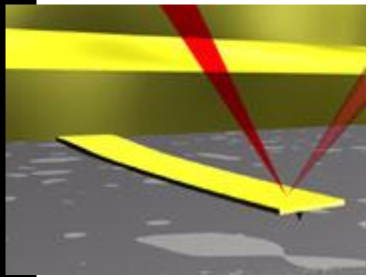
In situ real-time information about molecular absorption in terms of:

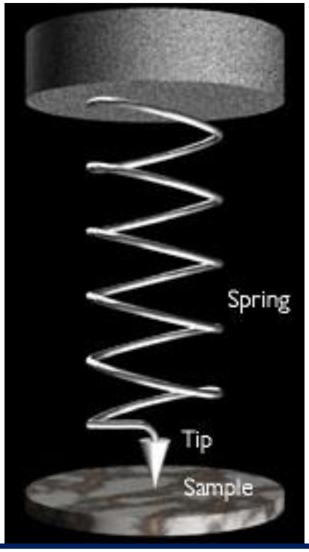
- strength of molecular interactions with polymer surfaces
- bacterial attachment and biofilm growth on the surface of an IRE or the coated polymer
- adsorption kinetics of model compounds, e.g., proteins, polysaccharides, humic and fulvic acids, to polymer surfaces.

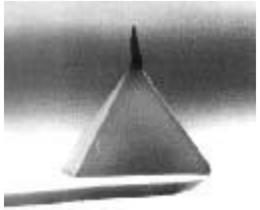
Surface characterization: morphology (AFM)

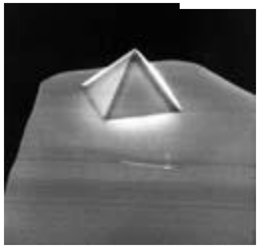
The magnitude of the deflection is captured by a laser that reflects at an oblique angle from the very end of the cantilever. A plot of the laser deflection versus tip position on the sample surface provides the resolution of the hills and valleys that constitute the topography of the surface.











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Surface characterization: morphology (AFM)

Contact mode

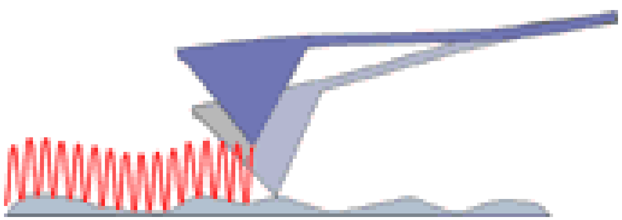
Advantages:

- High scan speeds
- Atomic resolution images

Disadvantages:

The combination of lateral forces and high normal forces can result in reduced spatial resolution and may damage soft samples (i.e. biological samples, polymers, silicon) due to scraping

Tapping mode



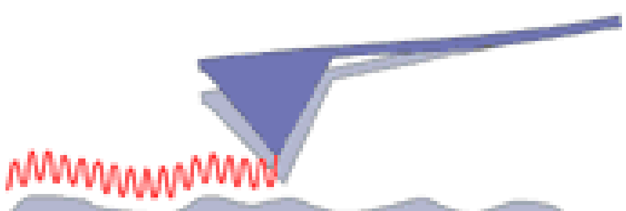
Advantages:

- High lateral resolution
- Lower forces and less damage to soft samples imaged in air
- Lateral forces are virtually eliminated so there is no scraping

Disadvantages:

Slightly lower scan speed than contact mode AFM

Non-contact mode



Advantages:

- The least sample disruption
- Charged surfaces

Disadvantages:

Difficult to control in ambient conditions

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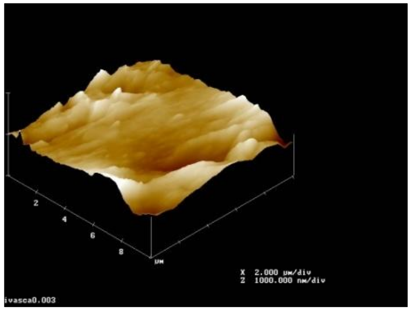
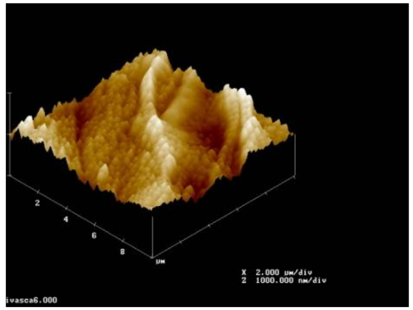
Surface characterization: morphology (AFM)

