Sensors and their integration

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Biosensors

Definition, components, classification, glossary, history, Performance factors & Desirable requirements, etc

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Definition

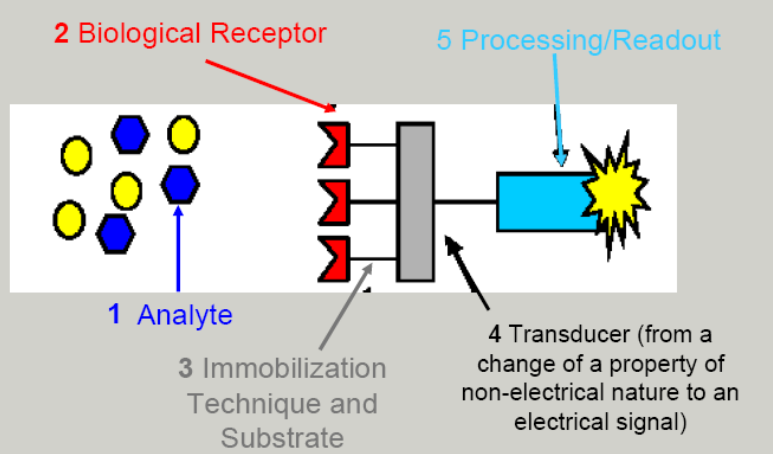
- **Physical sensors:** measuring physical quantities for their own sake
- **Chemical sensors:** responsing to a particular analyte in a selective way through a chemical reaction and can be used for qualitative/quantitative determination of the analyte [R.W. Catterall]

- Biosensors: devices for the detection of an analyte that combines a biological component (sensing element) with a physicochemical detector component (transducer). [International Union of Pure and Applied Chemistry]

- Biosensors2: devices that use specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals. [IUPAC Gold Book]

Function: Qualitative or quantitative determination of a particular analyte in a selective way through a biochemical reaction.

Name from the nature of the sensing element, not from the analyte



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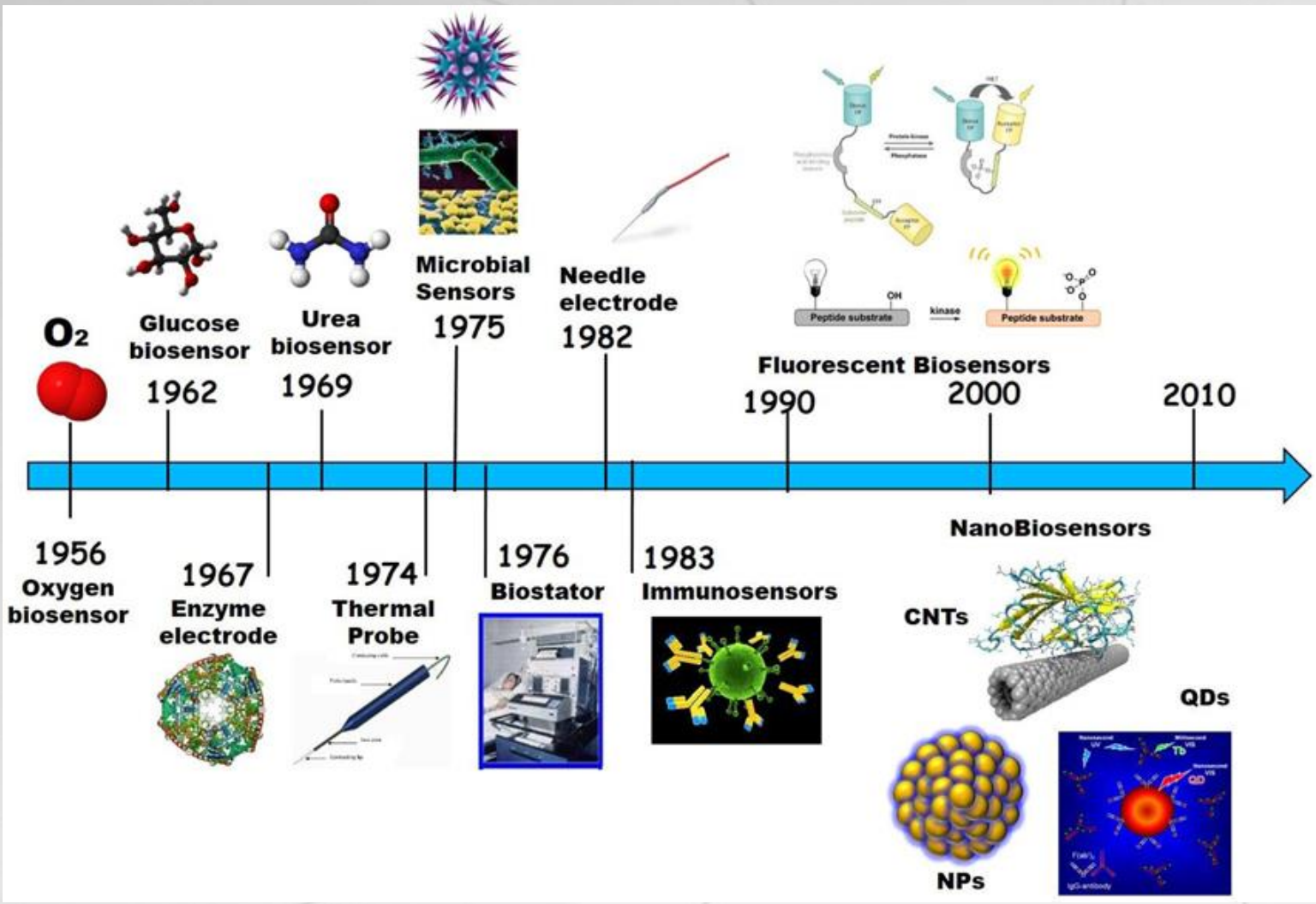
History of Biosensors



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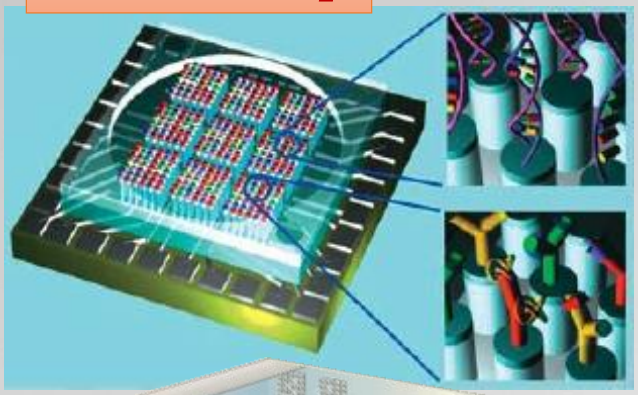
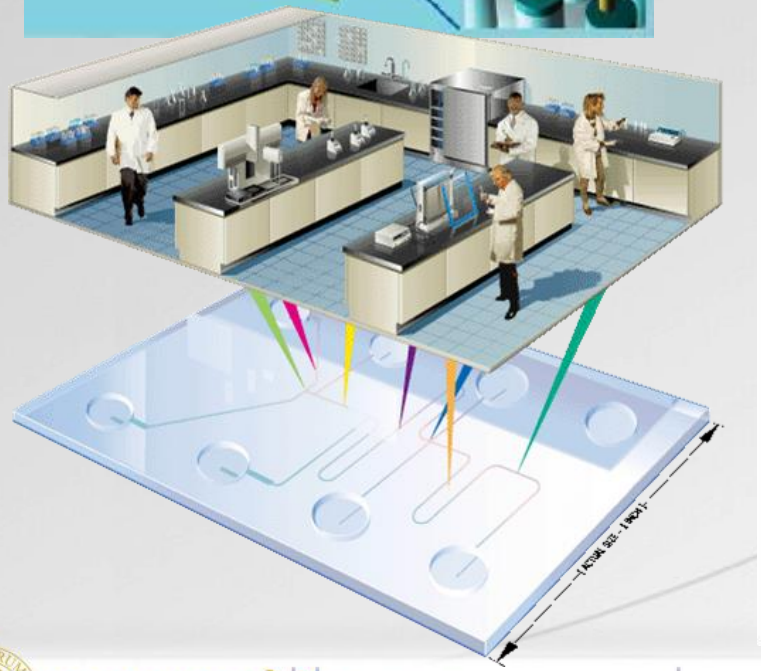
Integration and wearable sensors

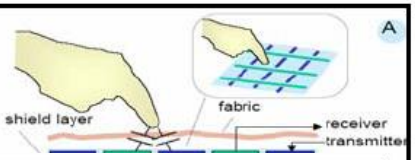
Lab on a chip





Wearable sensors

wearable by using gesture-based commands



Digital Finger Pulse Oximeter

http://www.nonin.com/products/9500.html

GPS Locator Watch For Alzheimer's Patients

http://www.wirelesshomesecurityalarmsystems.com/

Telemonitoring Services

Actiwatch Actigraphs

http://www.minimitter.com/products/Actiwatch/

Bracelet Vivago

http://www.vivago.org/home.htm

HEART RHYTHM (ECG) MEASUREMENT DEVICE

Exercise Set (extended exercise profiles)

http://www.polar.fi/polar/channels/eng/segments/Running/S810i/allfeatures.html

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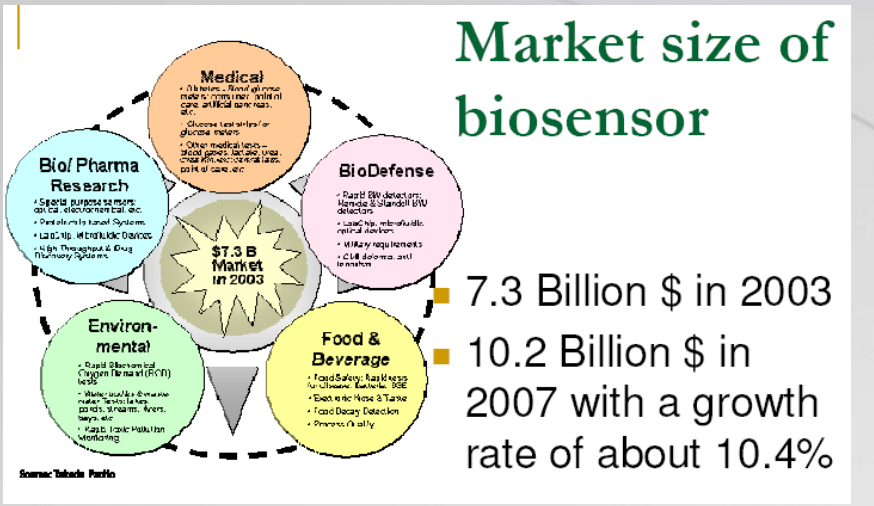
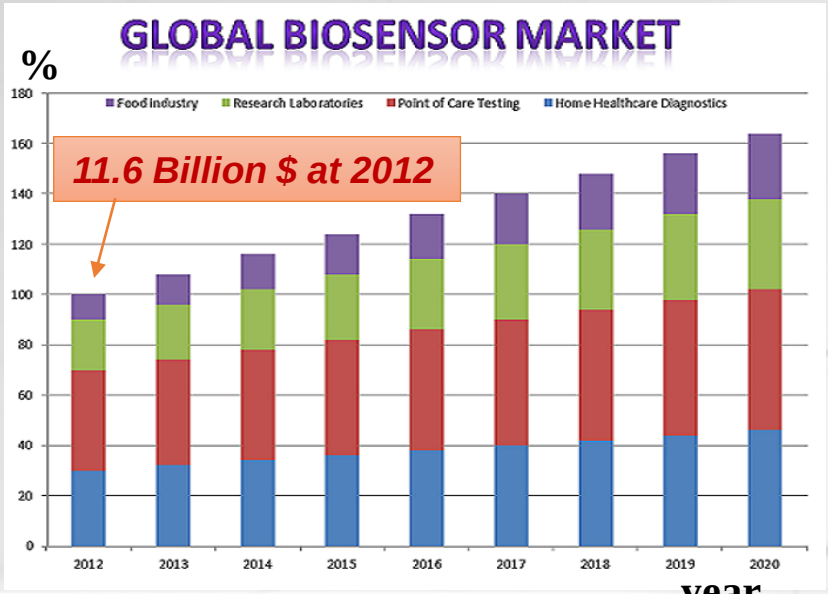
Market



Areas of application

- **Health care – measurements of blood, other** biological fluids, ions, metabolites to show a patient metabolic state
- **Industrial processes, e.g. Fermentation**
- **Warfare detection**
- **Environmental monitoring**
- **Food control**



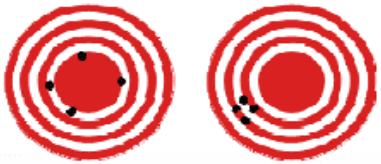
Common assays in medicine

Analyte	Method of assay
Glucose	Amperometric biosensor
Urea	Potentiometric biosensor
Lactate	Amperometric biosensor
Hepatitis B	Chemiluminescent immunoassay
Candida albicans	Piezo-electric immunoassay
Cholesterol	Amperometric biosensor
Penicillins	Potentiometric biosensor
Sodium	Glass ion-selective electrode
Potassium	Ion-exchange-selective electrode
Calcium	Ionophore ion-selective electrode
Oxygen	Fluorescent quenching sensor
pH	Glass ion-selective electrode

Accuracy & Precision

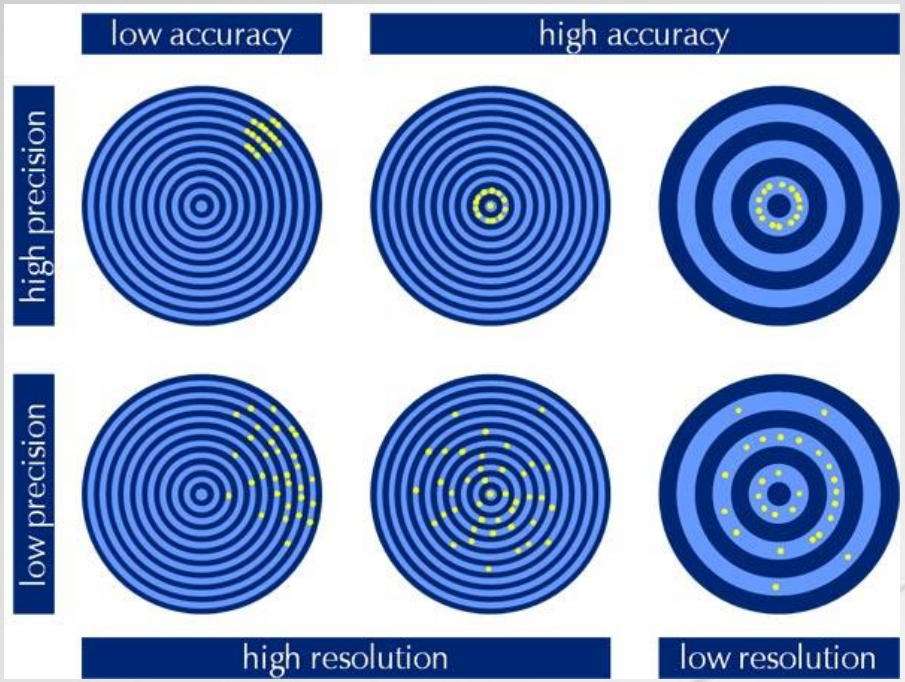
Accuracy: agreement between measured value and actual value.

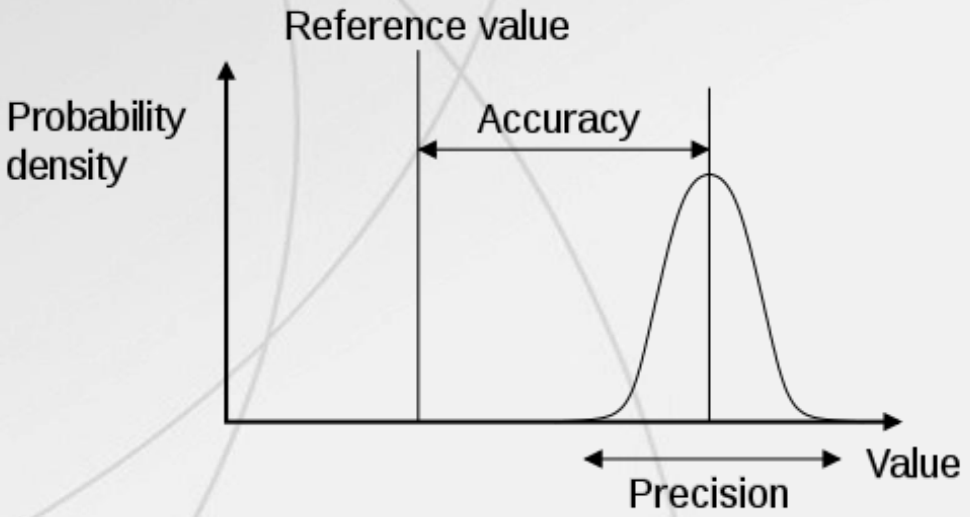
Precision: extent of random error in the measurement.



Accurate, but not precise

Precise, but not accurate





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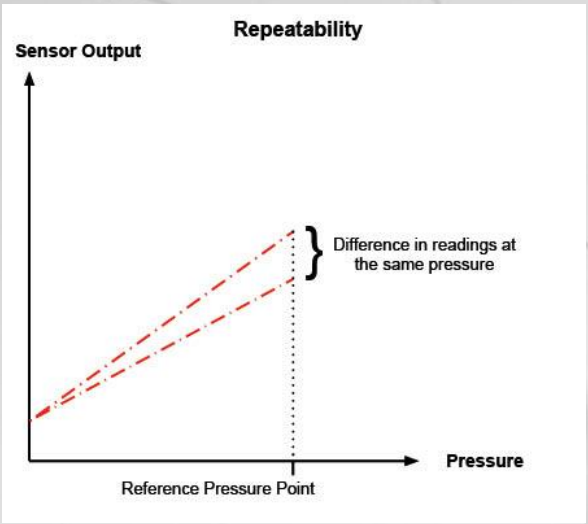
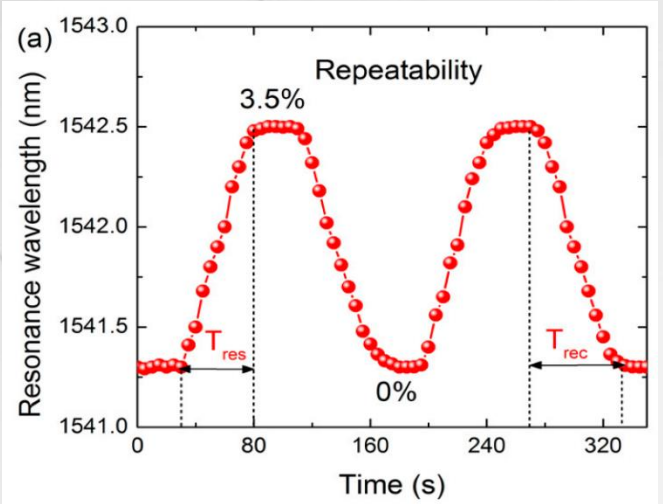
Repeatability/Reproducibility & Hysteresis

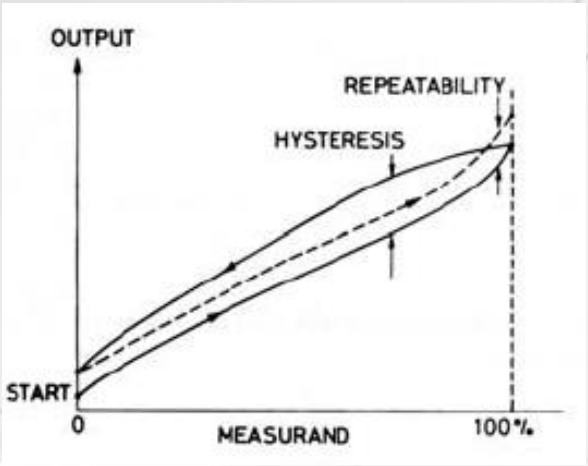
- **Repeatability: ability to reproduce output** readings repeating the exact measurement at exactly the same condition within short time period.
- **Reproducibility: same after longer period** of time or at a different conditions.

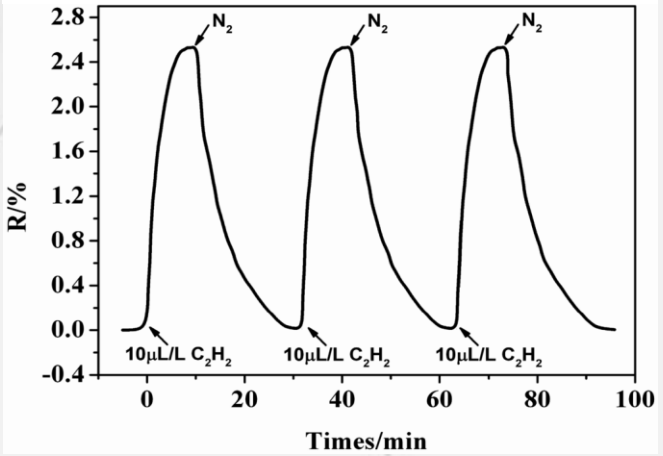




- **Reversibility:** Continuous measurements (no consumption of the analyte)

- **Hysteresis: maximum difference in output, at any measurand** value, when the value is approached first with an increasing and then decreasing measurand.





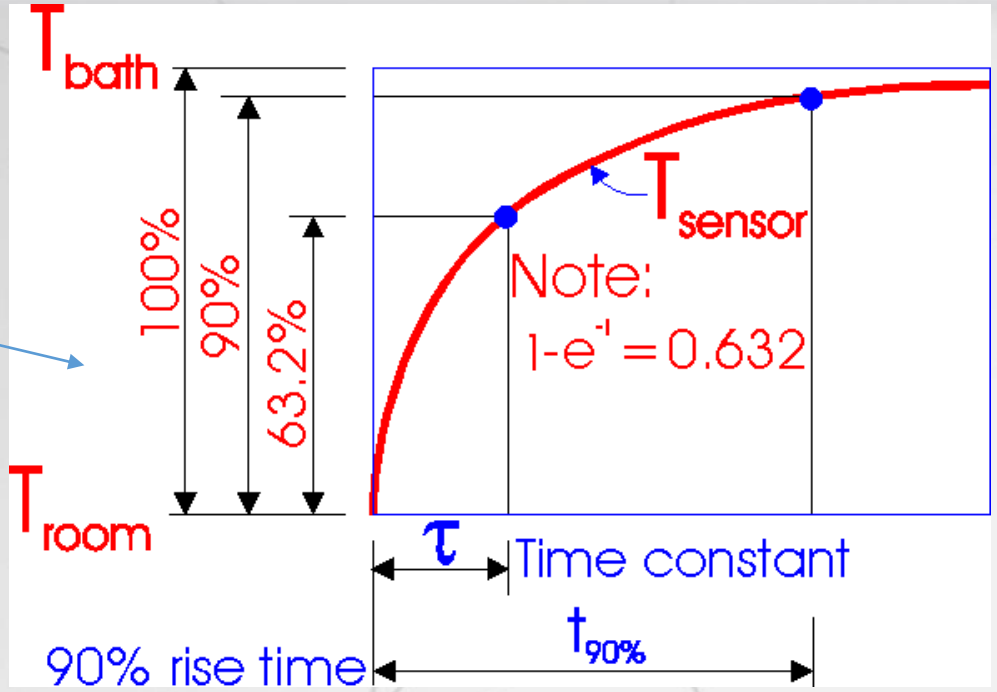
Time factors

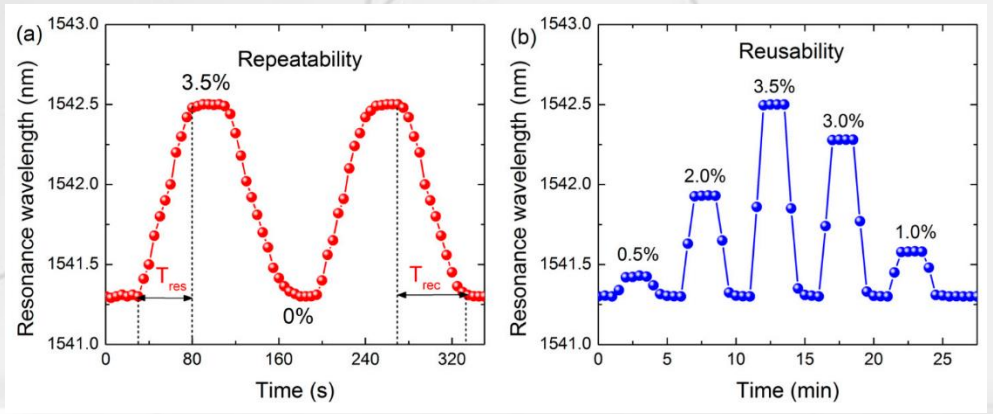
- **RESPONSE TIME: Time necessary for having 95% of** final value in response to a step change in the measurand response. A short response time is derirable for fast measurements and high sample throughput.

- **WORKING LIFETIME (and shelf time as well): minimum time over which** sensor will operate without changing performance characteristics beyond specified tolerance.

  – Degradation during continuous use
  – Storage in wet condition
  – Shelf life (in dry, in original packaging)

- **STABILITY: sensor ability to maintain its** performance characteristics for a time period.