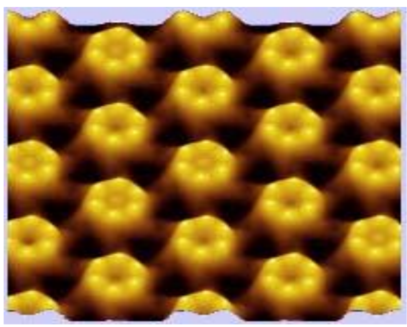
Surface morphology



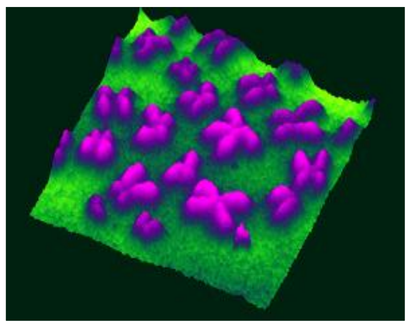
SCA untreated



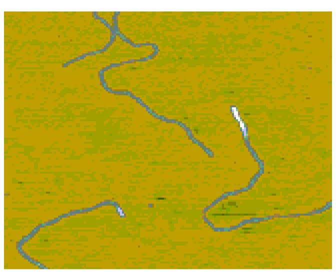
SCA plasma etched



Protein surface layer

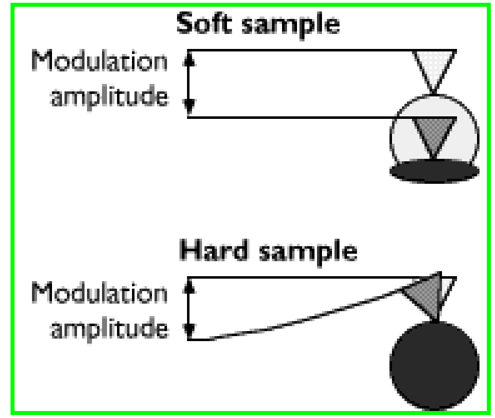


Human chromosomes

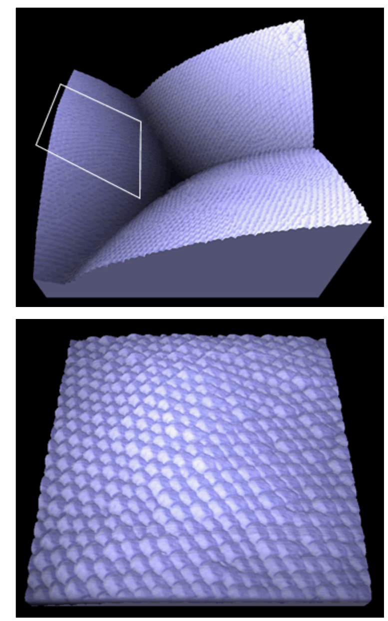


The AFM can feel the difference between double helical DNA (blue) and triple helical DNA, which is higher (white).