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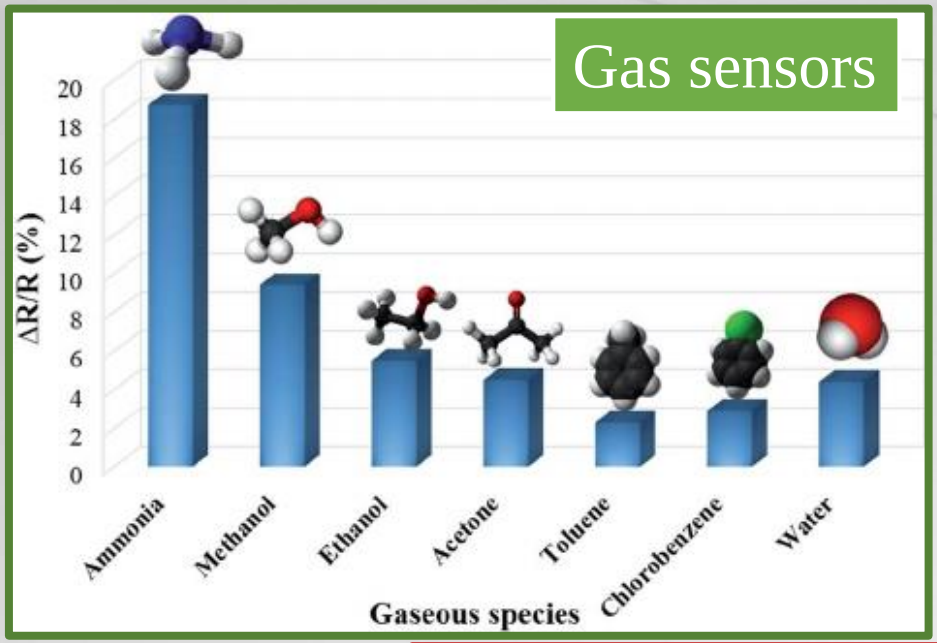
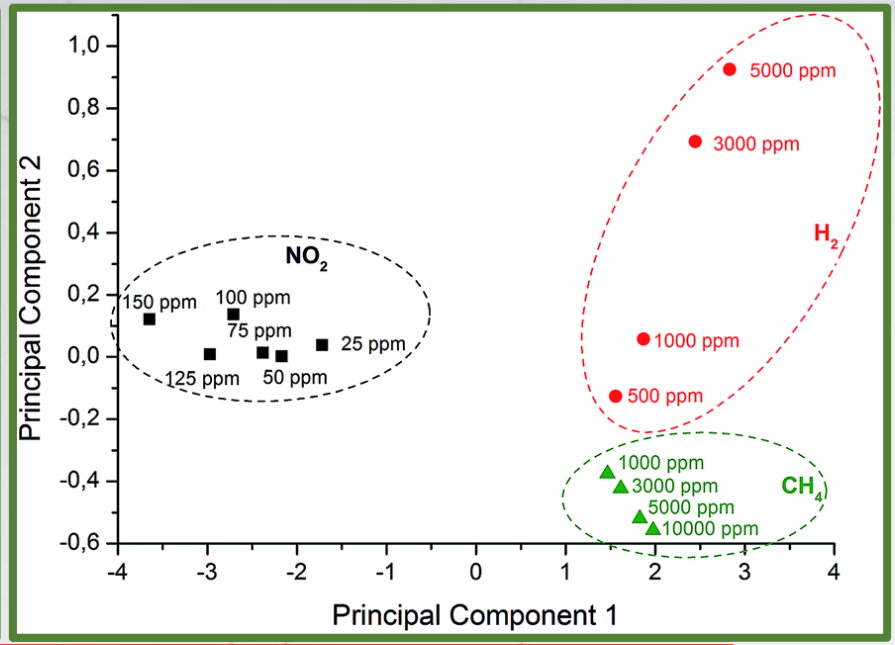
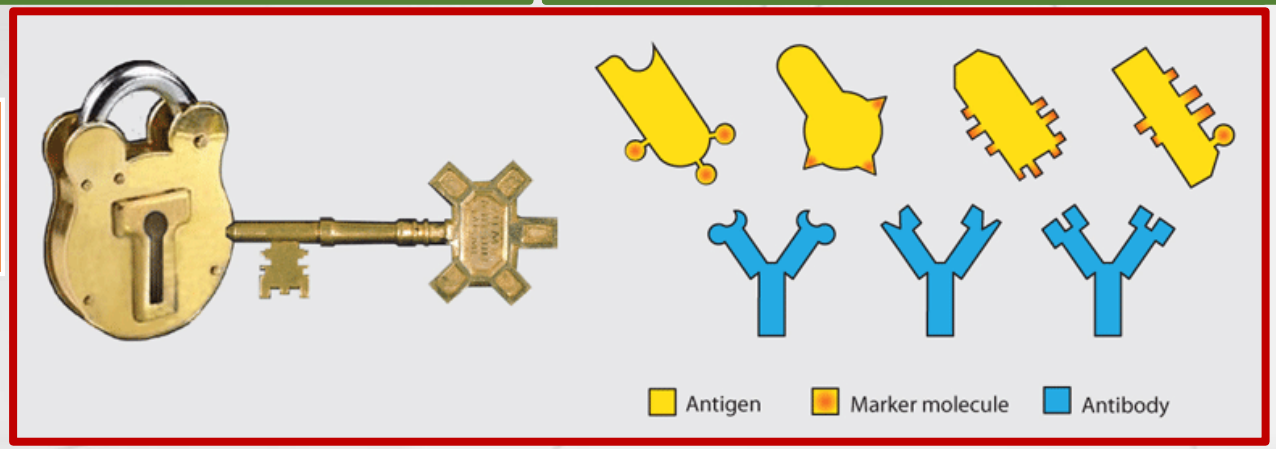
Specificity/Selectivity

Gas sensors





Molecular recognition



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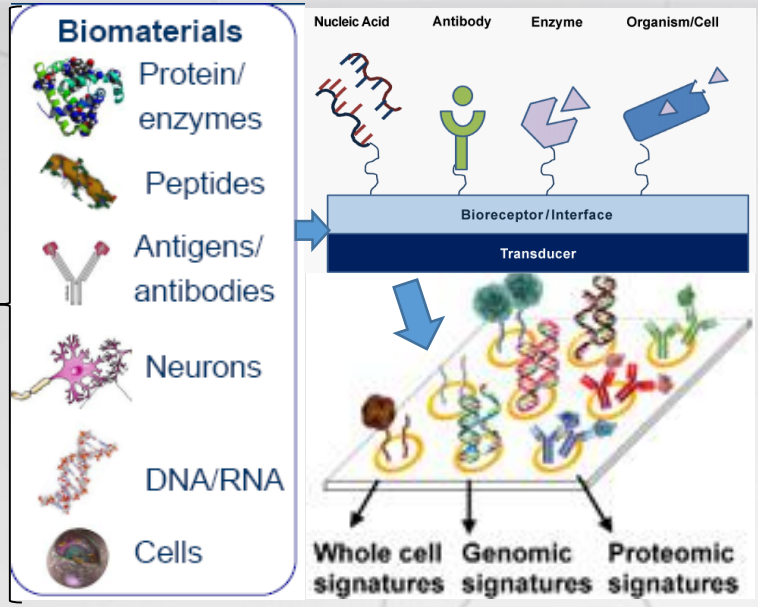
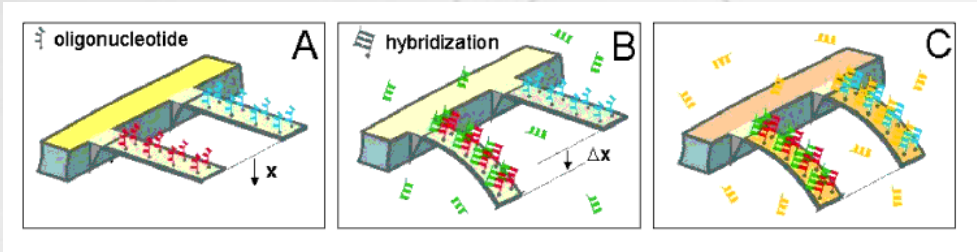
Classification of Biosensors

based on

Recognition Element: *Enzyme (e.g. glucose oxidase), DNA/RNA (gene), antibodies, receptor protein, whole-cell, tissue slice (liver, heart).*



Transduction mechanism: *Optical (light/matter interactions, light emission & luminescence, SPR), electrical/electrochemical (redox reactions or electrical signals), mechanical (gravimetric/mass, piezoelectric and cantilever), calorimetric, magnetoresistive transducers*

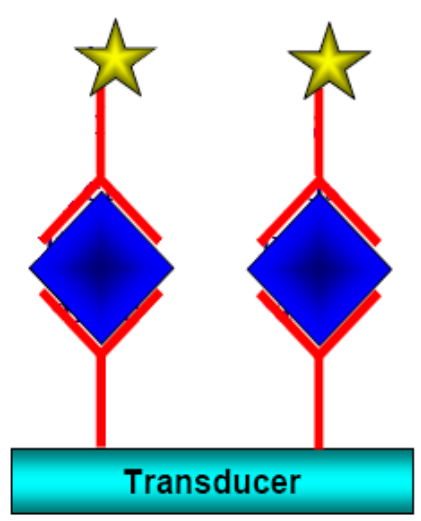


Placement: *In-vivo, in-vitro, point-of-care*

Two Principal Biorecognition Strategies

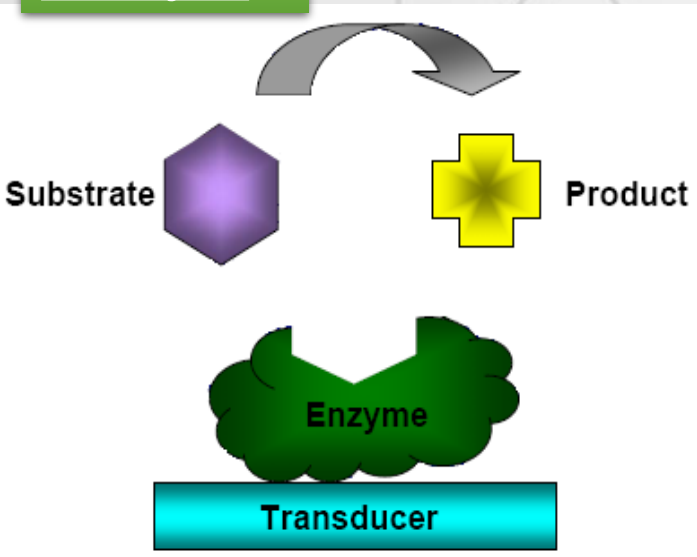
SENSING STRATEGY	REACTION
Insoluble salt-based sensors	$S^+ + R^- \rightleftharpoons$ (insoluble salt)
Bioaffinity sensors based on change of local electron densities	$S + R \rightleftharpoons SR$
Metabolism sensors based on substrate consumption and product formation	$S + R \rightleftharpoons SR \rightarrow P + R$

Non-catalytic



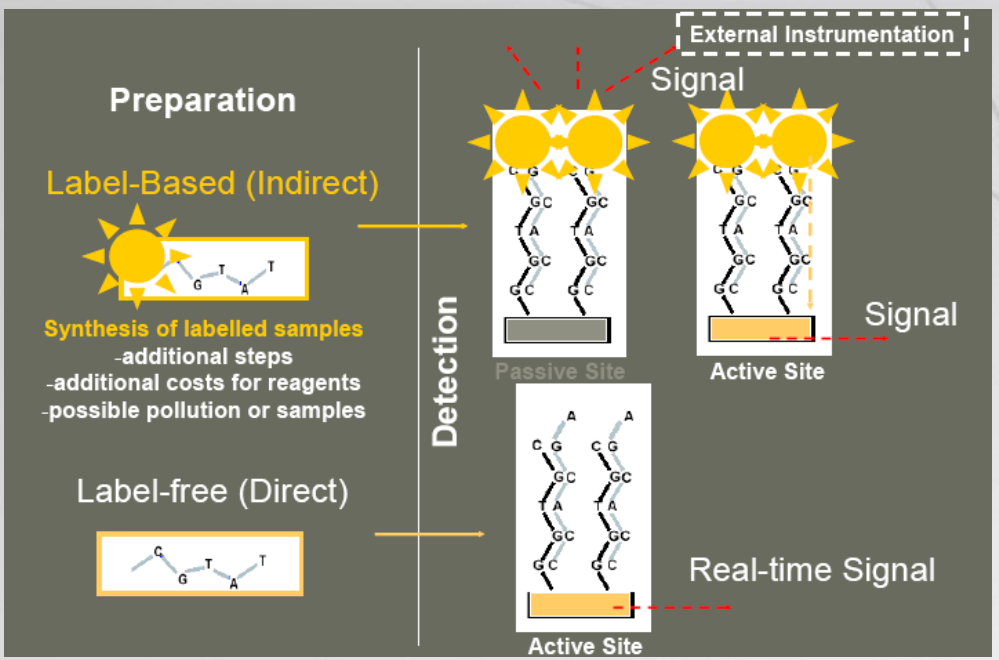
Biocomplexing or bioaffinity sensors (Antibody-based Detection)

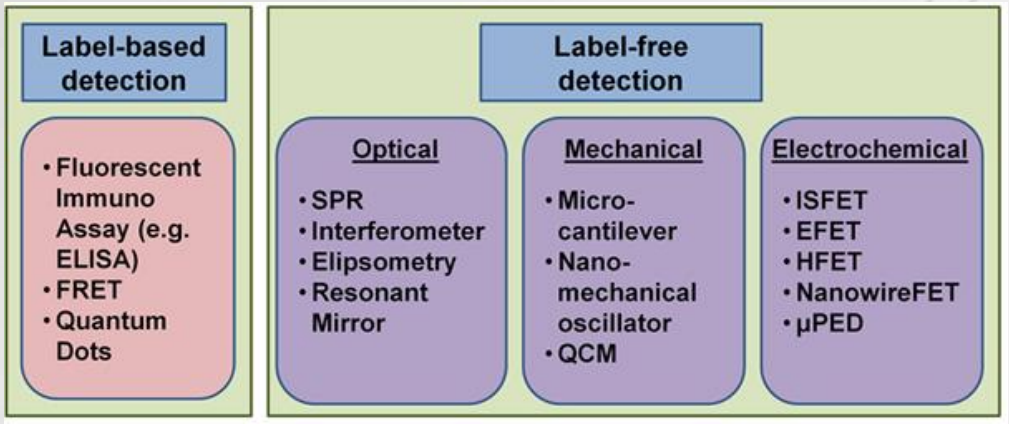
Catalytic



Biocatalytic sensors (Enzyme-based Detection)

Label-based vs Label-free Strategies

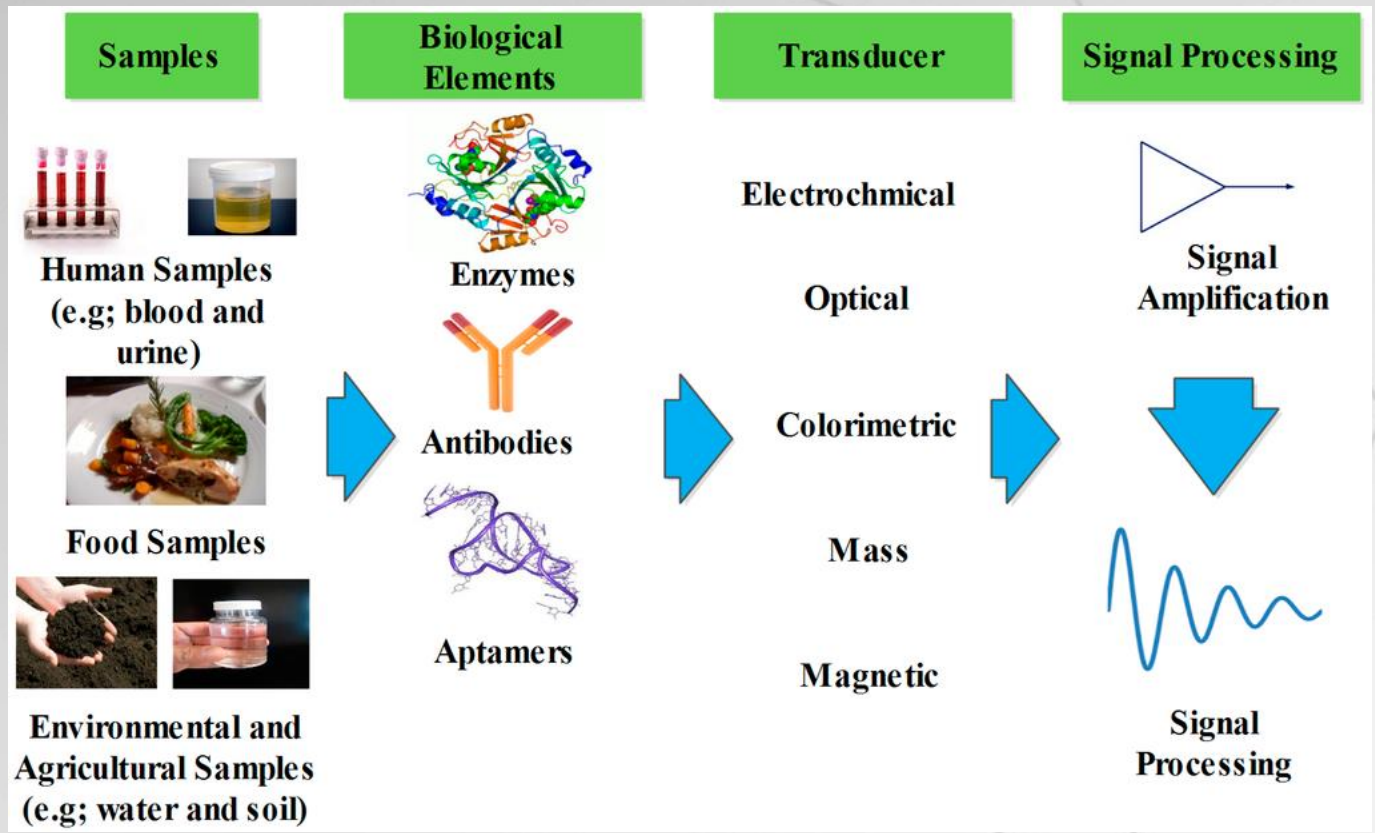




DISADVANTAGES OF "LABEL-BASED " TECHNIQUES

- extra-time and cost
- Labeling can interfere with molecular interaction by occluding a binding site (false negatives)
- background binding can interfere (false positive)
- fluorescent compounds are generally hydrophobic
- sometimes do not allow observation of binding kinetics

Biosensors



Transducers

electrochemical:
- potentiometric
- amperometric
- conductimetric
- voltammetric, polarographic
- impedimetric, capacitive
- piezoelectric

optical:
- transmission / absorbance / reflection
- dispersion, interferometric
- polarimetric
- circular dichroism, ellipsometry
- scattering
- emission intensity, photon counting (luminescence) decay time

calorimetric

acoustic / gravimetric:
- surface photo-acoustic wave
- quartz microbalance

Recognition elements

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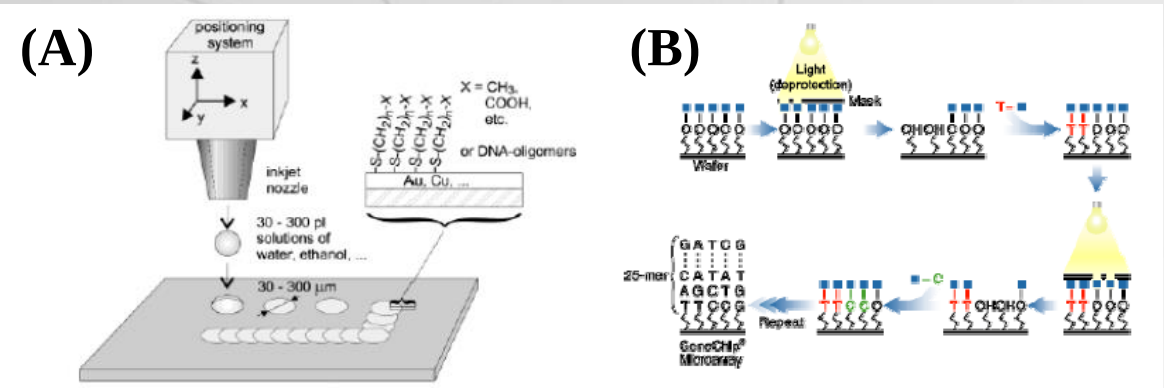
Development steps of the sensing surface

1 .Deliver or synthesize the bioreceptors

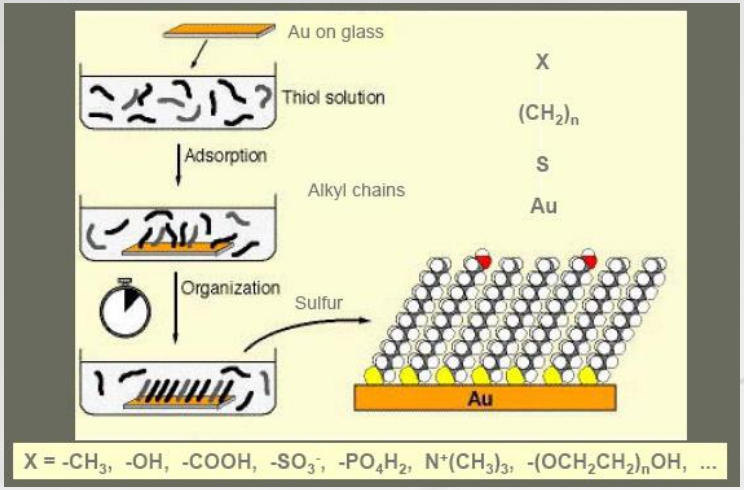
A. IBM and Agilent Technologies Inkjet printing of single stranded DNA

B. Photolithography patterning of DNA features Affymetrix, GeneChip® Santa Clara (California)



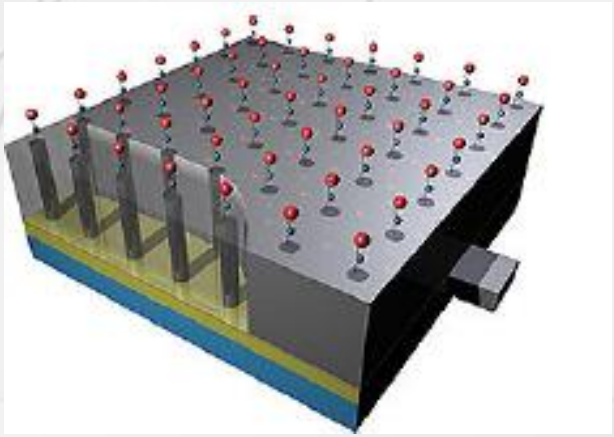
2. Immobilize bioreceptors

➢ Self-assembly of thiols on gold films



3. Passivate the surface

4. Stock in appropriate conditions



SAMs for biosensors

Platform for linking biomolecules either using direct chemical linkages or by encapsulation with the help of polymeric supports.

Advantages

1. ***Easy formations of*** ordered, pinhole free and stable monolayers.
2. Membrane-like microenvironment (cellular) ***suitable for biomolecule immobilization***.
3. ***Flexibility to design the head group*** of SAM with various functional groups in order to accomplish hydrophobic or hydrophilic surface as per the requirement.
4. Only ***minimum amount of biomolecule*** (monolayer) is needed for immobilization on SAM.
5. ***Reasonable stability*** for extended period, allowing several reliable measurements.
6. ***Ability to unravel molecular level information*** about phenomena such as protein adsorption, DNA hybridization, antigen–antibody interaction etc. using surface sensitive techniques such as AFM.

Disadvantages

1. ***Immobilized enzymes are very much sensitive towards*** changes in pH, ionic strength and temperature: a minor change in one of these parameters can sometimes be responsible to loose the biological activity.
2. ***The chemical stability*** of some of the SAMs is not very good as monolayer can be chemically oxidized during the course of investigations.
3. Electric field induced and thermal ***desorption of monolayers*** is detrimental to biosensor applications.
4. Due to high surface energy, ***hydrophobic SAM surface can accumulate several contaminants*** and hence unwanted impurities can adsorb and block the analyte recognition sites.

Strategies - Functional groups

- Many technologically relevant materials possess ***well-defined surface chemistries***, including metals, semiconductors, oxides, and other complex materials such as superconductors
- a variety of heteroatom containing molecules have been shown to self-assemble on such substrates.