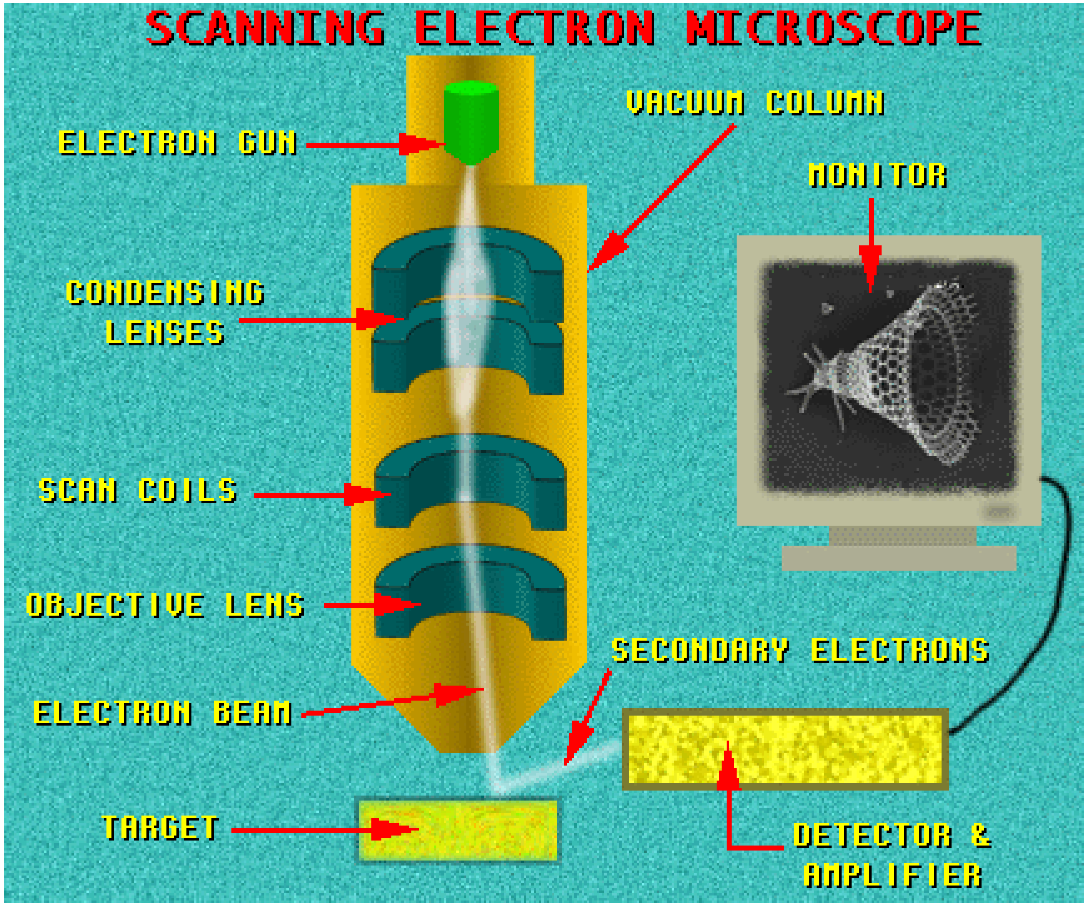
Sample elasticity



Moth's eye



Surface characterization: morphology (SEM)



The SEM uses a beam of electrons to scan the surface of a sample and to build very detailed image of the specimen. When the electron beam hits the sample, the interaction of the beam electrons from the filament and the sample atoms generates a variety of signals.

It is very similar to scan dark room from side to side using a flashlight or to the image building on a TV monitor).

Surface characterization: morphology (SEM)

TOPOGRAPHY few nanometers

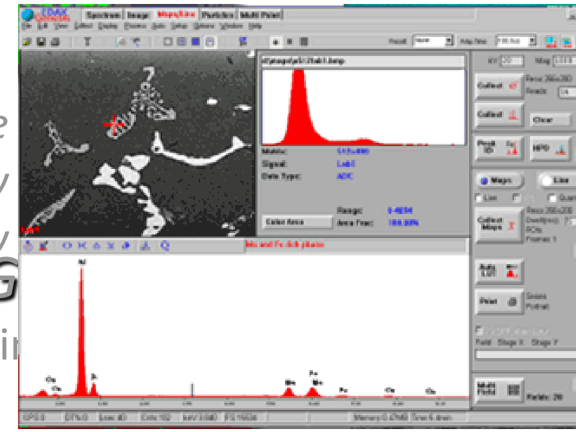
hair

cornea

MORPHOLOGY few nanometers

COMPOSITION in areas ~ 1 micrometer in diameter

Energy-dispersive X-ray spectroscopy



CRYSTALLOG

only useful on si

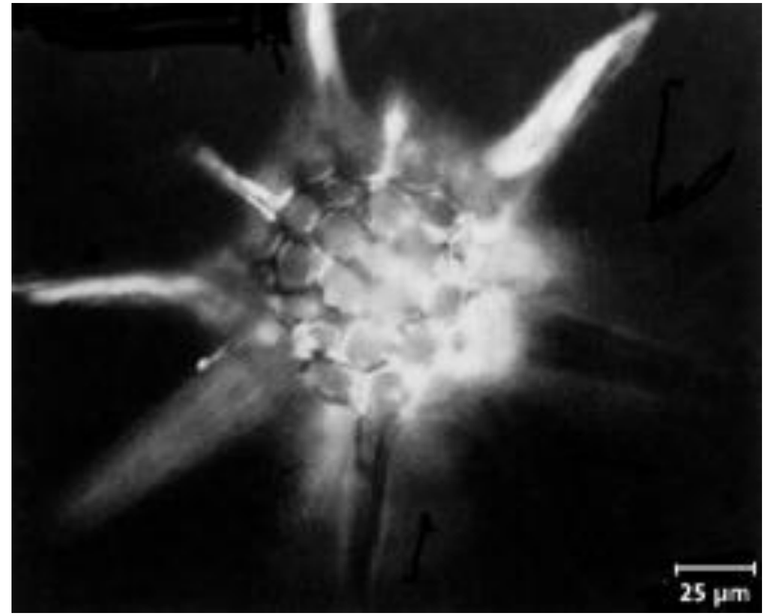
micrometers

Red blood cells

Haemophilus influenza

Different crystals from calcite

Roughness/morphology: SEM *vs* LM



SEM
- Magnification
- Resolving Power
- Depth of Field

LIGHT MICROSCOPY
- No vacuum
- *in situ*
- No conductive coating
- Relatively cheap
- Easy for maintenance