Chemical systems of adsorbates and substrates that form SAMs

Surface	Substrate	Adsorbate(s)	Selected reference(s)
Metal	Au	R–SH, R–SS–R, R–S–R, $R–NH_2$, R–NC, R–Se, R–Te	[17,75–78]
	Ag	R–COOH, R–SH	[18,79]
	Pt	R–NC, R–SH	[80–82]
	Pd	R–SH	[83]
	Cu	R–SH	[84]
	Hg	R–SH	[85]
Semiconductor	GaAs (III–V)	R–SH	[86,87]
	InP (III–V)	R–SH	[88]
	CdSe (II–VI)	R–SH	[89]
	ZnSe (II–VI)	R–SH	[90]
Oxide	Al_2O_3	R–COOH	[14]
	TiO_2	R–COOH, $R–PO_3H$	[91,92]
	$YBa_2Cu_3O_{7-\delta}$	$R–NH_2$	[93,94]
	Tl–Ba–Ca–Cu–O	R–SH	[95]
	ITO	R–COOH, R–SH, $R–Si(x)_3$	[96,97]
	SiO_2	$R–Si(x)_3$	[5]

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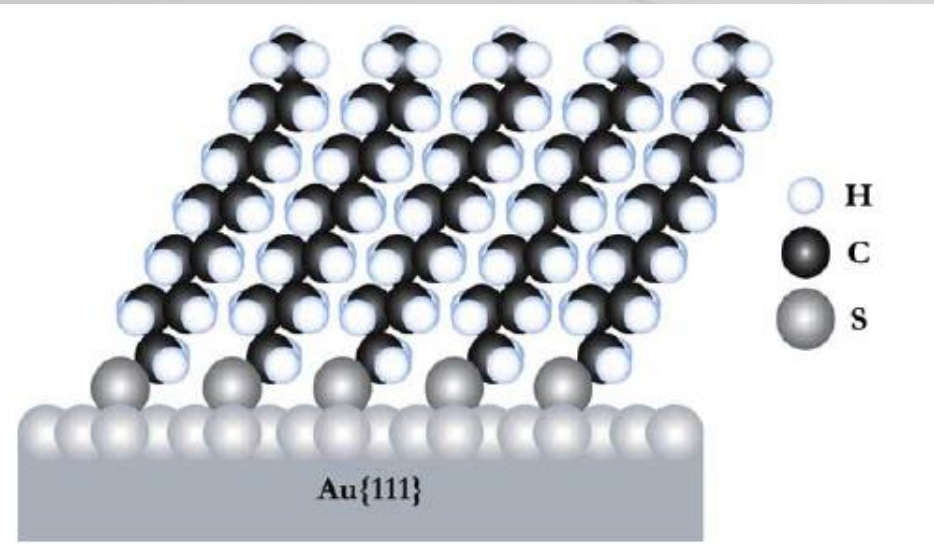
The n-alkanethiolate SAM



n-dodecanethiolate monolayer self-assembled on an atomically flat gold substrate

Thiol-based SAMs are attractive structures for several reasons. Well-ordered SAMs can be formed from a variety of sulfur containing species (i.e., thiols, sulfides, disulfides [3]), molecules are stable once adsorbed on the surface

Alkanethiolates on noble metal surfaces

The structure as well as the placement of alkanethiolate films on surfaces other than gold has been studied and reported, including platinum [82,132], palladium [105,133], silver [111,134], and copper [111,135].

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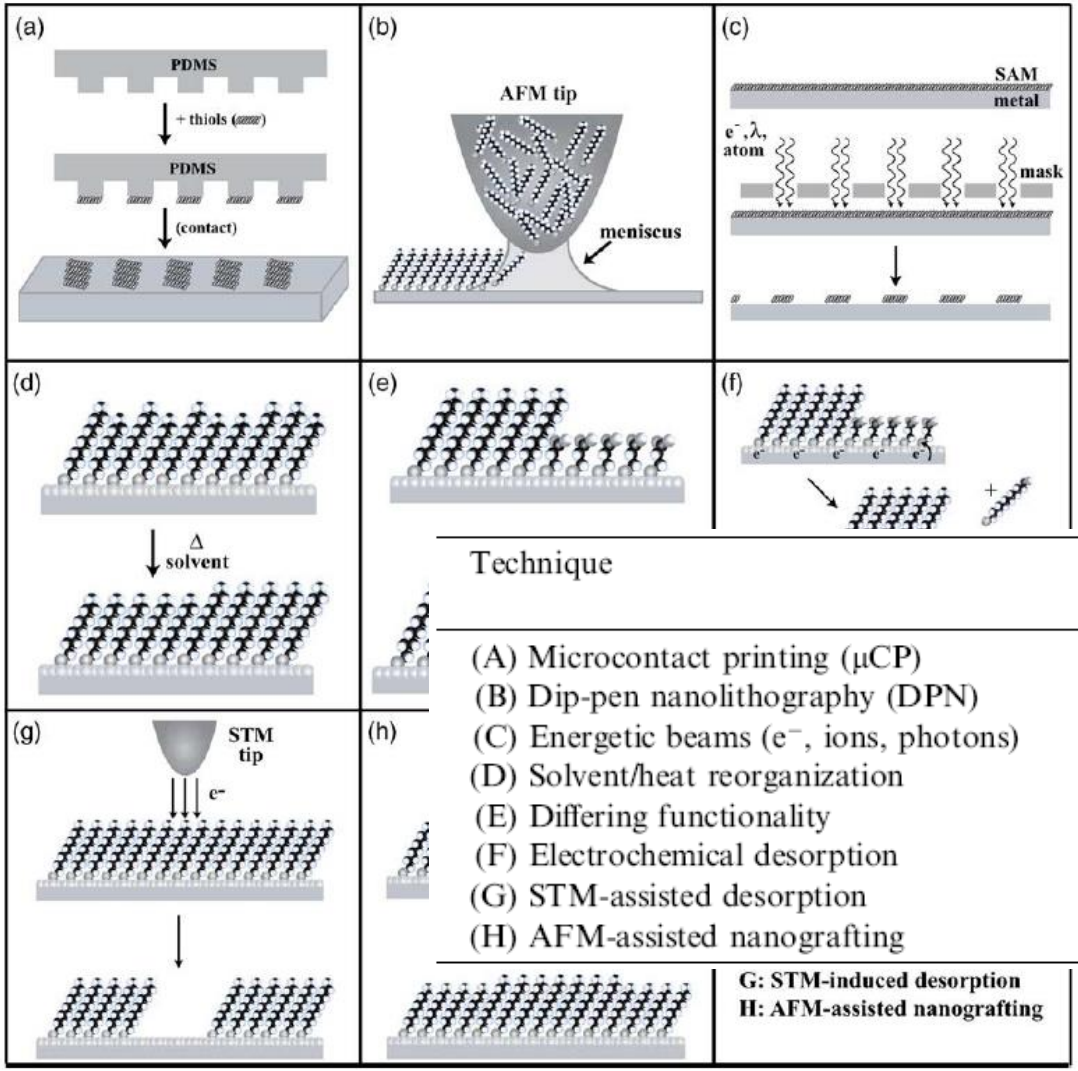
Techniques for SAM patterning



Technique	Approximate resolution (nm)	Area	Comments
(A) Microcontact printing (μCP)	30	>cm²	Fast, parallel
(B) Dip-pen nanolithography (DPN)	10	<mm²	Slow, serial
(C) Energetic beams (e^-, ions, photons)	100	>cm²	Fast, parallel
(D) Solvent/heat reorganization	100–500	>cm²	IC
(E) Differing functionality	10–50	>μm²	NC
(F) Electrochemical desorption	10–50	>μm²	IC
(G) STM-assisted desorption	>1	nm²–μm²	Slow, serial
(H) AFM-assisted nanografting	>1	nm²–μm²	Slow, serial

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Molecularly imprinted polymers

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Calorimetric/ Thermometric sensors

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Calorimetric readouts

- Measurement of **heat generated by an enzymatic reaction** (enthalpy change).
- **Enzyme provides selectivity** and reaction enthalpy cannot be confused with other reactions.

$$\text{glucose} + O_2 + H_2O \xrightarrow[\Delta H = -80\ \text{kJ mol}^{-1}]{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$CO(NH_2)_2 + H_2O \xrightarrow[\Delta H = -6.6\ \text{kJ mol}^{-1}]{\text{urease}} CO_2 + 2NH_3$$

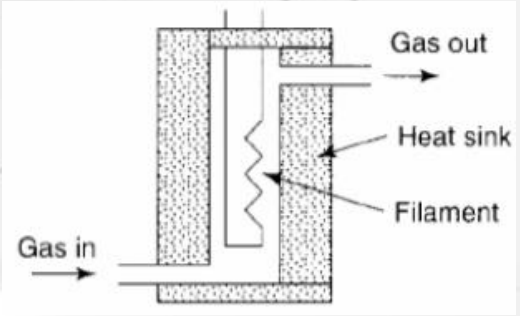
Enzyme reaction

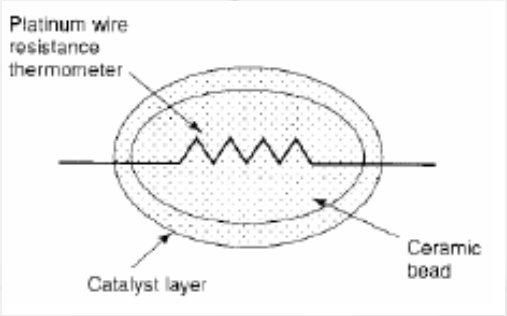
$$CH_4 + 2O_2 \xrightarrow[\Delta H = -800\ \text{kJ mol}^{-1}]{} CO_2 + 2H_2O$$

Catalytic gas sensor

- Ideally **total heat evolution** by a calorimetric measurement but **always heat loss** in real systems (not adiabatic process) → **temperature difference** before and after evolution measured most often.
- **heat capacity** of specimen and container assumed constant over small temperature range measured.
- **Simplest transducer**: thermometer coated with the enzyme.
- **Thermistors** typically utilized to transform heat into electrical signal (change of resistance with temperature). The change of resistance of certain oxides is much greater than the change of length of a mercury column or the microvolt changes of thermocouple junctions.

Thermal conductivity devices
(typically gas chromatography)





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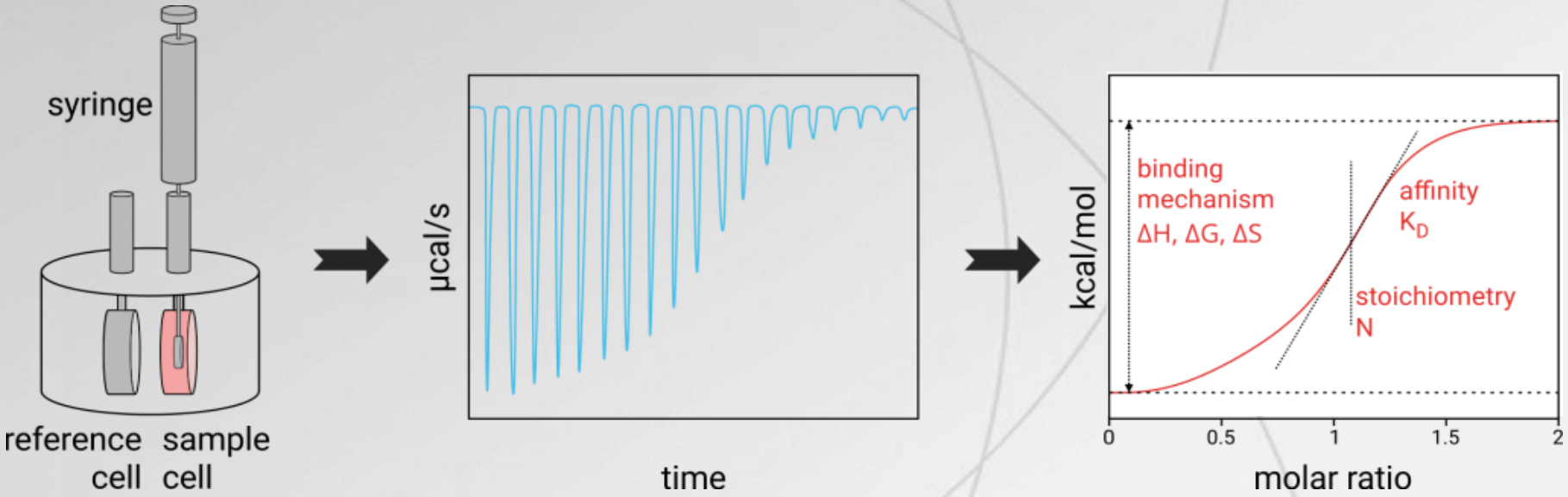
Isothermal titration calorimetry (ITC)

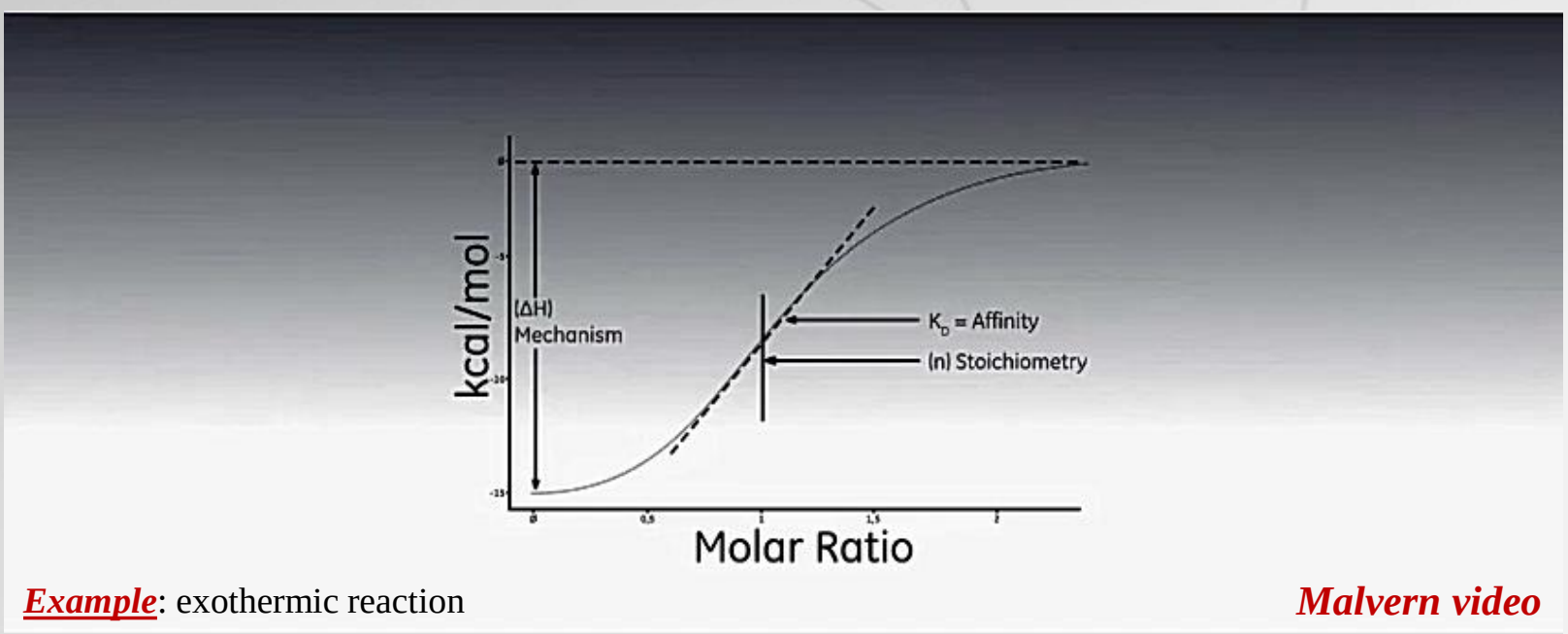
Measurement of ***reversible reactions among biomolecules*** → determination of ***binding constants***, ***reaction stoichiometry*** and the ***thermodynamic profile (enthalpy and entropy)*** of the interaction in a single experiment ***without*** immobilization/modification of reactants and/or labels.



- ***Two cells kept at the same temperature***: heat sensor detects temperature difference and feedback to the heaters which compensate to return the cells at equal temperature
- ligand solution titrated into a well-insulated, stirred cuvette containing a receptor kept at constant temperature.
- As heat is released or absorbed during a molecular interaction, a **BINDING ISOTHERM** is obtained as a plot of the heat change versus the molar ratio of ligand to receptor.
- control experiments **to compensate for bulk effects** such as heat of dilution of ligand and receptor and heat of mixing

Isothermal titration calorimetry (ITC)

Measurement of ***reversible reactions among biomolecules*** → determination of ***binding constants***, ***reaction stoichiometry*** and the ***thermodynamic profile (enthalpy and entropy)*** of the interaction in a single experiment ***without*** immobilization/modification of reactants and/or labels.



Example: exothermic reaction

Malvern video

- Two cells kept at the same temperature: heat sensor detects temperature difference and feedback to the heaters which compensate to return the cells at equal temperature
- ligand solution titrated into a well-insulated, stirred cuvette containing a receptor kept at constant temperature.
- As heat is released or absorbed during a molecular interaction, a **BINDING ISOTHERM** is obtained as a plot of the heat change versus the molar ratio of ligand to receptor.
- control experiments **to compensate for bulk effects** such as heat of dilution of ligand and receptor and heat of mixing

Mechanical/Mass sensors

- **QCM & BAW** (Bulk Acoustic Wave), **SAW** (Surface Acoustic Wave) : they are sensors of **Mass and Pressure.**

- **MicroCantilever** : they are sensors of **Mass** and **Surface Stress**

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QCM, BAW, SAW sensors

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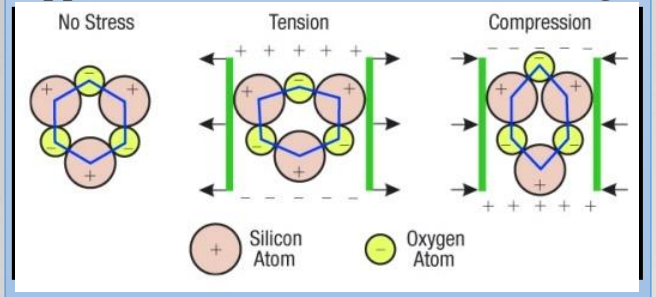
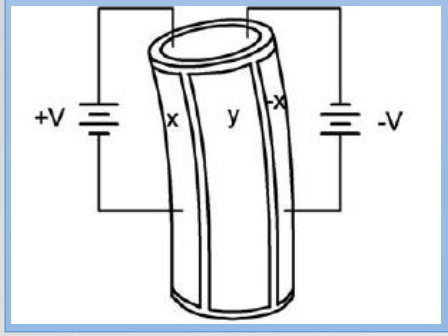
Piezoelectric materials and their applications

The Piezoelectric effect:

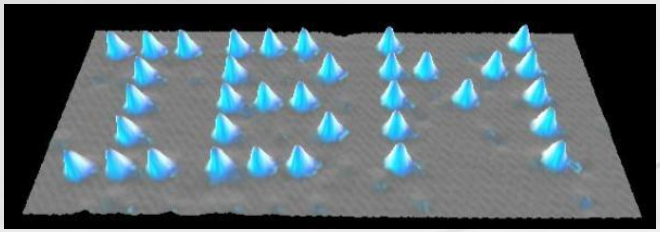
Applied force → surface electrical charge



Inverse-Piezoelect. Effect



Piezoelectric ceramics for hi-resolution (<nm) mid-range (100 μm) actuators



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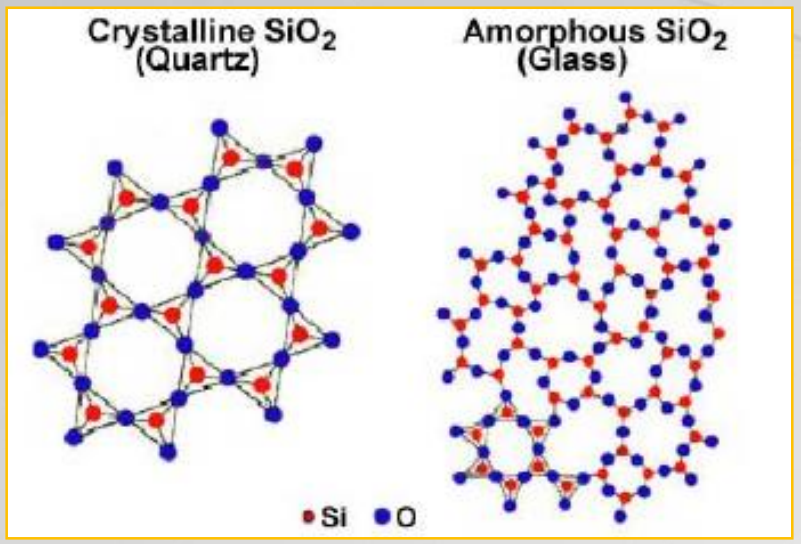
Quartz Crystal – Crystalline SiO_2



Only specific materials, belonging to certain crystal classes, show the piezoelectric effect: quartz, cadmium sulphide, lithium niobate, lithium tantalate, zinc oxide, lead zirconium titanate (PZT), III-nitrides group (GaN, AlGaN).

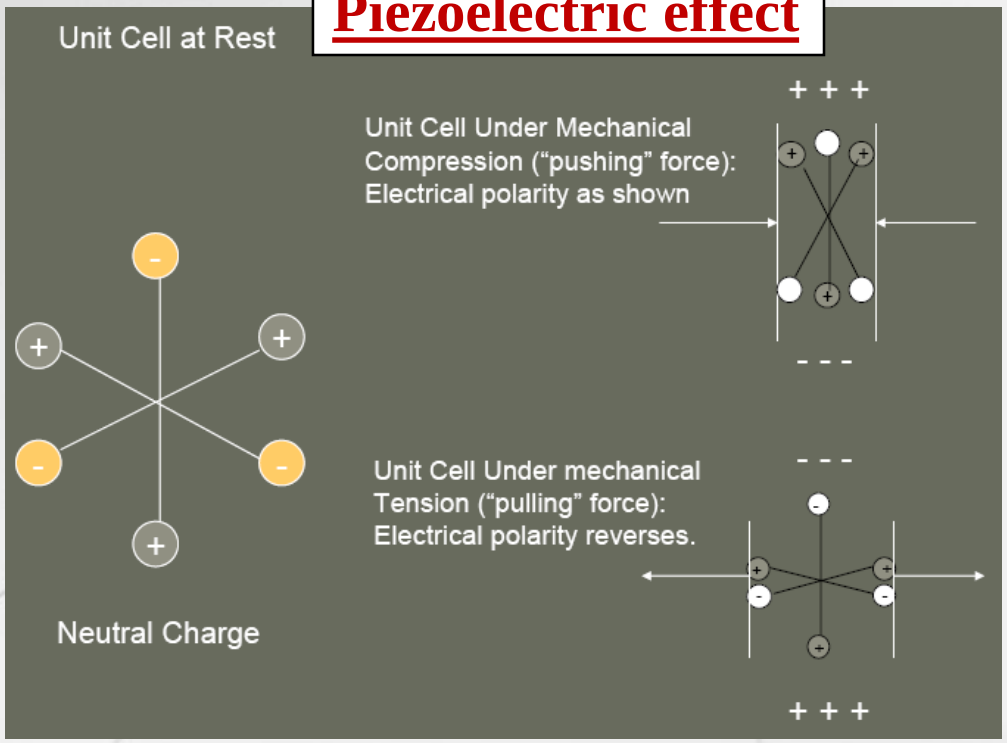
Constitutive equations :

$$T = cS - e^T E \equiv \text{applied stress}$$

$$D = eS + \varepsilon E \equiv \text{electric displacement (polarization)}$$

$S \equiv$ induced strain
$E \equiv$ electric field
$c \equiv$ stiffness coefficient matrix
$e \equiv$ piezoelectric constant matrix
$\varepsilon \equiv$ permittivity (dielectric constant) matrix

Piezoelectric effect



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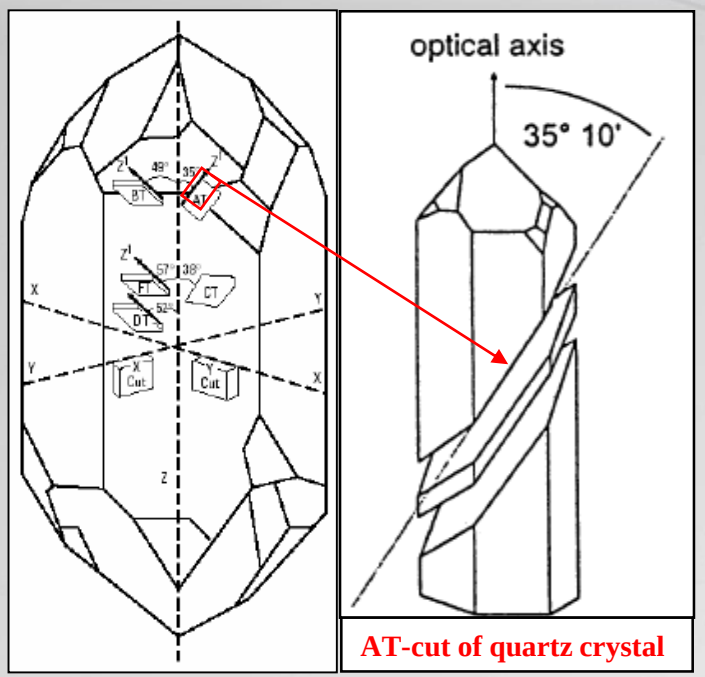
Quartz Crystal – cut & AW generation

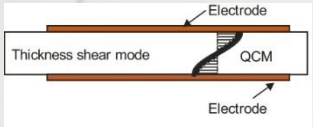


AT-cut of quartz crystal

First piezoresonator employed as a chemical sensor was a ***AT-quartz resonator***

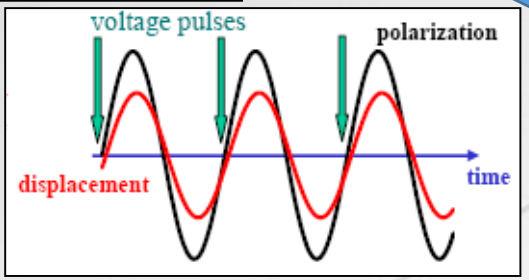
- *X plate crystals*: large voltage generated when compressed and decrease in frequency with T increases
- *Y plate crystals*: large voltage generated by shear stress and increase in frequency with T increases

- *X cuts* exhibits an extensional vibration mode with AC voltage
- ***AT cuts (35 degrees off the Y axis)*** vibrates in the ***thickness shear mode***



Acoustic wave generation

1. ***Applied V_{ac}*** → time-varying mechanical ***displacement*** along field direction → ***generated acoustic wave*** propagates until reaching a ***boundary*** where is ***reflected***.
2. Piezoelectricity → reflected wave generates charge separation → ***time-varying electric field*** in phase with mechanical displacement → electric potential ***back in the electrode regenerating an acoustic wave***

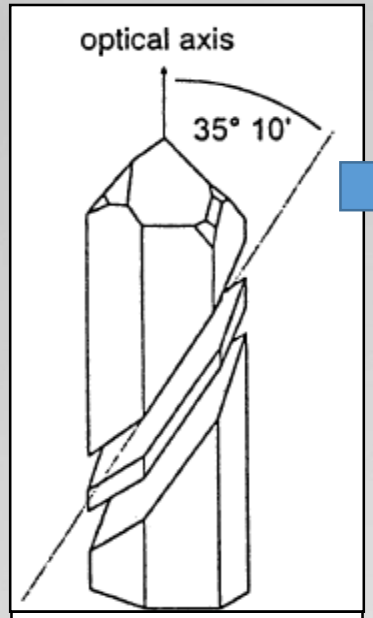


RESONANCE if *this induced electric field is* ***reapplied*** *to the device* ***in phase with the displacement*** → ***standing wave***

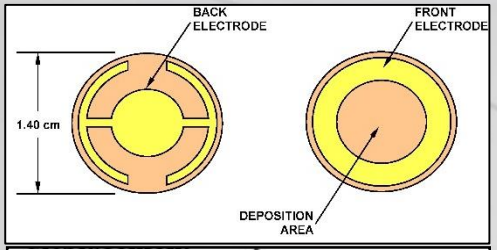
no net gain or loss in energy through the process of generation, propagation, and regeneration. ***Due to the damped wave motion*** (frictional losses, …), an ***extra electric energy*** has to be supplied to each regenerated wave.

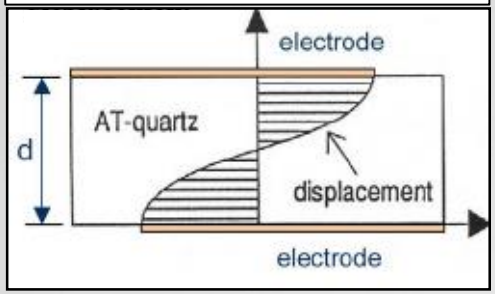
- Two metal electrodes on a thin slice of quartz cut with a specific orientation relative to the crystallographic axes (AT-cut)
- Displacement in a thickness-shear mode, in which entire material subject to displacement

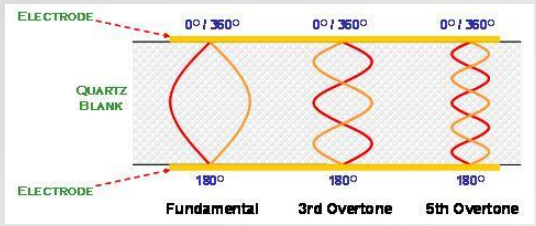
Quartz Crystal Microbalance (TSM-BAW)



AT-cut of quartz crystal







- First piezoresonator employed as a chemical sensor was a AT-quartz resonator
- Two metal electrodes on a thin slice of quartz cut with a specific orientation relative to the crystallographic axes (AT-cut)
- Displacement in a thickness-shear mode, in which entire material subject to displacement

Resonance condition: thickness equal to an odd number of a half wavelength

$$d = \frac{\lambda}{2} n \quad \Rightarrow \quad \lambda = \frac{2d}{n}, \qquad n = 1,3,5\ldots$$

$v_b \equiv$ shear wave velocity (~ 3200m/s for quartz)

Typical d = 0.2 mm → f_0 ~ 9MHz

$$f_0 = \frac{v_b}{\lambda} = n\frac{v_b}{2d}$$

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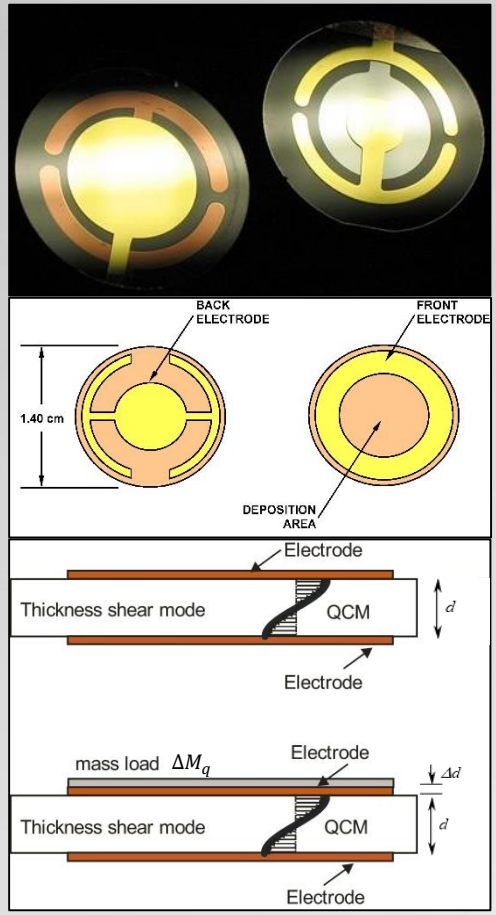
Mass sensitivity of QCM



Sauerbrey (1959) - For small mass changes, the added mass can be treated as an additional mass of quartz with its corresponding added thickness.

$$f_0 = \frac{v_b}{\lambda} = n\frac{v_b}{2d}$$

- The change in thickness, Δd, *causes a change in the* oscillation frequency, Δf_0.
- *An increase in thickness* and mass produces a decrease in frequency.

$$M_q = \rho_q V = \rho_q A d$$

$$\Rightarrow d = \frac{M_q}{\rho_q A} \;\&\; \Delta d = \frac{\Delta M_q}{\rho_q A}$$

$$\frac{\Delta f_0}{f_0} = -\frac{\Delta d}{d} = -\frac{\Delta M_q}{\rho_q A}\frac{\rho_q A}{M_q} = -\frac{\Delta M_q}{M_q}$$

$$= -\frac{\Delta M_q}{\rho_q A d} = -\frac{\Delta M_q}{\rho_q A}\frac{2f_0}{v_b}$$

$$f_0 = \frac{v_b}{2d}$$

$$\Delta f_0 = -2 f_0^{\,2}\frac{1}{\rho_q v_b}\frac{\Delta M_q}{A}$$

For quartz, $\rho_q = 2.64\ g/cm^3$

$v_b = 3{,}33\ cm/s$

f_0=10MHz, A = 1cm^2

$$\frac{\Delta f_0}{\Delta M_q} = 230\ Hz/\mu g$$

Sauerbrey equations describe relationship between resonant frequency and mass deposited.

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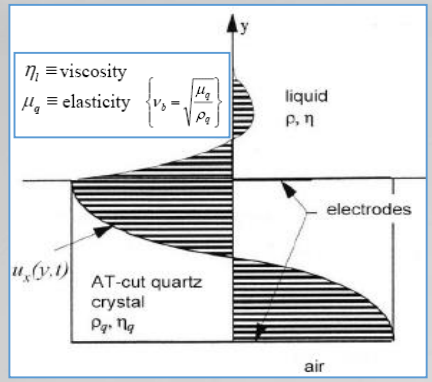
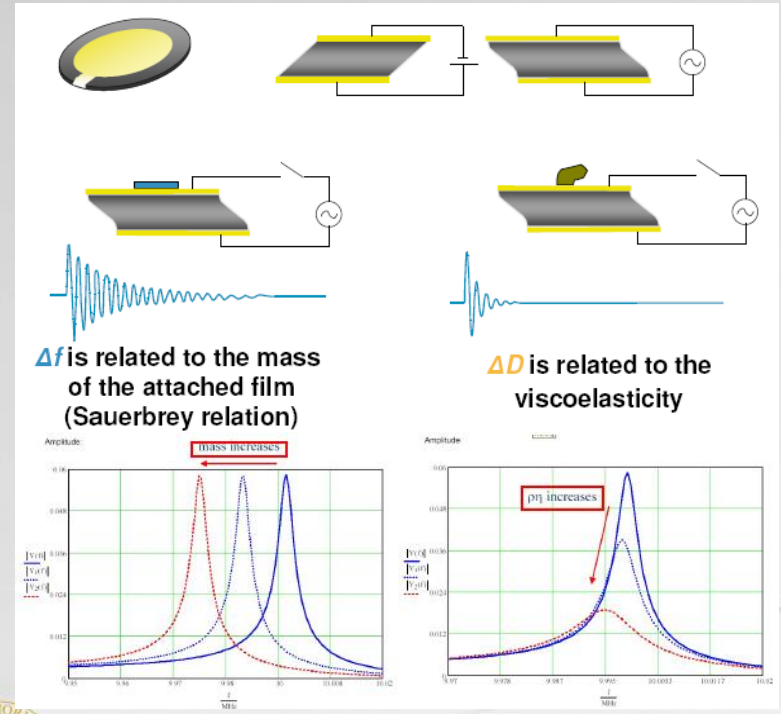
QCM - Liquid operation



Initially believed impossible due to excessive wave damping by acoustic coupling into the liquid, but stable resonance can be obtained if only one face of the QCM resonator is in contact with the liquid.

- Due to the strong adhesive forces between the liquid phase and the surface there is a strong coupling of the wave motion from the surface into the liquid
- Due to the moderate cohesive forces in the liquid (much smaller than in a solid), the motion damping is very accentuated.

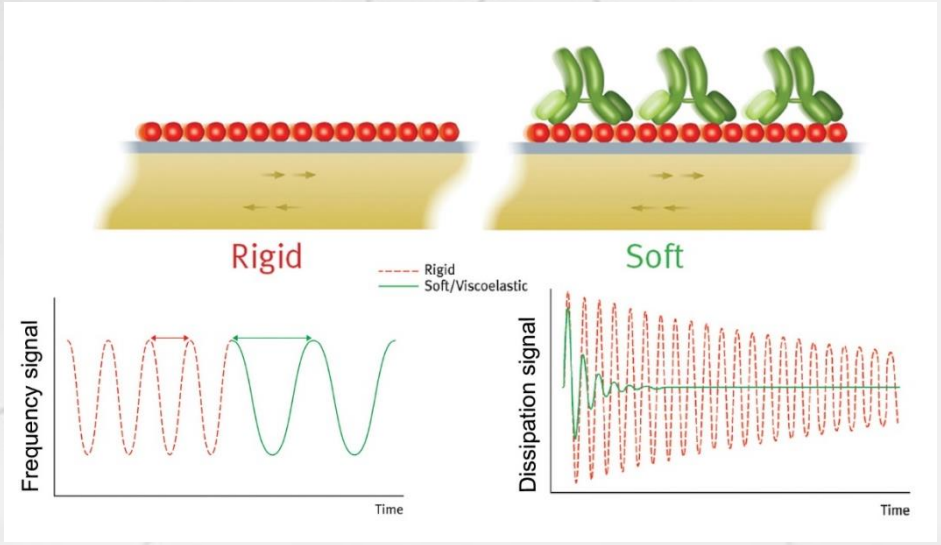
decay rate of liquid compressional waves and amount of liquid-coupled energy depends on ***liquid viscosity***. The lost of acoustic energy results in a change in frequency/attenuation



frequency shift

$$\Delta f_0 = -f_0^{3/2} \sqrt{\frac{\eta_l \cdot \rho_l}{\pi \rho_q \mu_q}}$$

l, refers to liquid



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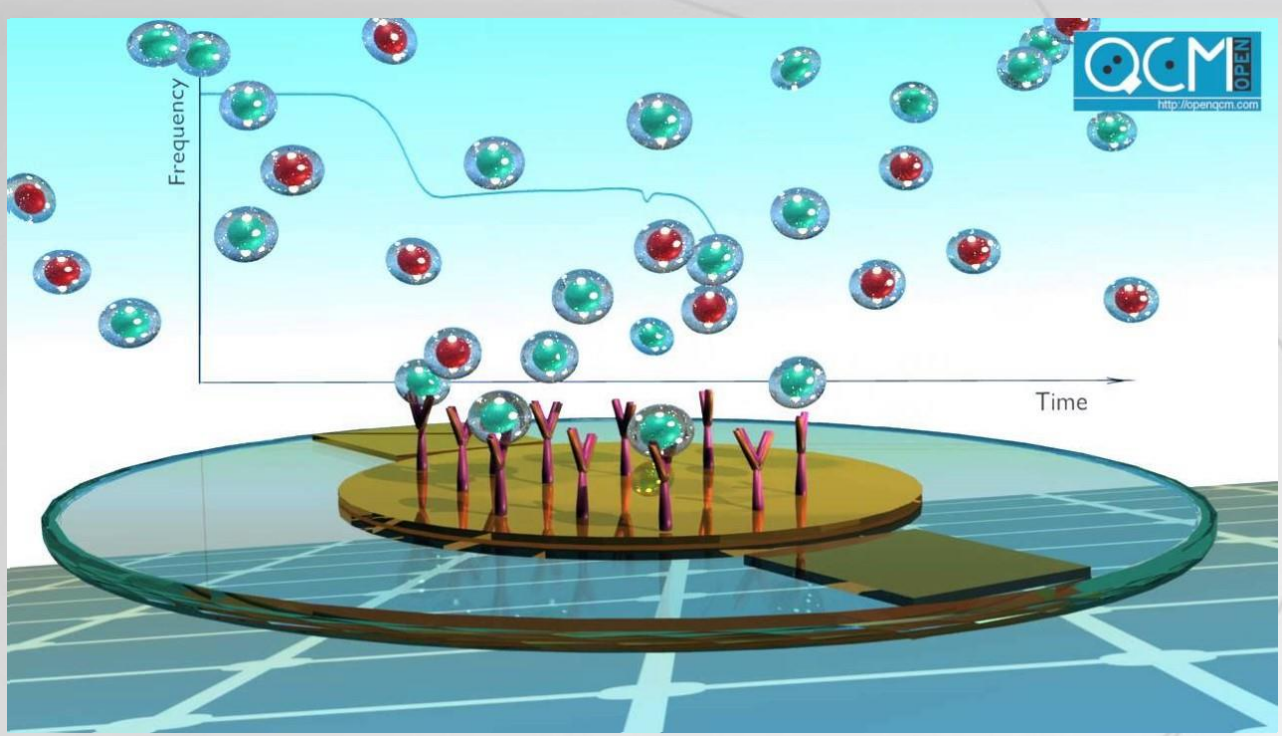
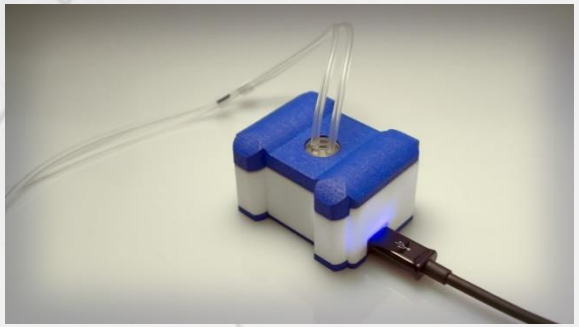
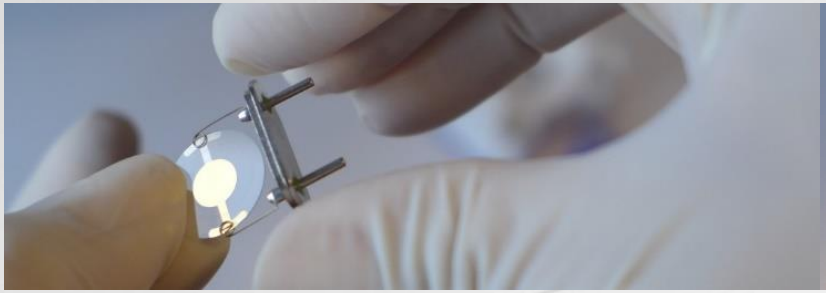
Quartz Crystal Microbalance (TSM-BAW)







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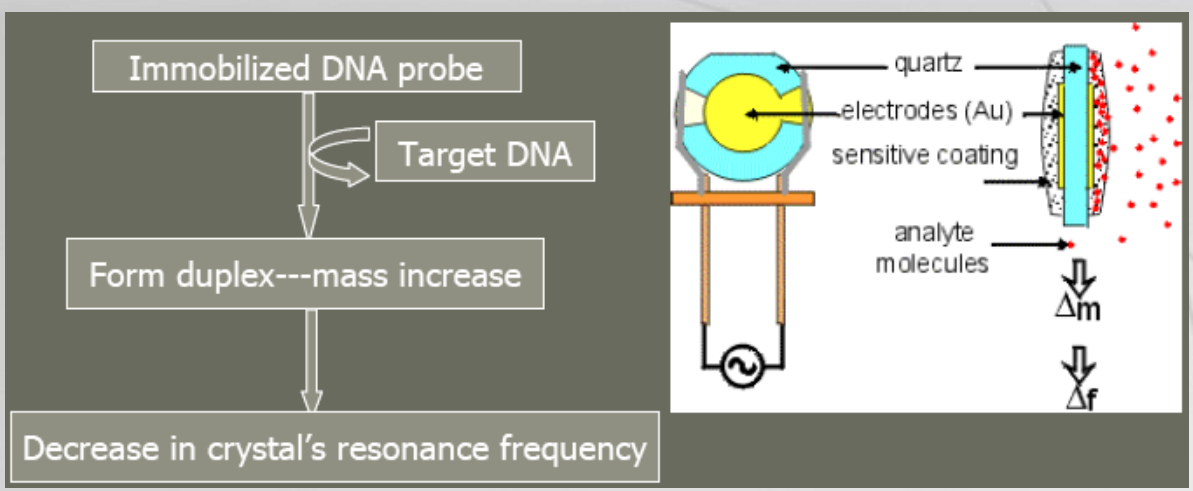
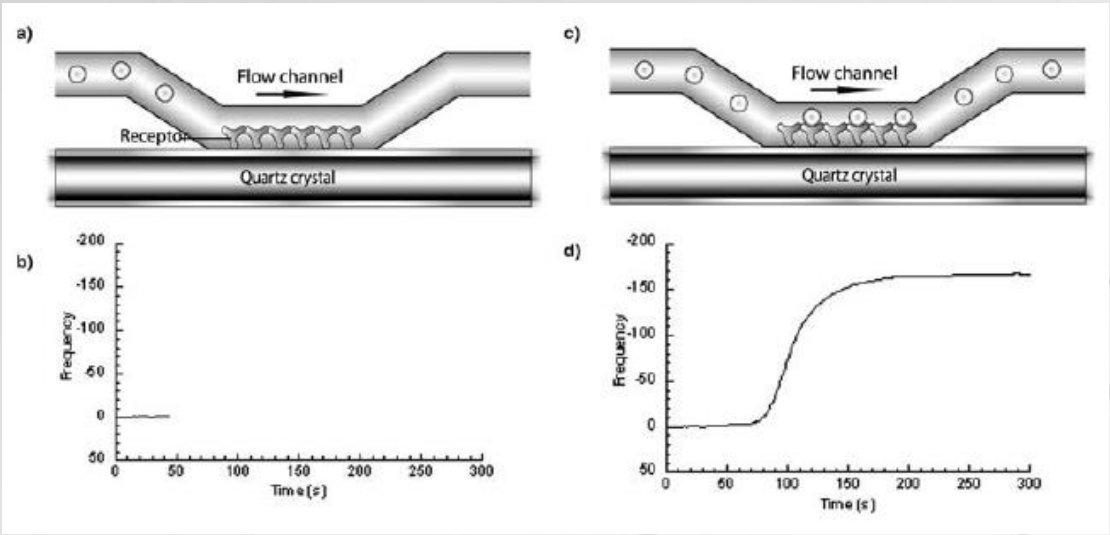
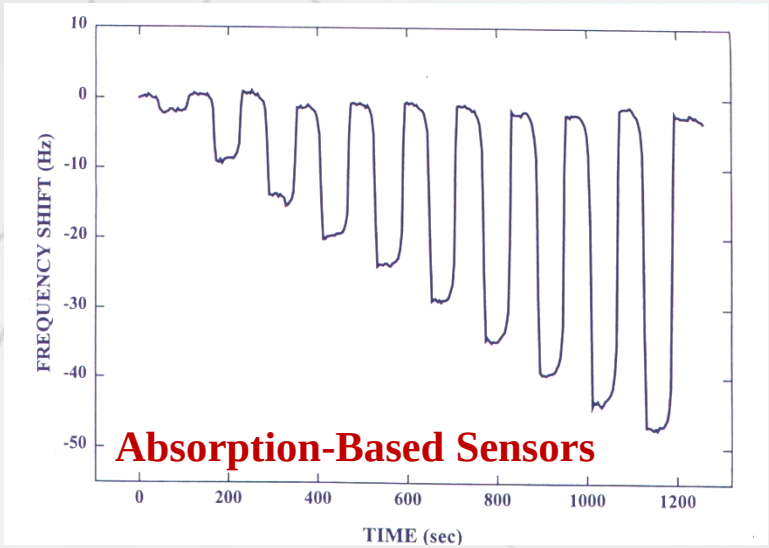
QCM transducers



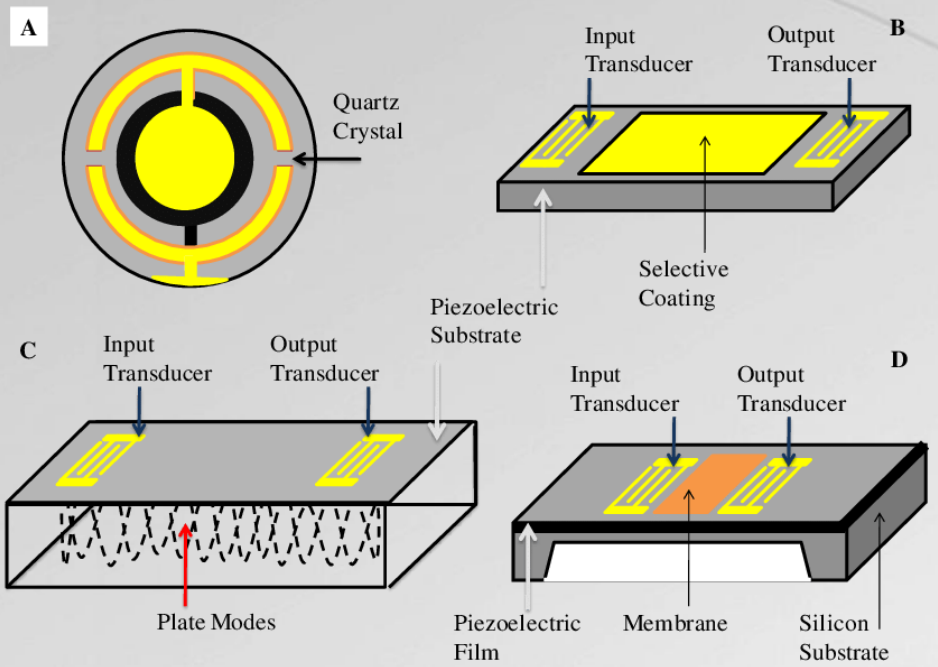
Quartz plate coated with gold electrode plus a sensitive layer (e.g. DNA probes)





Acoustic Wave Sensors

Mechanical shift of resonance for detection of mass change (due to adsorption or chemical reaction)



External parameters changing resonant frequency by modifying the characteristics of the propagation path:

- mass of landing material (coating or particles) on the surface
- pressure
- temperature
- geometrical properties

Frequency range of generated elastic wave: from 1MHz to few GHz

Acoustic waves:

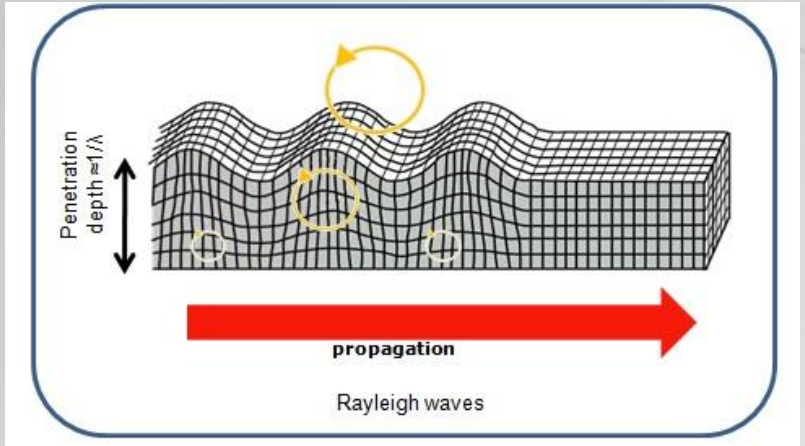
- Bulk Acoustic Wave *(through the material)*
  - TSM (thickness shear mode-QCM),
  - SH-APL(Shear-horizontal acoustic plate mode)
- Surface Acoustic Wave *(on the surface)*
  - SH-SAW (Shear-horizontal acoustic wave) or STW
  - Vertical SAW

LIQUID ENVIRONMENT (see later)

If amplitude normal to surface, energy radiated into the liquid can cause excessive dumping and energy losses. **Mode propagation adapted to liquid environment:**

- **TSM (BAW)**
- **SH-APM (BAW)**
- **SH-SAW (SAW)**

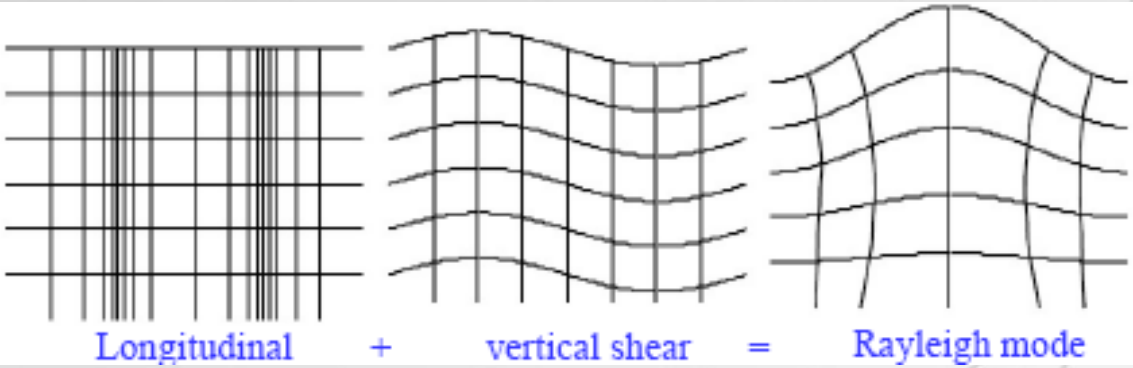
Surface Acoustic Waves



SAW properties

$$v_{group} = v_{acoustic} = \frac{\omega}{k_{wave}} = \frac{\omega\lambda}{2\pi}$$

- Wave bound to the surface → effectively is an evanescent wave
- Small amplitude (~ 10Å) compared with wavelength and falls off exponentially with distance from surface
- Penetration depth of wave into the substrate varies inversely proportional with frequency



· A Rayleigh wave is composed of a longitudinal and a vertical shear component

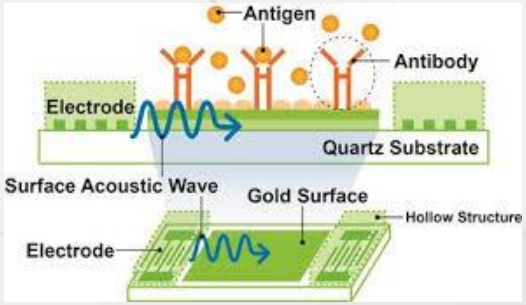
Lord Rayleigh, 1881: "On waves propagating along the plane of an elastic solid"

Bulk longitudinal wave
v = 4000-12000 m/s

Bulk transverse wave
v = 2000-6000 m/s

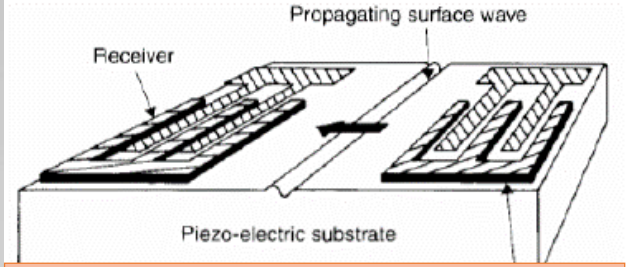
Surface (Rayleigh) wave
v = 2000-6000 m/s

- For f_0~200MHz, 3 pg of biomolecules are detectable
- 3 order of magnitude more sensitive than BAW

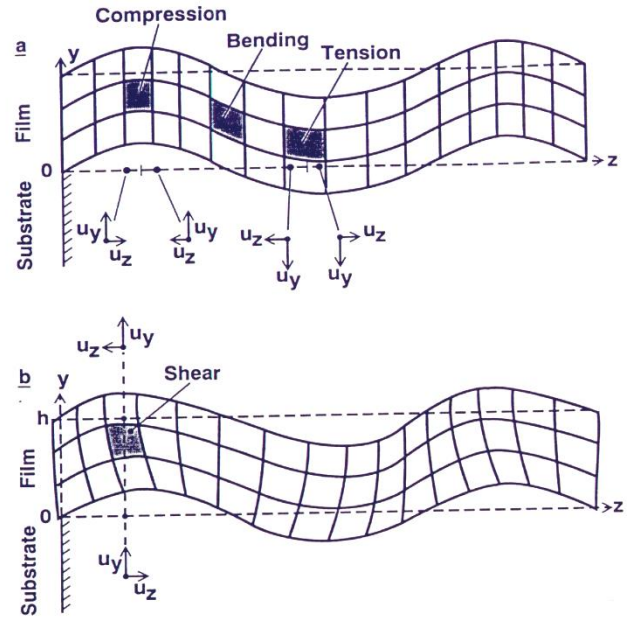


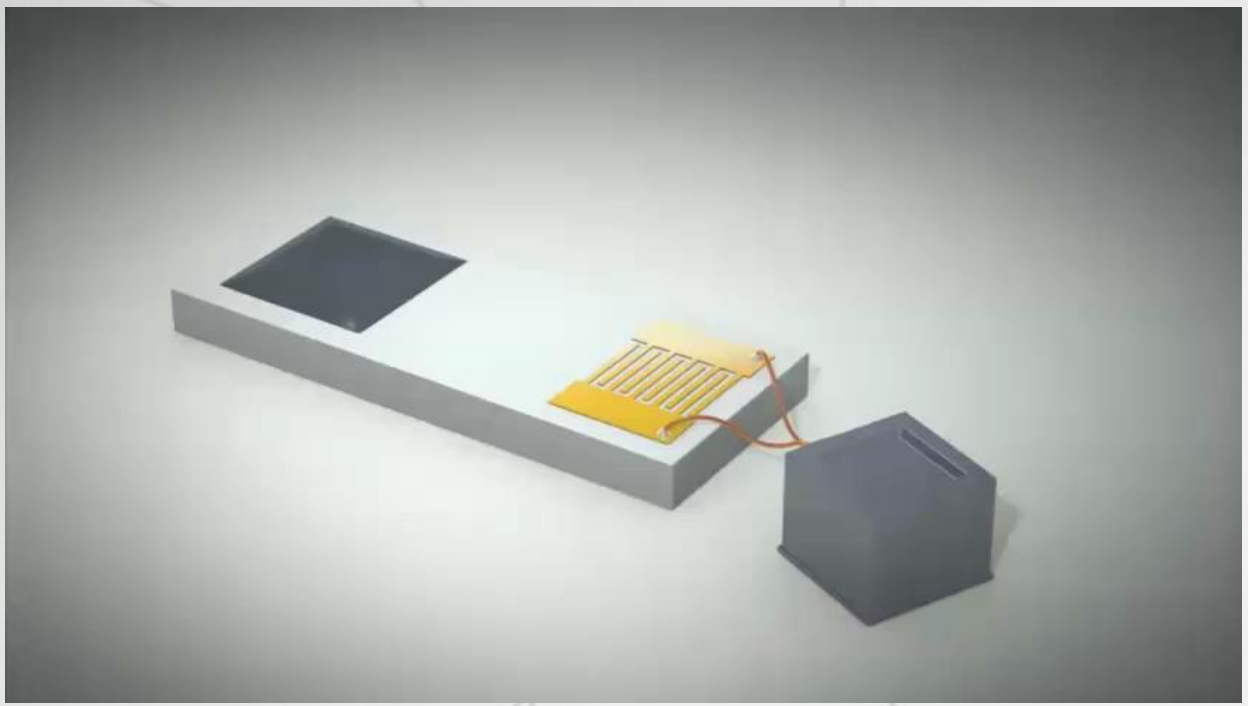
Surface Acoustic Waves

SAW Excitation and Detection



Elastic and Viscoelastic Films on a SAW Device





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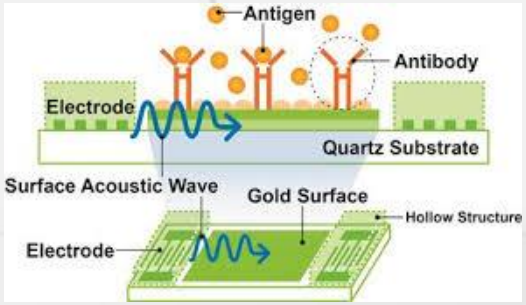

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Surface Acoustic Waves - Applications

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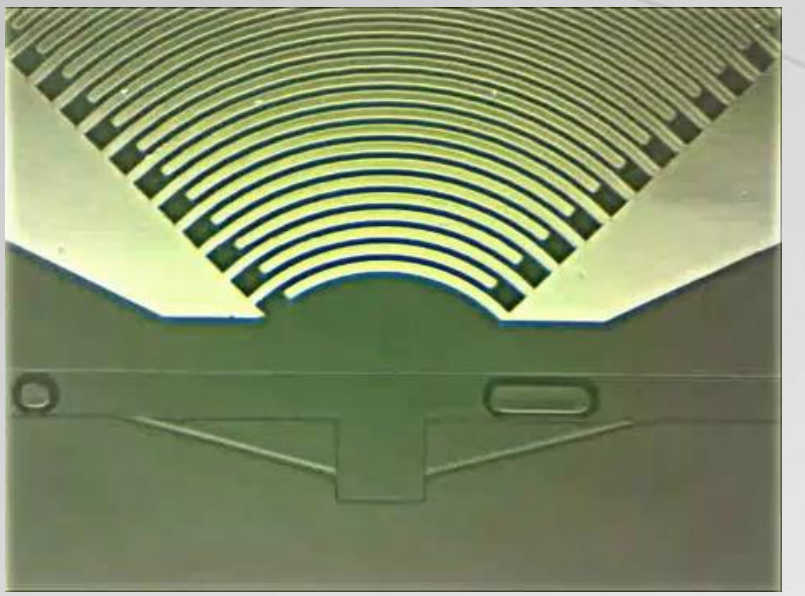
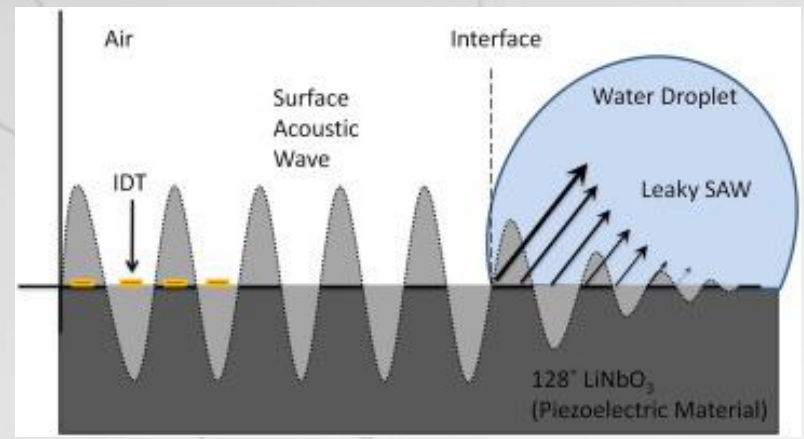
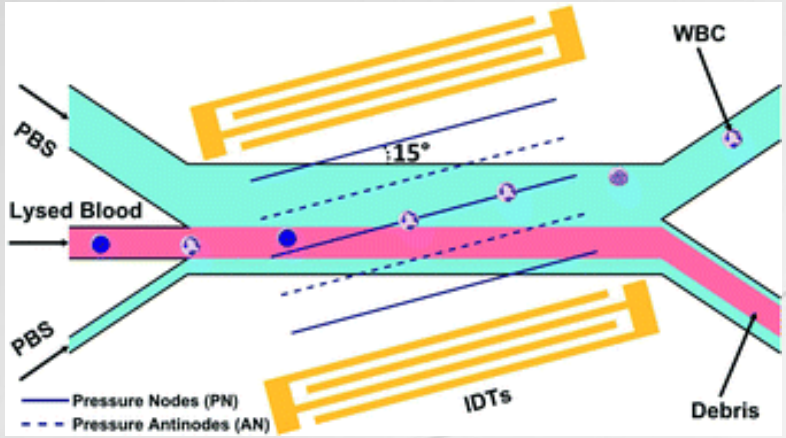
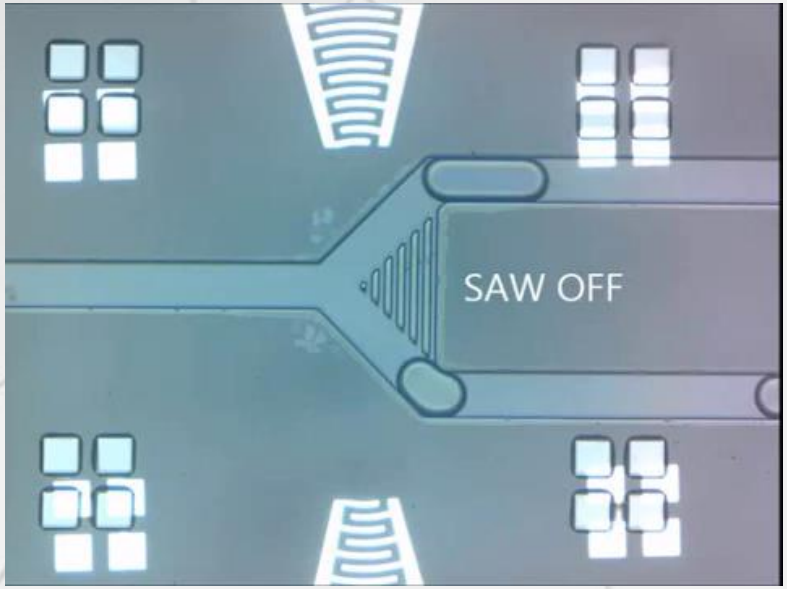
Surface Acoustic Waves - Applications









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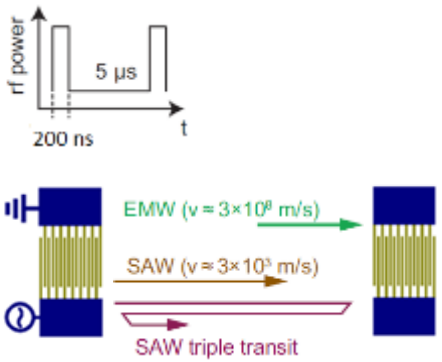
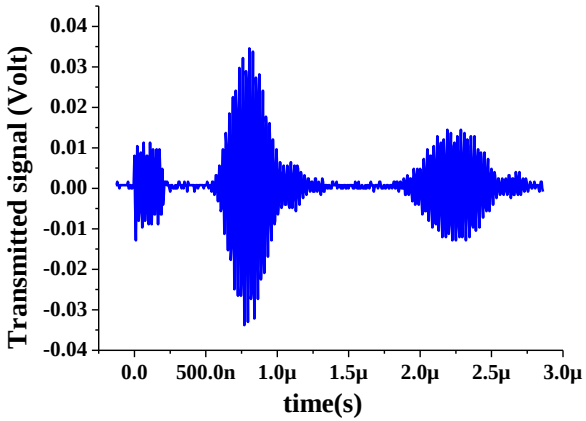
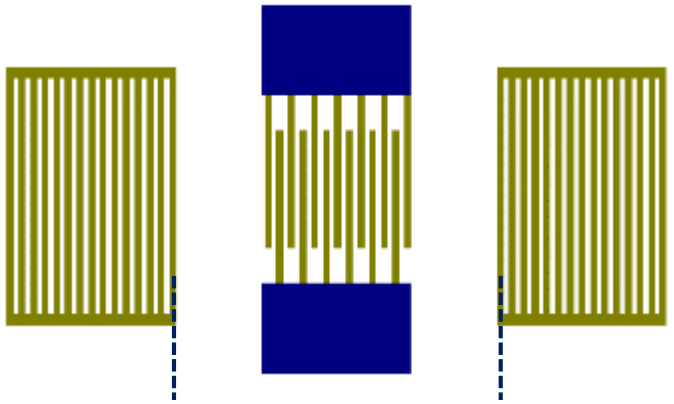
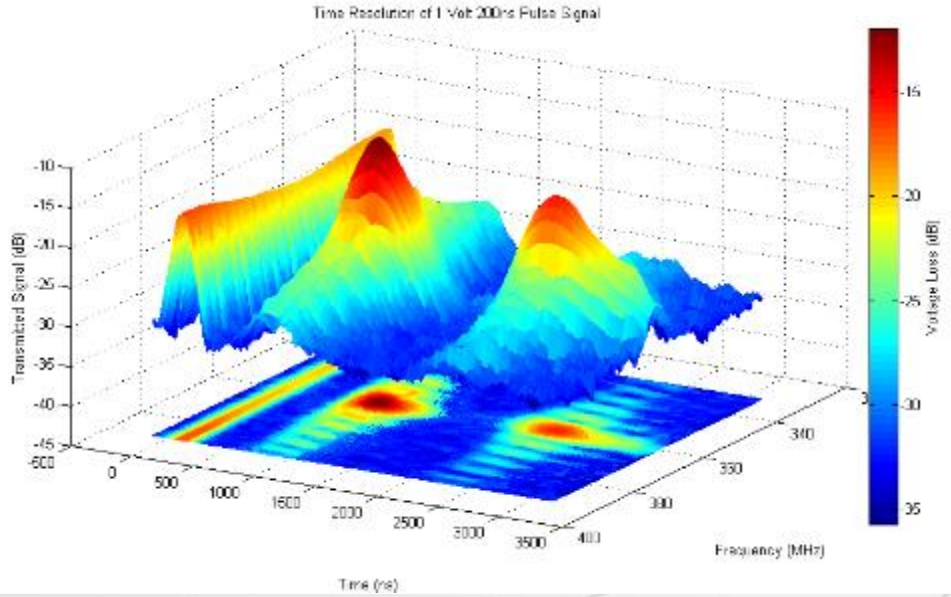
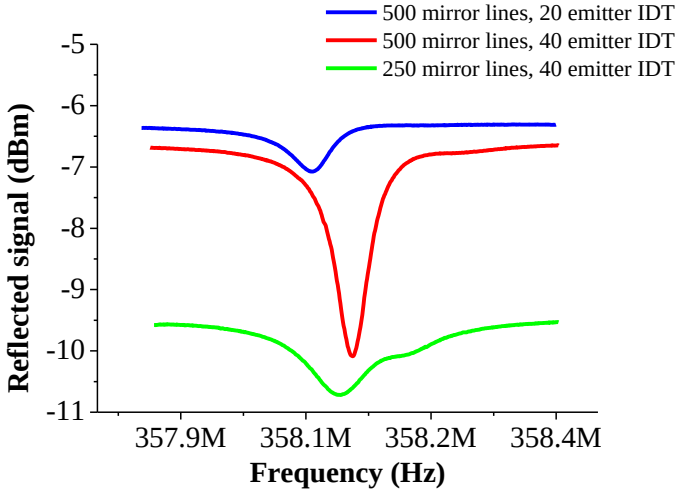
SAW devices in Lecce

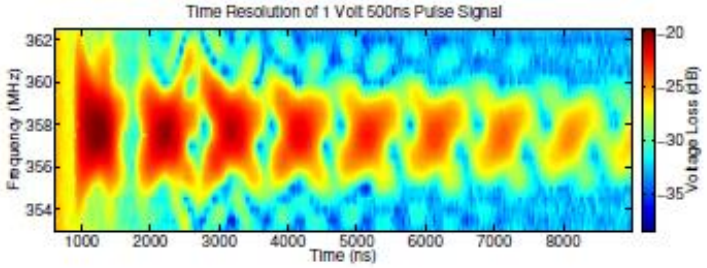












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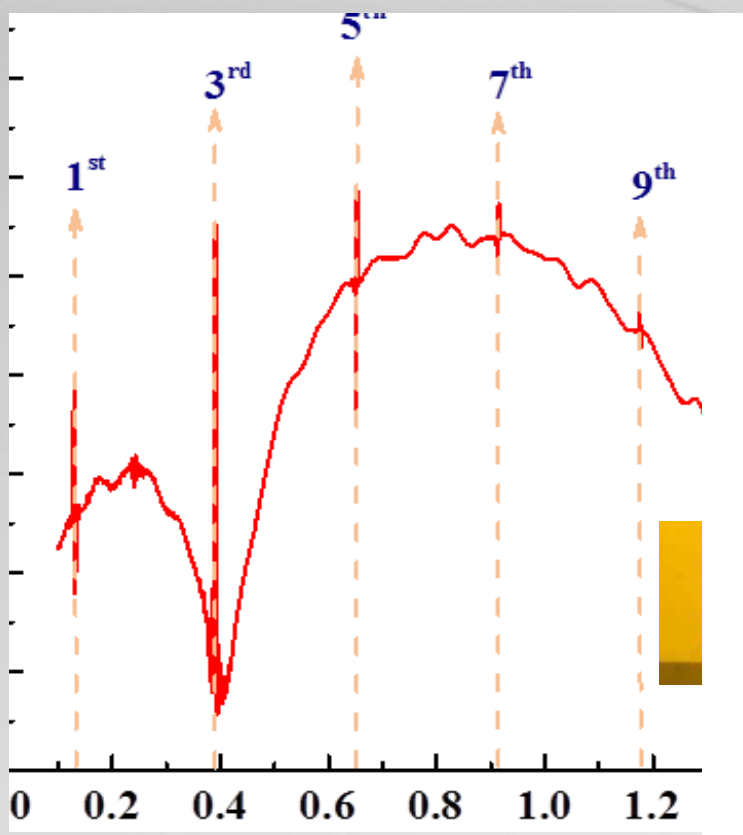
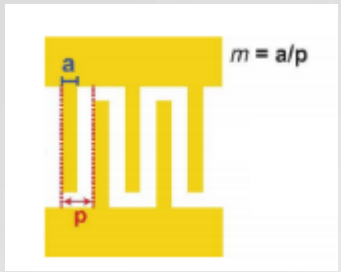
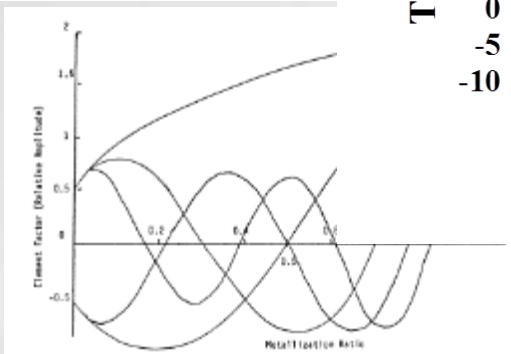
SAW Delay lines : High Order Harmonic Mode excitation

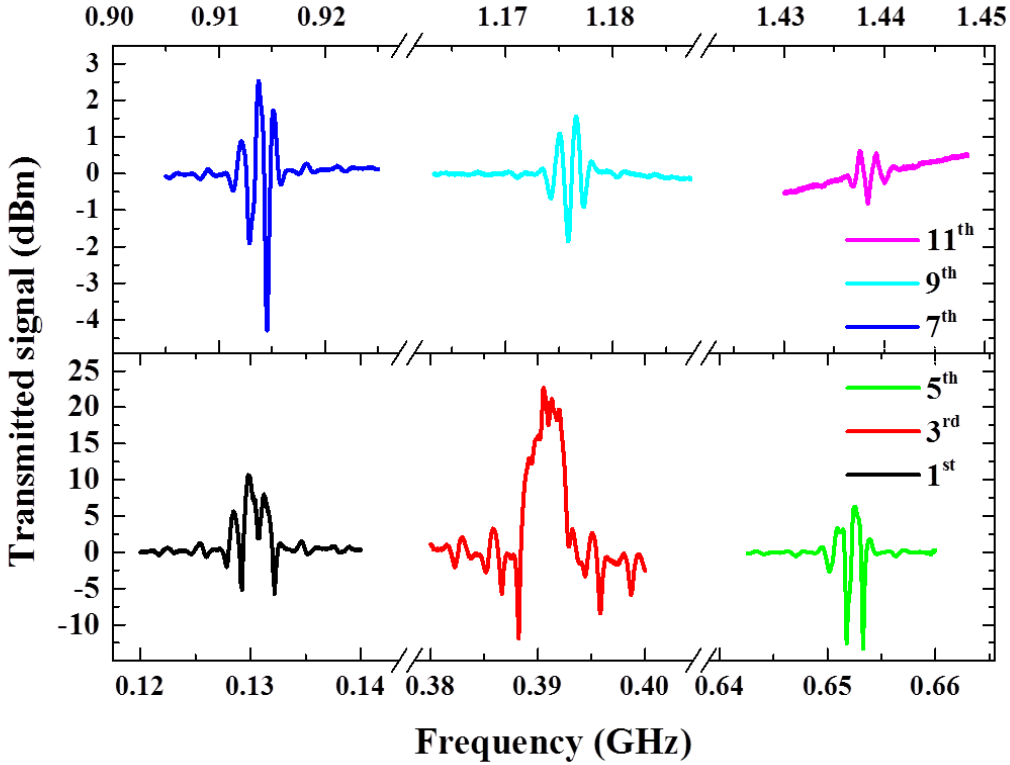






Applications

- **Sensing**



S.Rizzato at al., *J. Micromechanics and Microengineering* *27*, 125002 (2017)

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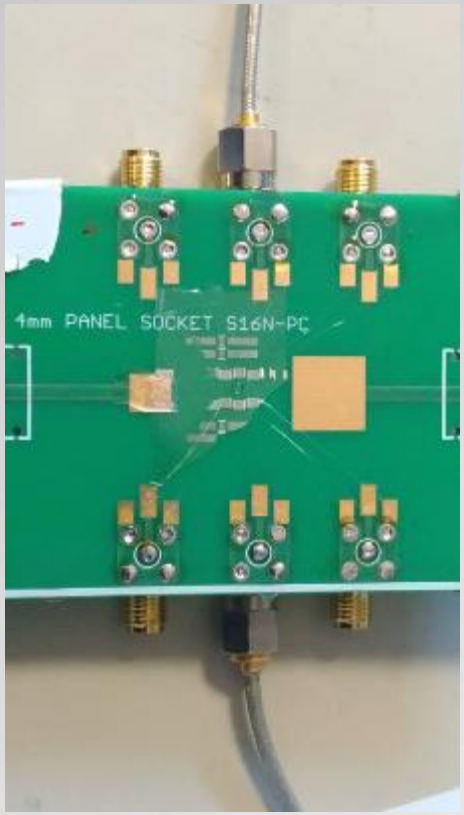
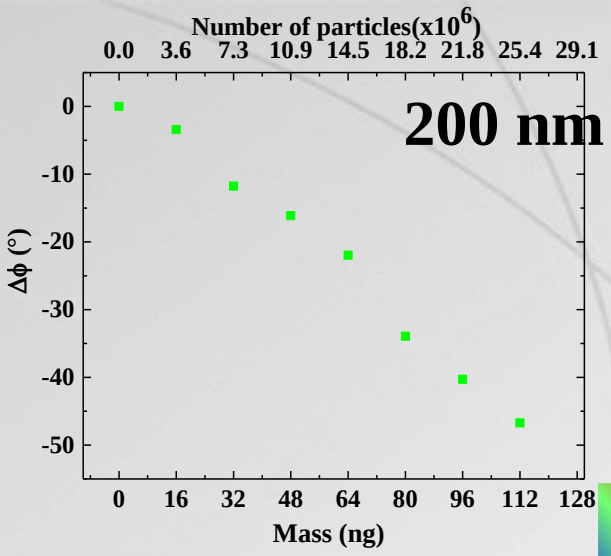
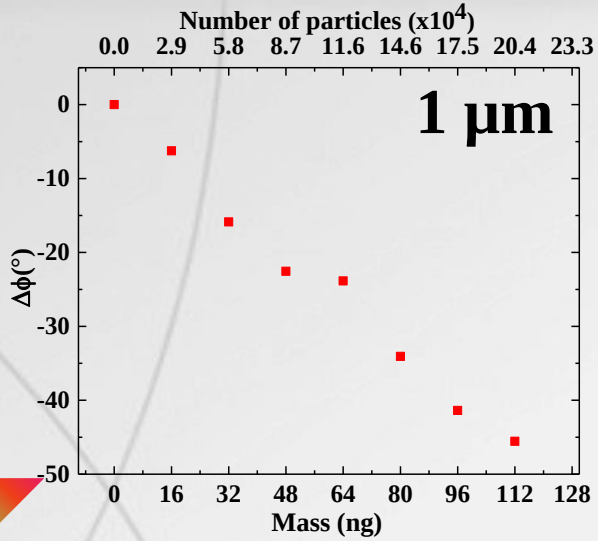
SAW-based sensor for the detection of nanoparticles

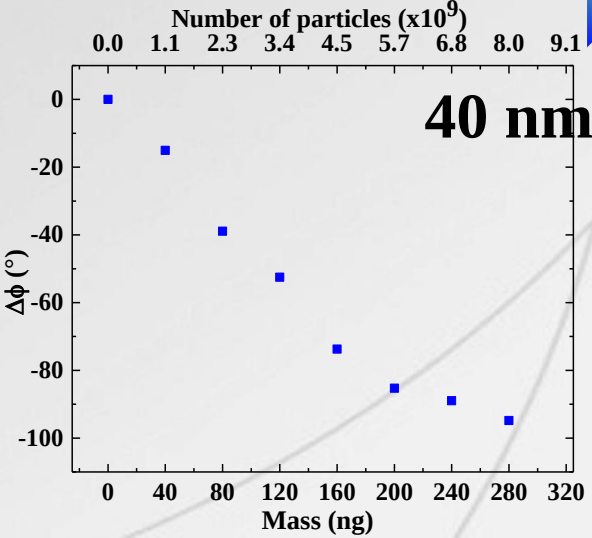
Quart

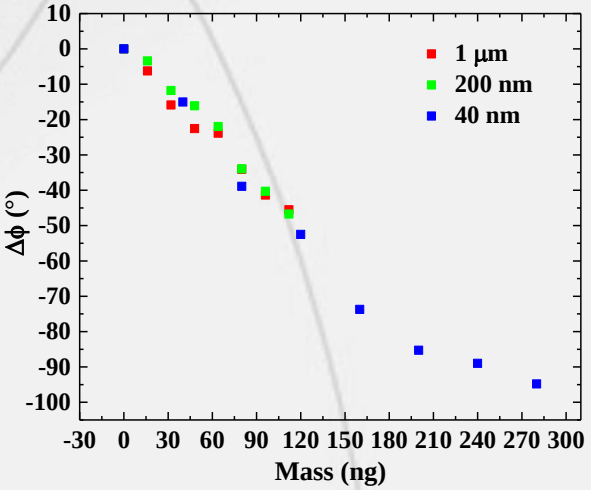
z











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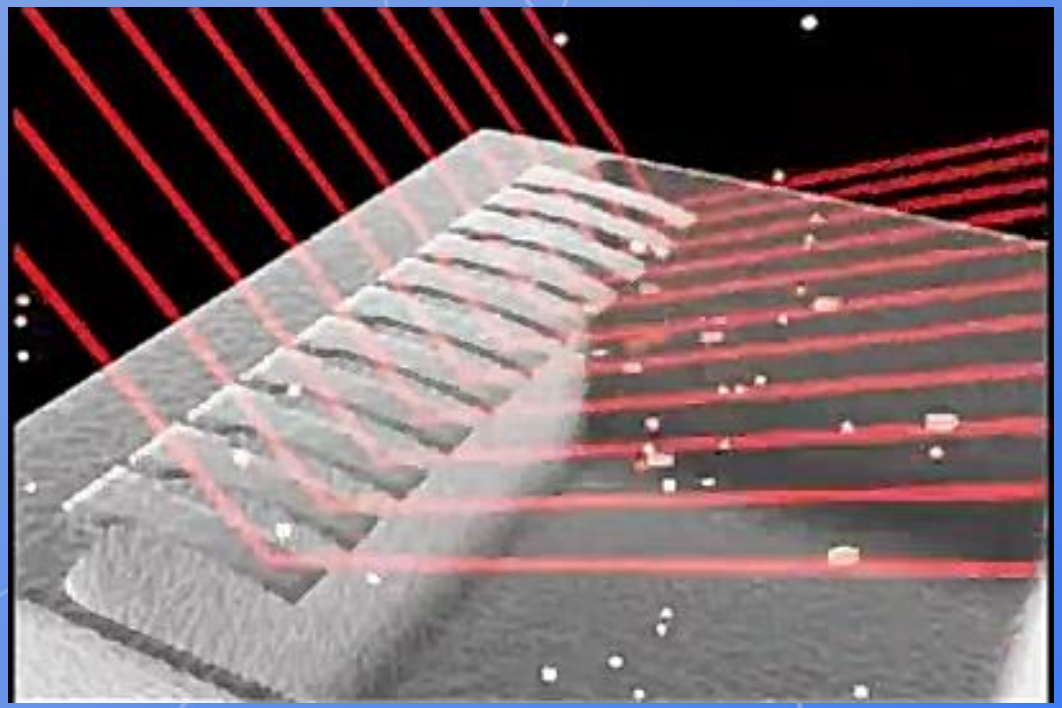
Cantilever-based sensors

Read-out schemes:

1. Static bending
2. Frequency change

Reference required in both cases



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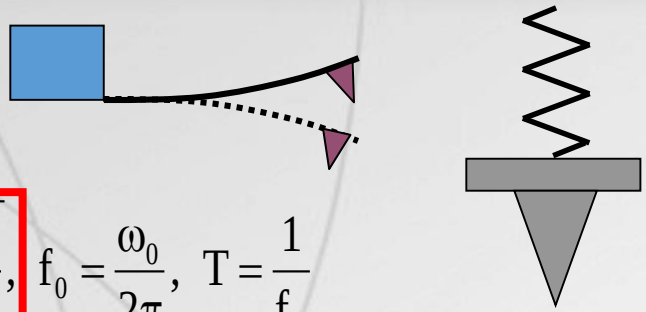
Cantilever as a spring

Harmonic motion, damped by sample interaction

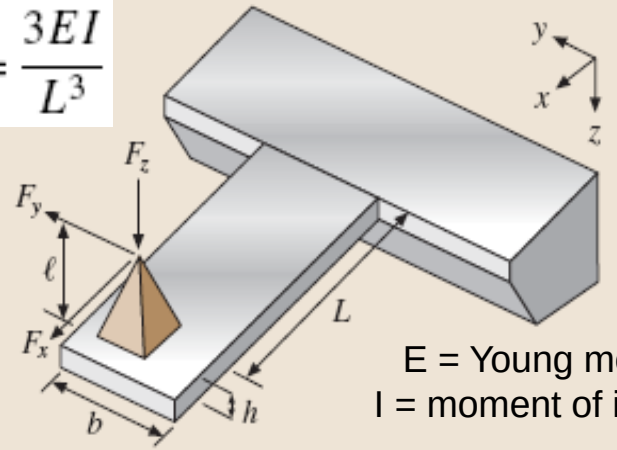
$$m\ddot{z} = -kz \Rightarrow \ddot{z} = -\frac{k}{m}z$$

test solution: $z = A\sin\omega_0 t \Rightarrow \ddot{z} = -\omega_0{}^2 z \Rightarrow \boxed{\omega_0 = \sqrt{\frac{k}{m}}}, \quad f_0 = \frac{\omega_0}{2\pi}, \quad T = \frac{1}{f_0}$



spring constant (stiffness) in normal direction

$$k = \frac{3EI}{L^3}$$



E = Young modulus
I = moment of inertia.

effective mass $m \Rightarrow m_{eff}$

resonance frequency $f_0 = \frac{1}{2\pi}\sqrt{\frac{k}{m_{eff}}}$

For rectangular cross section: $\boxed{I = \frac{bh^3}{12}}$

$$f_0 = \sqrt{\frac{Ebh^3}{4L^3 m_{eff}}} = \left(\frac{\sqrt{5}}{3}\sqrt{\frac{E}{\rho}}\right)\frac{h}{L^2}$$

Ex. ***h= 0.5 μm, w= 10 μm, l = 100 μm***
E = Young modulus: SiO_2 = 0.6 10^{11} N/m²
ρ = density : SiO_2 = 2.2 10^3 kg/m³

→ **f_0 ~ 250 kHz**
(typical in air)

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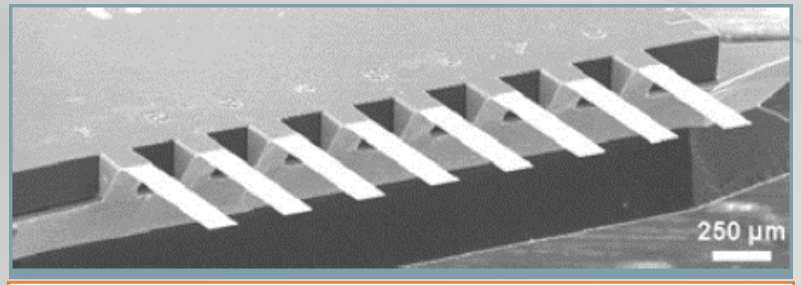
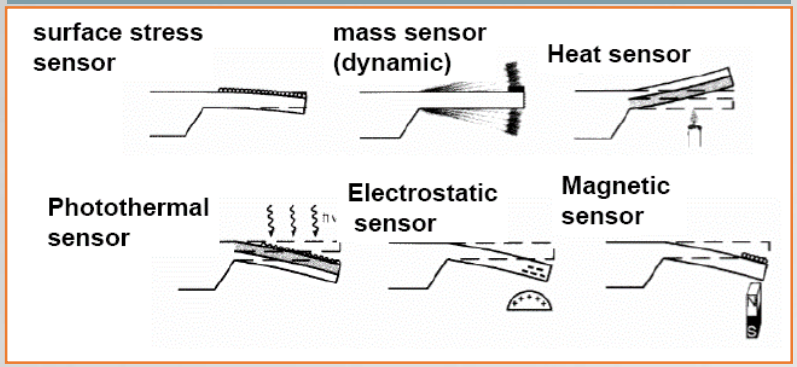
Cantilevers

produced by ***microfabrication using dry- and wet-ething***

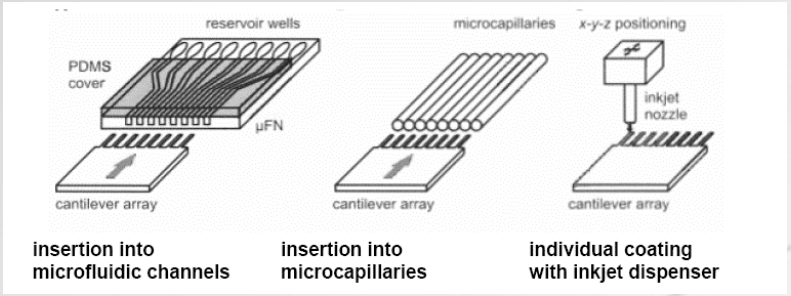


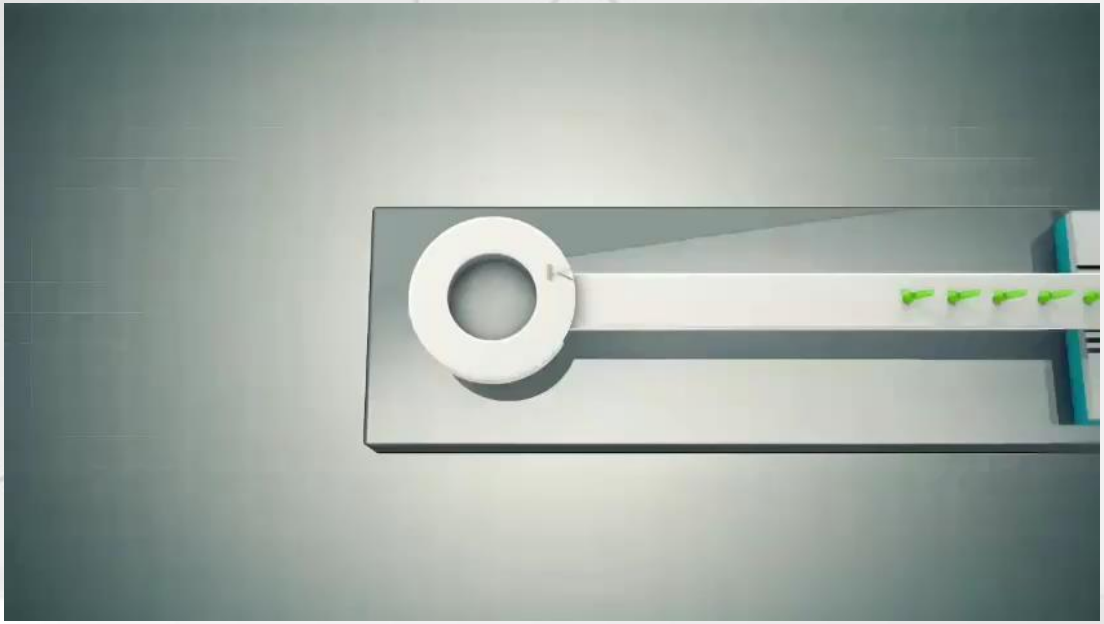


Read-out strategies

- Mechanical Displacement (Bending)
  - *induced by **surface stress***
  - *detect a single base mismatch between two 12-mer olinucleotides*
  - *analogous to the effect on the lateral tension of a lipid bilayer produced by the interaction between membrane molecules*
- Change in the resonance frequency
  - *induced typically by mass changes*
  - *analogous to QCM sensor but more easily miniaturizable and suitable for high density array (a different probe for each cantilever)*

Functionalization





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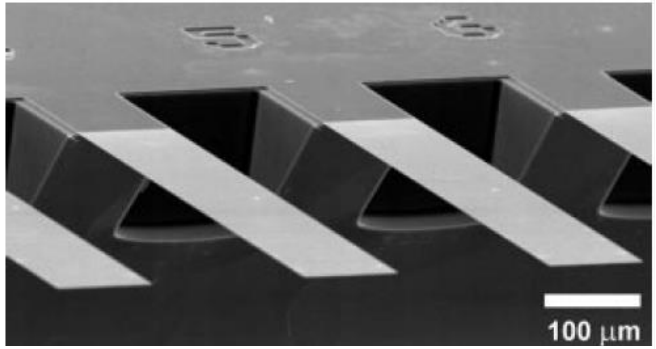
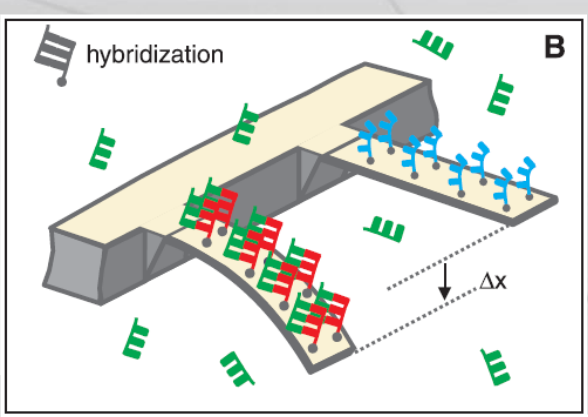
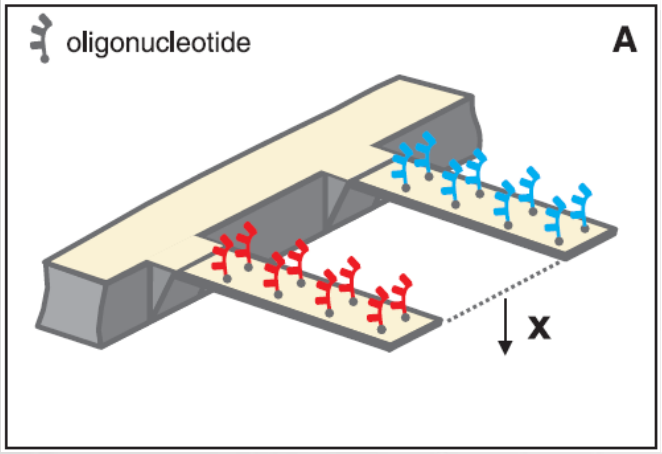
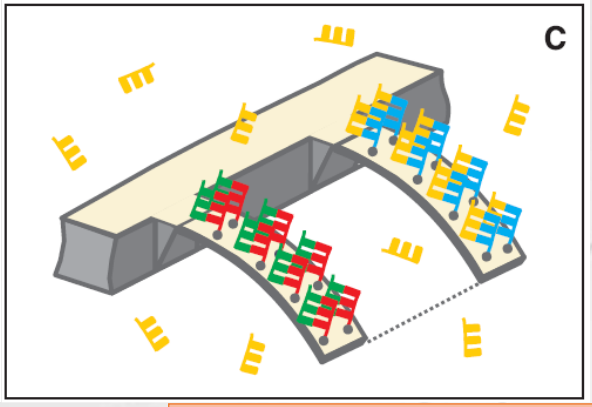
Mechanical Displacement

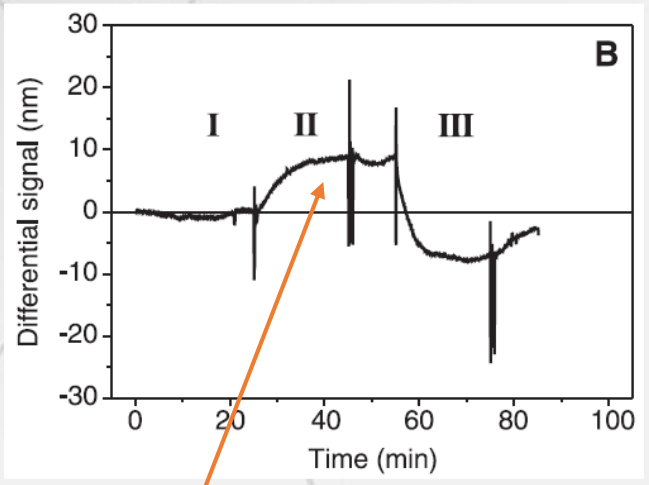








Asymmetric Surface Stress between the functionalized part and the opposite one



Differential deflection: about 10 nm for a 16-mer oligonucleotide target.

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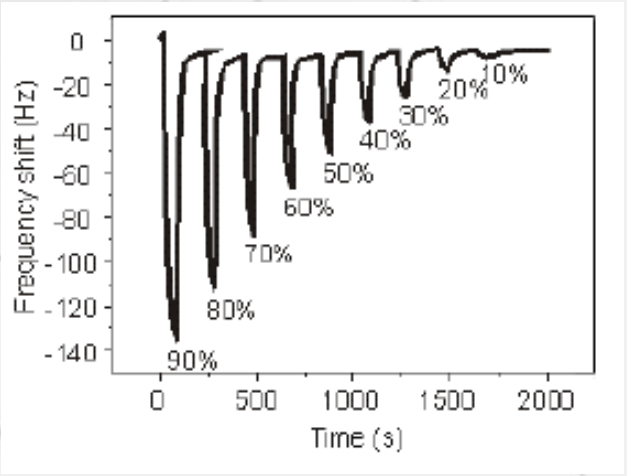
Fritz et al. Science 288, 316 (2000)

Change in resonant frequency

A cantilever array oscillated at its resonance frequency can be used as a mass detector. Molecules from the environment diffuse into the coating of the cantilever. The mass change can be determined from the shift in resonance frequency.

A typical mass change of 1 pg/Hz is observed.

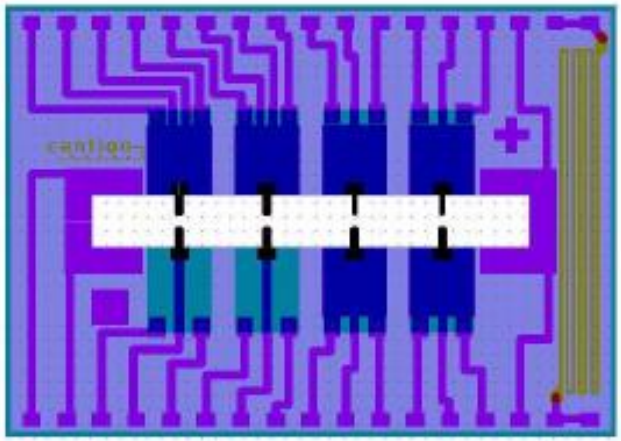
Tipical resonant frequency: few hundreds kHz.

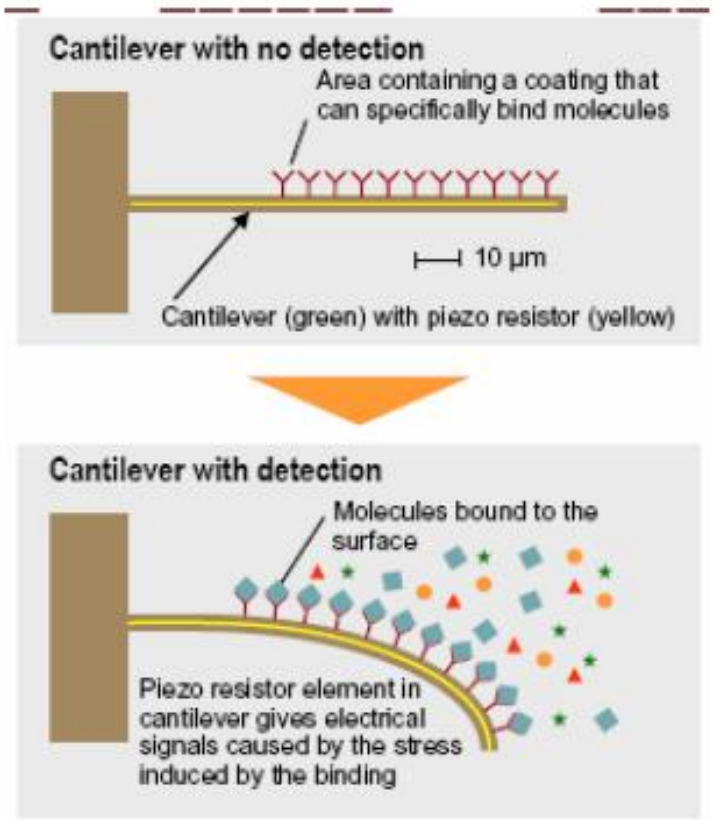


Piezoresistive cantilevers

- Canteon technology (NanoNord)

•Static bending is detected
•Piezoresistive cantilvers
•Can be used in referenced mode
•Placed in a fluidic catridge





EC sensors

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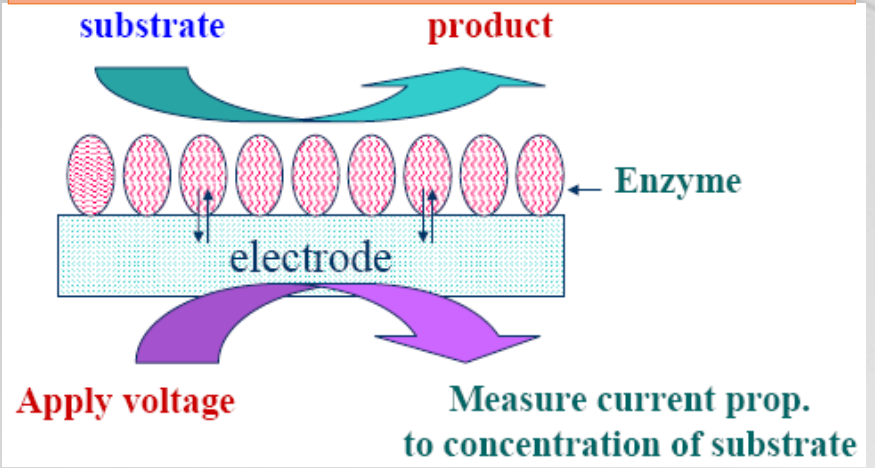
History of Biosensors

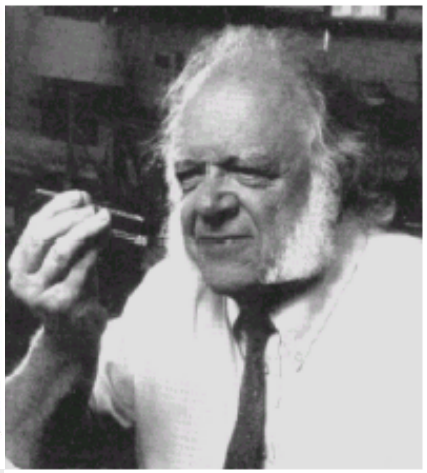
Principle of electrochemical Biosensors



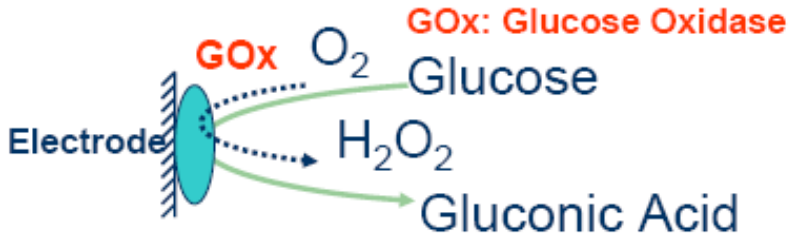
1956: Clark paper on the oxygen electrode.

1962: "how to make electrochemical sensors more intelligent" by adding "enzyme transducers as membrane enclosed sandwiches". → First biosensor where an amperometric oxygen electrode was immobilized with an enzyme (glucose oxidase) [1].

1918–2005



the blood glucose biosensor



$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 \xrightarrow[0.6\ \text{V vs. SHE}]{\text{Pt}} 2H^+ + O_2 + 2\,e^-$$

L. C. Clark and C. Lyons, *Ann. N.Y. Acad. Sci.*, *1962*, ***102, 29–45.***

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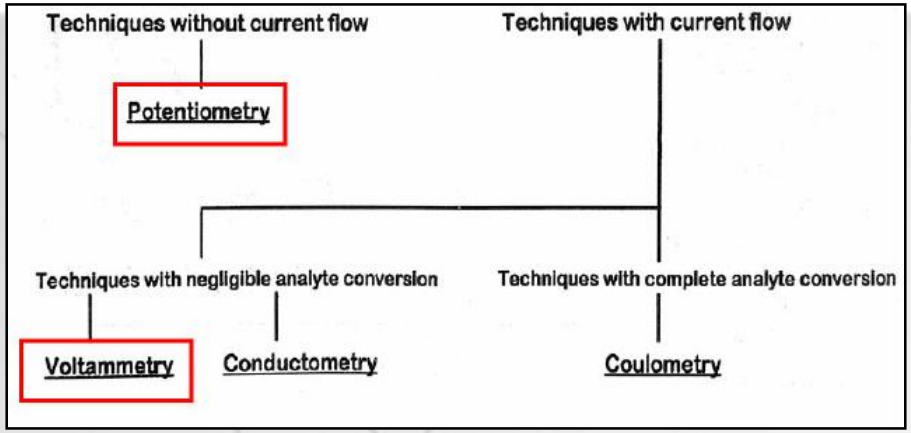
Electrochemical methods/read-out



- ***Potentiometry:*** Electrode and solution are in chemical equilibrium, current flow is near zero, and the ***the cell emf (potential) is measured*** relative to a reference electrode. The emf is proportional to the logarithm of the concentration of the substance being determined.
- ***Voltammetry (Amperometry): increasing (decreasing) potential applied to the cell*** until oxidation (reduction) of substance to be analyzed occurs and there is a sharp rise (fall) in the current. Height of peak current is directly proportional to concentration of electroactive material. If appropriate oxidation (reduction) potential is known, one may step the potential directly to that value and observe the current.
- ***Chronoamperometry:*** concerning ***monitoring of a RC electrical decay*** of the cell after an input voltage step.
- ***Conductometry: Most reactions involve a change in the*** composition of the solution. This will normally result in a change in the electrical conductivity of the solution, which can be measured electrically. All ions in solution contribute to conductance, so it is not specific.
- ***Coulorometry:*** analyte specifically and completely converted due to direct or indirect electrolysis, and the quantity of electricity (total charge in coulombs) consumed in the reaction is measured.
- ***Impedance Spectroscopy:*** involving ***complex impe***
- ***Field-Effect Device: A transistor is adapted to b***

potentiometric signals, produced by a bio-process o

Advantages of electrical output

- Fast measurements
- Sensitive: low detection limits typically ~10^{-9}M
- Electrical signal better suited for signal transmission; multitude of microelectronic circuits available for amplification, filtering, modulation, etc.; many options for information display and or recording

Electrochemical Processes

At an electrode surface, two fundamental electrochemical processes can be distinguished:

- **CAPACITIVE PROCESS.**
  - *no reaction involved but capacitive currents caused by the (dis-)charge of electrode surface as a result of changes in area size (dropping mercury electrode), by a potential variation, or by an adsorption process.*
  - ***Under potentiostatic conditions**, this process tends to be very fast → resulting current usually expires in a few milliseconds and can thus be reduced by choosing slower scan rates or pulse widths of longer duration.*
  - ***NB.** In high resistance media, capacitive current will need a substantially longer period to fall off (time constant: RC, where R = resistance and C= capacitance).*
- **FARADAIC PROCESSES.**
  - *Faradaic currents I_F as a result of electrochemical reactions at the electrode surface.*
  - *Measuring I_F → useful parameters as concentration and diffusion coefficient of a species.*
  - *Peak potential (i.e. position) → nature of the species.*
  - ***Usually under potentiostatic conditions**, faradaic currents are slower to diminish than capacitive currents. However, when reactant depletion occurs, a faradaic current will also decrease with time. The scan rate/pulse duration should therefore be chosen slow/long enough to reduce the charging current, without letting the magnitude of the faradaic current decline below noise level.*
  - ***To study electrode kinetics** another strategy must be followed. Due to limiting effect of mass transfer, influence of reaction rate, or corrosion resistance only expressed on short time scales, making necessary to employ short pulses, fast scan rates, or high frequencies. In such cases, there might be an overlap with capacitive current. Usually in these cases, one will employ a range of scan rates, pulse duration's, or frequencies allowing for a detailed analysis of the electrochemical components and their impedance's.*

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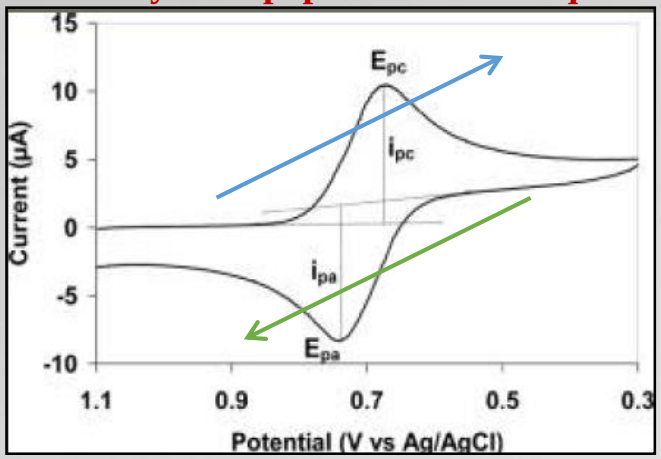
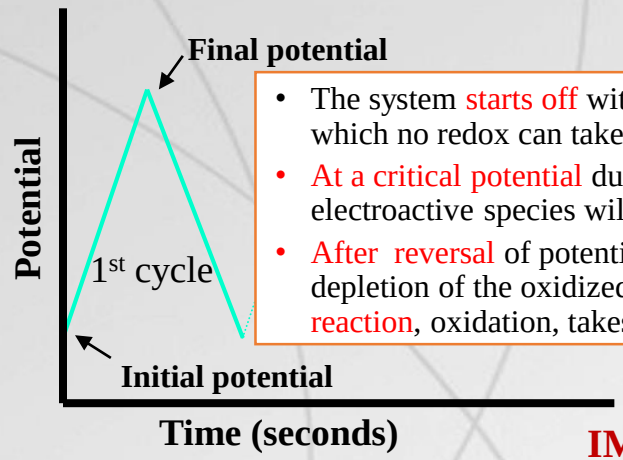
Cyclic voltammetry

- **Probably most popular EC technique** for solid electrodes. CV traces **electron transfer during a redox reaction**.





- The system starts off with an initial potential at which no redox can take place.
- At a critical potential during the forward scan, the electroactive species will begin to be reduced.
- After reversal of potential scan direction and depletion of the oxidized species the reverse reaction, oxidation, takes place.

IMPLEMENTATION

- Electrodes in cell containing **analyte** (undergoing redox reaction) and **supporting electrolyte**.
- Cell also contains **two tubes** connected to N_2 tank used to purge out oxygen-containing air from cell.
- Inclusion of **backward scan** (2 vertex potentials instead of start- and end-potential).
- Often more cycles repeatedly in sequence as part of electrode conditioning process, or to monitor EC processes with time.

ADVANTAGES

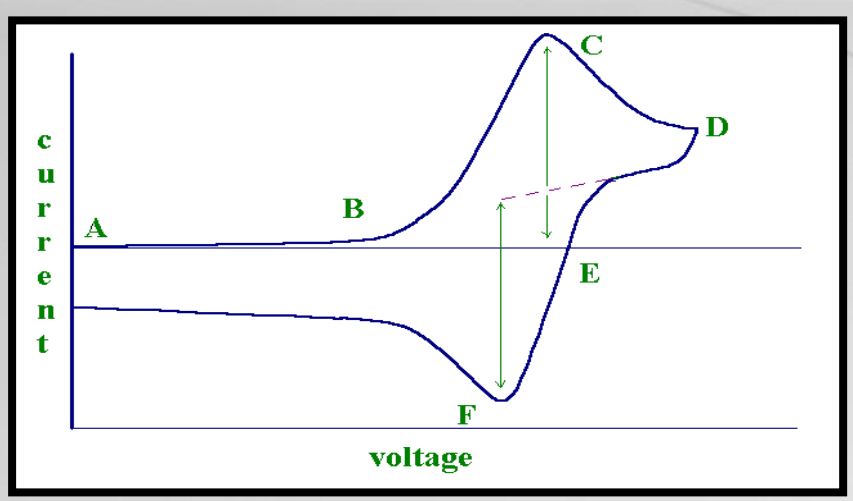
- reproducible results (invaluable for relatively badly defined electrode surfaces).
- reduction/oxidation waves observable simultaneously (helpful in the investigation of electrode processes).
- Several electrode kinetic and electrosorption processes can be studied in detail from the analysis of cyclic voltammograms recorded at various scan rates.

Term	Definition
Switching potentials	initial and final potentials of working electrode (cycled potential = V_{WE}-V_{RE}).
E_{pc} ; i_{pc}	cathodic peak potential; highest current value
E_{pa} ; i_{pa}	anodic peak potential; lowest current value

SOME APPLICATIONS:

- Probe coupled chemical reactions: particularly to determine mechanisms and rates of oxidation/reduction reactions.
- Study electrode surfaces.

Analysis of an Example $K_3Fe(CN)_6$



- Starting at initial voltage (A), then potential scanned.
- At B, potential negative enough to start cathodic current between species, reducing the analyte at working electrode.
- Reaction continues until most of the species has been reduced, peaking the cathodic current at (C).
- Current then decays until potential scan reversed (D).
- Scan in positive direction proceeds similarly: cathodic current continues to slowly decay until the potential reaches a point to start oxidation of analyte (E).
- Anodic current then measured as the concentration of the reduced species is significantly diminished (F).
- Anodic current then decays from this peak.

Case study:

Feasibility study of capacitive biosensor for direct detection of DNA hybridization

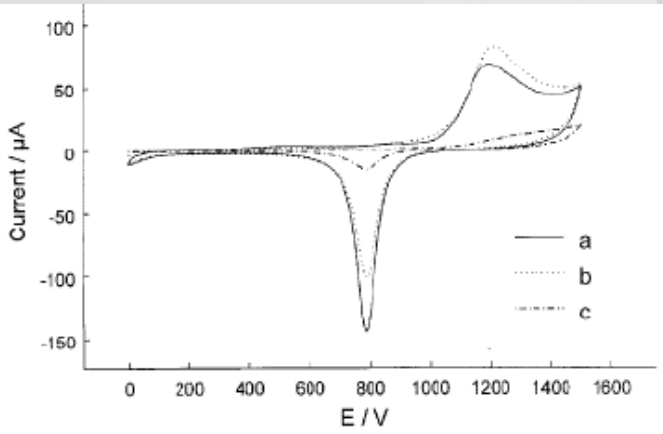


Fig. 1. Cyclic voltammograms for a) a bare gold electrode, b) a CMV SH self-assembled gold electrode and c) a 1-dodecanethiol self-assembled gold electrode. All scans were performed in 0.1 M H_2SO_4, with scan rate of 100 mV/s.

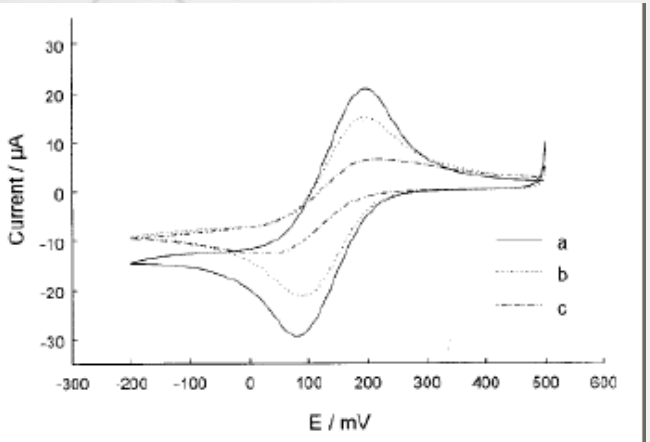


Fig. 2. Cyclic voltammogramms for a) a bare gold electrode, b) a gold electrode modified with self-assembled CMV-SH and c) the same as b) but with additional 1-dodecanethiol treatment. All scans were performed in aqueous 5 mM $K_3Fe(CN)_6$ solution, with a scan rate of 10 mV/s.

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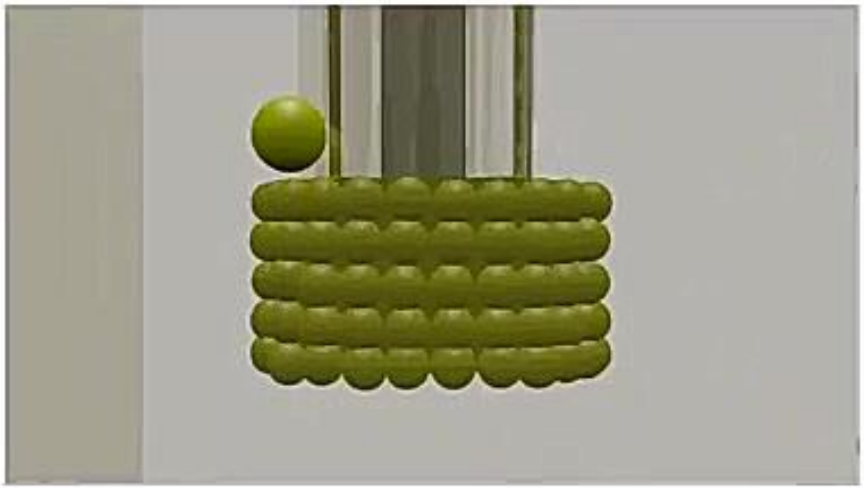
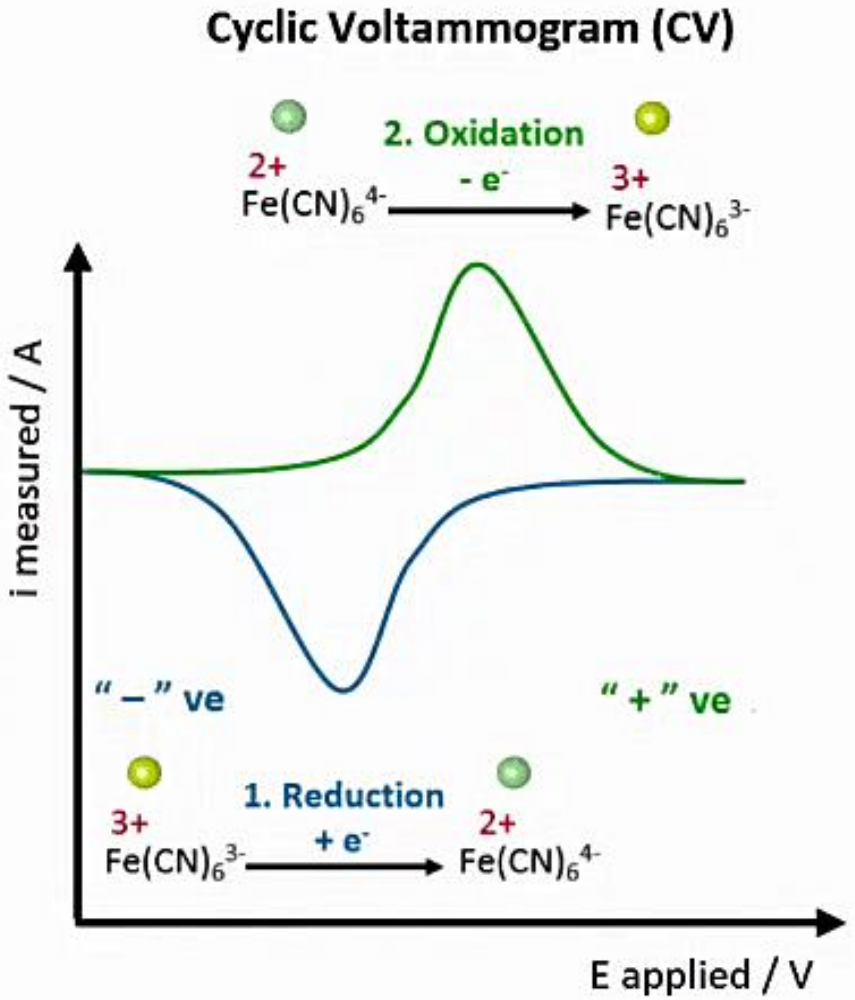
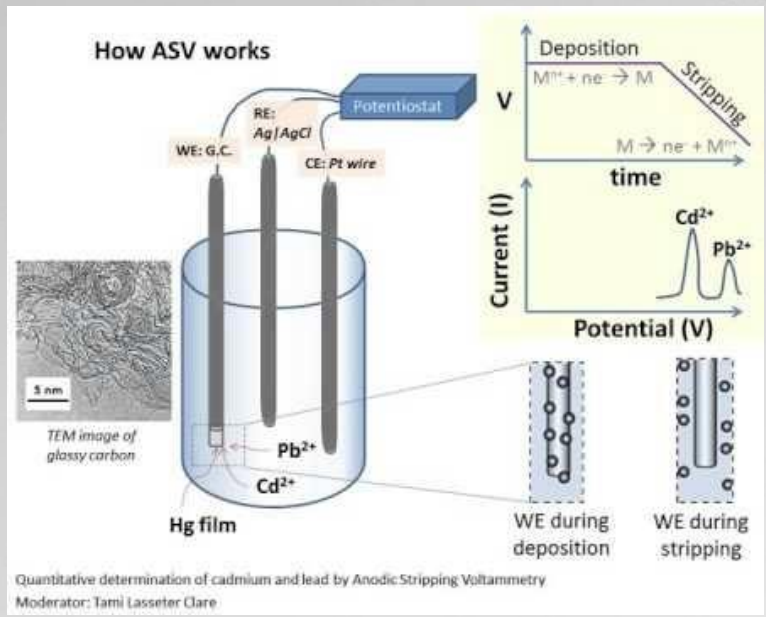
Cyclic voltammetry





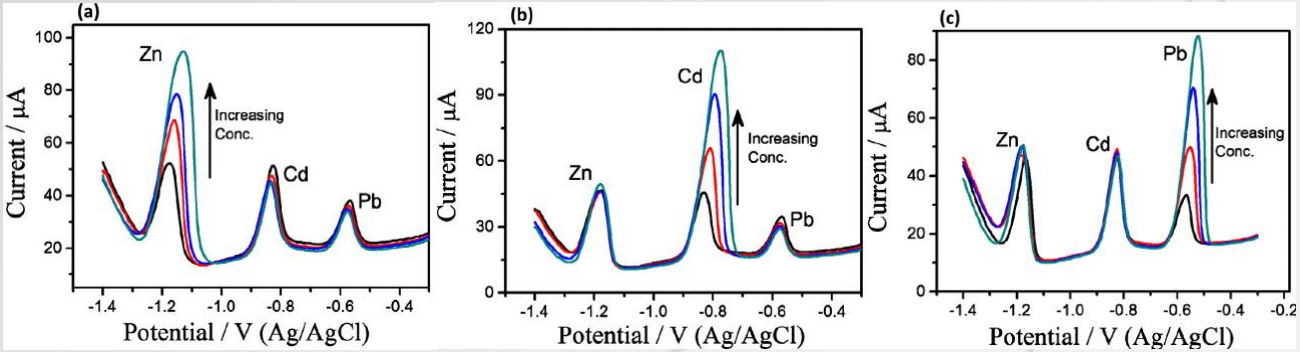
Anodic Stripping Voltammetry

for quantitative determination of specific ionic species.

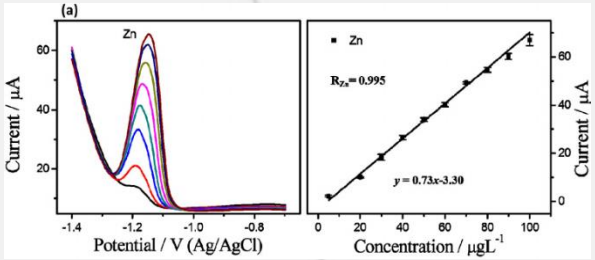


Two steps:

- ***during the deposition step:*** the analyte is electroplated on the working electrode
- ***during the stripping step***: the analyte is oxidized from the electrode.
- The ***current*** is measured during the stripping step.
- The oxidation of species is registered as a ***peak*** in the current signal ***at the potential at which*** the species begins to be oxidized.
- The stripping step can be ***either linear, staircase, squarewave, or pulse***.



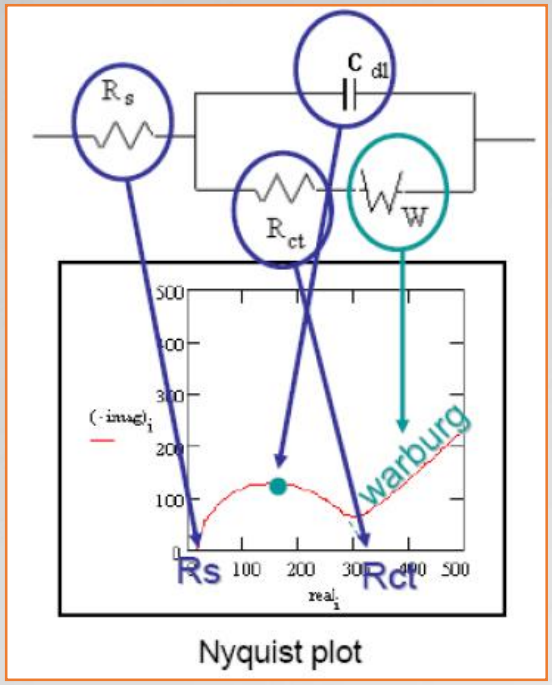
Example: DP-ASV response of the AG-NA/Bi nano composite modified GCE

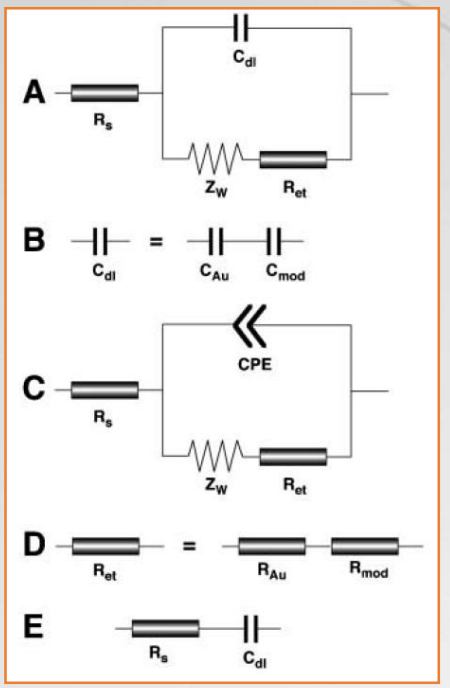


Randles equivalent circuit

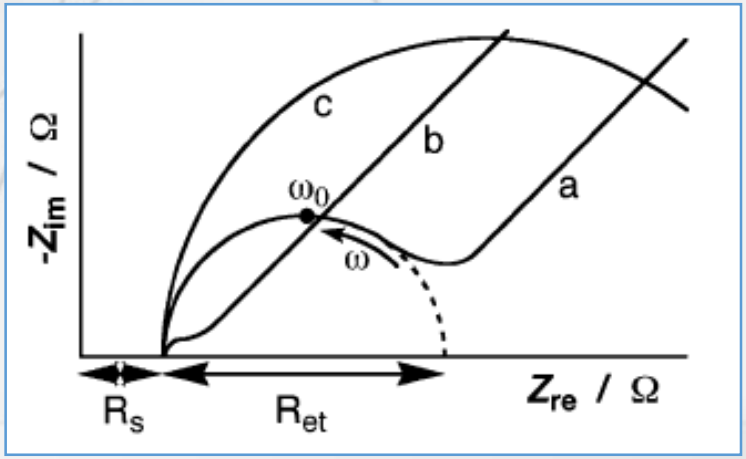
1947: Randles proposed an equivalent electric circuit for the a cell containing an electrolyte in water solution:

- Double layer capacitance C_{dl} in parallel with interface charge transfer resistance R_{ct} and Warburg impedance Z_W which is related to the diffusion process of ions towards the electrode. This combination is in series with the resistance of the solution R_s.





Scheme 1. A) General equivalent circuit for impedance spectroscopy measurements in an electrochemical cell. B) Equivalent circuit corresponding to the double-layer capacitance, C_{dl}, that includes the variable component, C_{mod}, controlled by the modifier layer. C) General equivalent circuit for the impedance spectroscopy with the constant phase element (CPE) depending on the roughness of the electrode surface. D) Equivalent circuit for the electron transfer resistance, R_{et}, that includes the variable component, R_{mod}, corresponding to the different modifier states. E) Equivalent circuit for non-Faradaic impedance spectroscopy measurements in the absence of the redox probe.



Scheme 2. Schematic Faradaic impedance spectra for a modified electrode where :

a) Z is controlled by redox probe diffusion (low frequencies) and interfacial electron transfer (high frequencies).
b) Z is mainly controlled by redox probe diffusion.
c) Z is controlled by interfacial electron transfer within the entire frequency range.

Arrow shows direction of frequency increase.

Resistance of the electrolyte solution, R_s, and electron transfer resistance, R_{et}, are shown.

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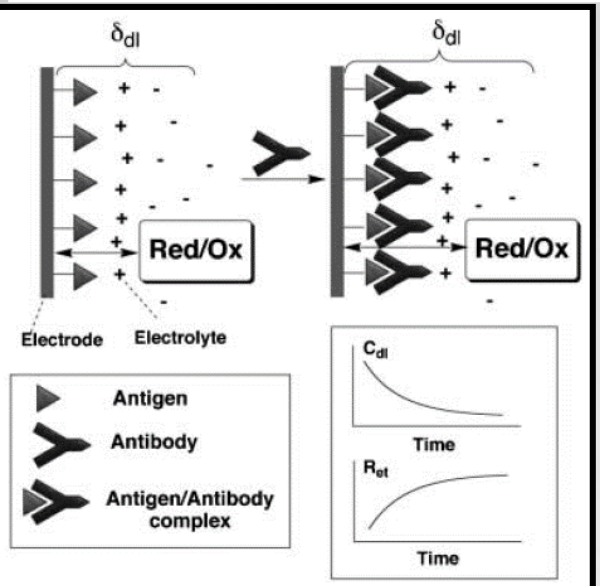
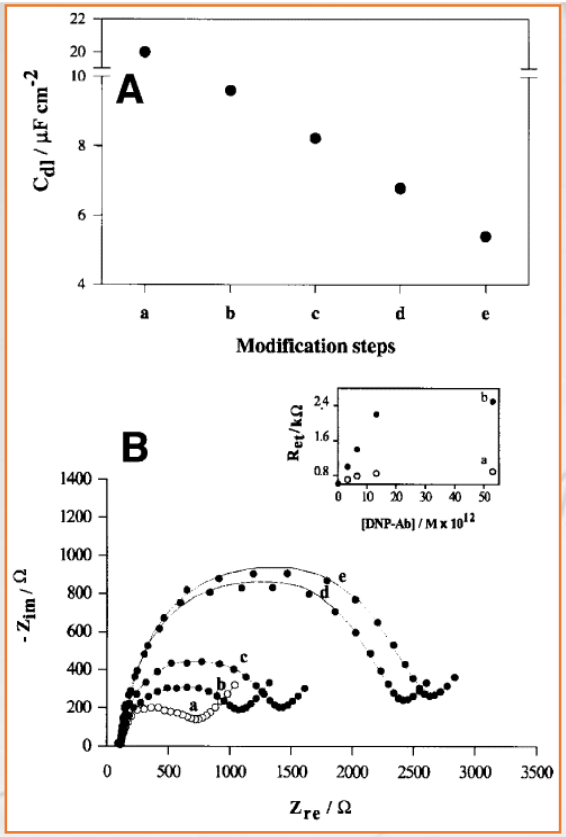
EIS Immunosensors

Katz and Willner, Electroanalysis, 15, 913 (2003)

- impedance spectroscopy (EIS), including non-Faradaic impedance measurements resulting in capacitance sensing, is an attractive EC tool to characterize biomaterial films associated with electronic elements, thus,allowing transduction of biorecognition events at respective surfaces.
- different kinds of EIS, such as non-Faradaic capacitance measurements, Faradaic impedance spectroscopy in presence of an external redox label, in-plane alternative voltage conductivity measurements, and transconductance measurements with field-effect transistors.



Interfacial impedance immunosensing: A bioaffinity interaction between an antibody and an antigen-functionalized electrode increases double-charged layer thickness, dl, and inhibits interfacial EC process of redox probe. Electrode capacitance, C_{dl}, and Electron transfer resistance, R_{et}, are changed respectively.



Non-Faradaic and Faradaic impedimetric sensing of DNP-Ab :

(A) Double-layer capacitance, C_{dl} for:

a) bare Au electrode.
b) DNP-antigen monolayer electrode.
c) DNP-antigen/DNP-Ab layered assembly.
d) DNP-antigen/DNP-Ab/anti-DNP-Ab-HRP layered assembly.
e) After biocatalyzed precipitation of insoluble product 7 on electrode.

(B) Faradaic impedance measurements: DNP-antigen monolayer electrodes treated with

- different concentrations of DNP-Ab for 5 min;
- then anti-DNP-Ab-HRP conjugate for 5 min.
- then allowed to stimulate biocatalyzed precipitation of insoluble product (7). Impedance spectra corresponding to
  - (a) DNP-antigen-functionalized electrode and to primary interaction of DNP-antigen monolayer with DNP-Ab at :
  - (b) 0.5 ng mL^{-1};
  - (c) 1 ng mL^{-1};
  - (d) 2 ng mL^{-1};
  - (e) 8 ng mL^{-1}.

Inset: Calibration plots: R_{et} at DNP-antigen monolayer-functionalized electrodes upon the analysis of different concentrations of the DNP-Ab: a) Ret observed by the direct interaction of the DNP-Ab with the sensing interface, b) Ret observed by the amplification of the initial binding of the DNP-Ab with the biocatalyzed precipitation of 7. $[Fe(CN)6]^{3-/4-}$, $1x10^{-2}$ M was employed as redox probe.

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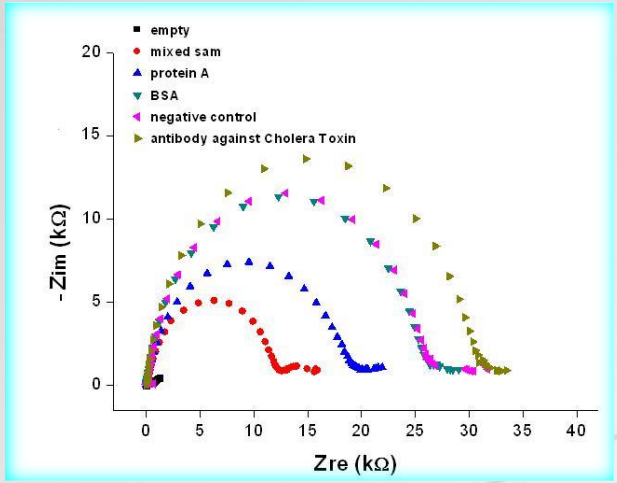
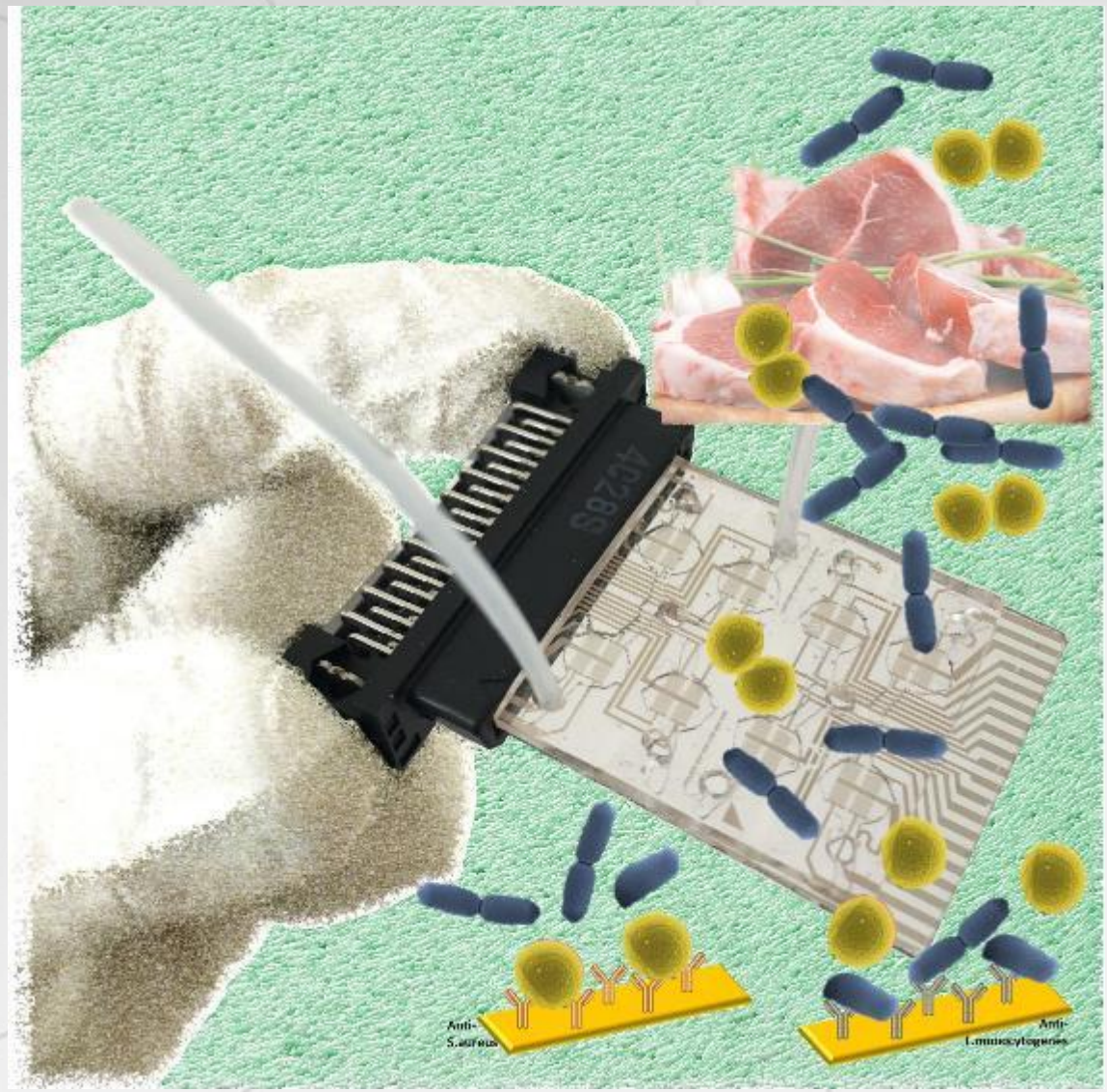
EIS Biosensors & biochips

Microfluidic module with four separate sensing areas,

- **20 µL chambers** with inlet and outlet microchannels for fluid handling
- each one containing an **array of 4 gold IDEs** (with 10 µm spacing and width)





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Chiriacò et al., Lab on a chip, 11, 658, (2011)

XMR sensors

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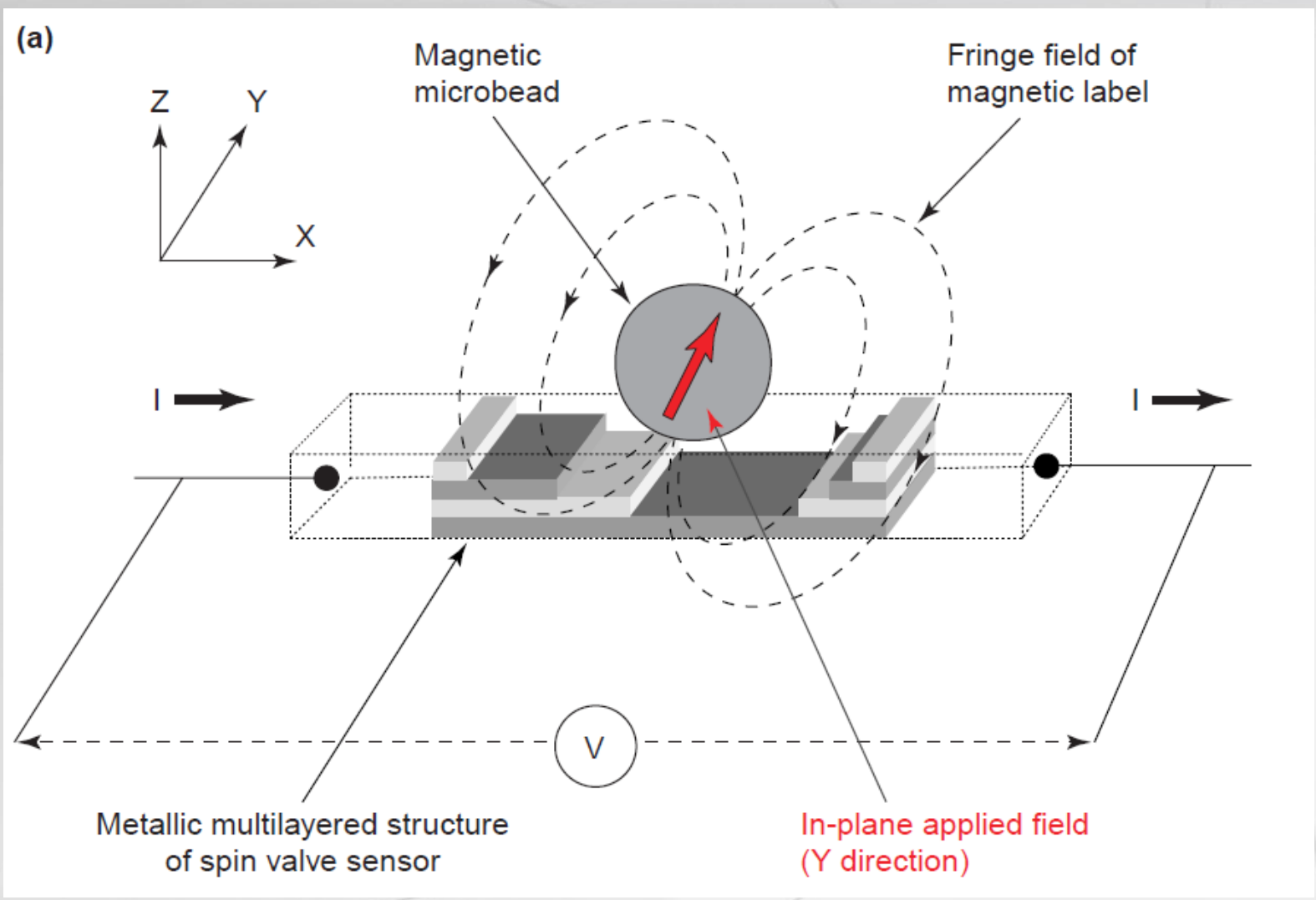
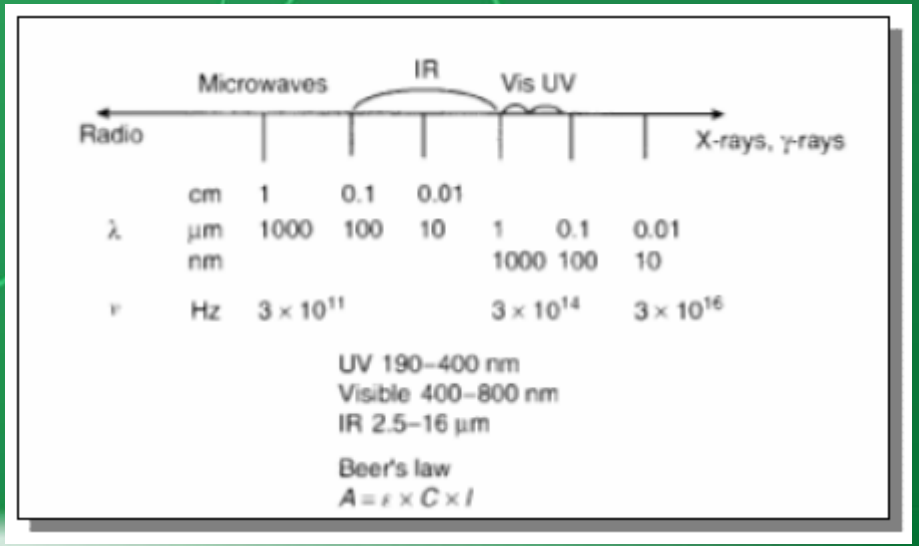
XMR-biosensor



Optical sensors

What they can be based on:

- Absorption spectroscopy (UV-VIS, IR)
- Fluorescence/phosphorescence spectroscopy
- Bio- and chemiluminescence
- Refractive index sensing
- Laser light scattering



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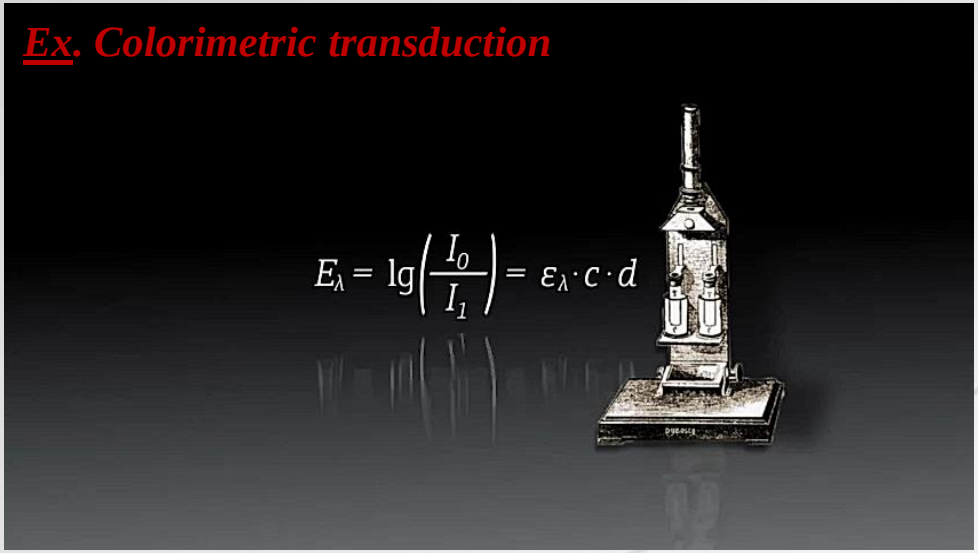
Optical, Optoelectronic Transducers

CONCEPT: Capture analyte and detect binding by ***optical tag or binding-sensitive optical phenomenon***
TECHNIQUES: absorption spectrophotometry, fluorimetry including fluorescence quenching, and reflectometry.
- Detection surface can either be planar (identification based on x-y location of tag; ex. gene chips) or composed of capture particles (faster kinetics of binding due to reduced distances to be traveled by analyte; identification based on particle-specific labeling (challenge))
- In most systems a **permselective membrane coating** allows the detected species to penetrate the dye region.

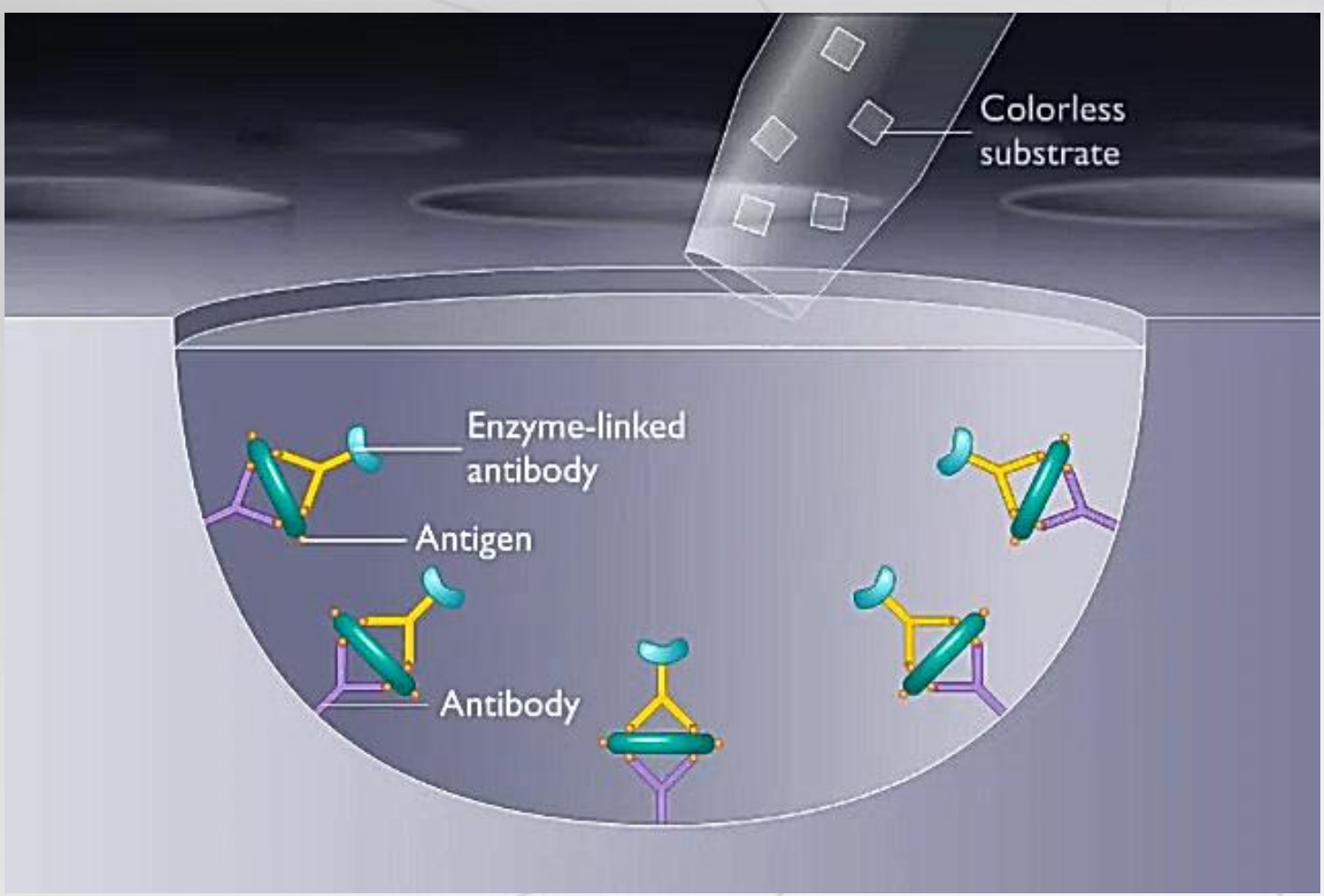
Advantages/disadvantages
- Pros
  - Fast measurements
  - Sensitive
- Cons
  - Cannot perform detection on turbid solutions

Ex. Colorimetric transduction



READ-OUT:
- Macroscopic ***fluorescence***, ***diffraction*** or ***interference***
- ***Optical bar-coding*** (ex. QDs loaded capture agent with size-dependent luminescence)
- Optical absorption (***colorimetric***)
- ***Surface plasmon resonance*** and SPR arrays
- ***Optical fiber-based*** (most widely published optical sensors use a miniature reagent contained/immobilized at the tip of an optical fiber)
- ***Ellipsometry*** is a reflectance technique that depends on the optical constants and thickness of surface layer. For colorless layers, a **polarized light beam** will change its plane of polarization upon reflection by the surface film. The thickness can sometimes be determined when optical constants are known or approximated by constants of the bulk material. **Antibody-antigen surface reaction** can be detected this way.

ELISA - Enzyme Linked Immunosorbent Assay



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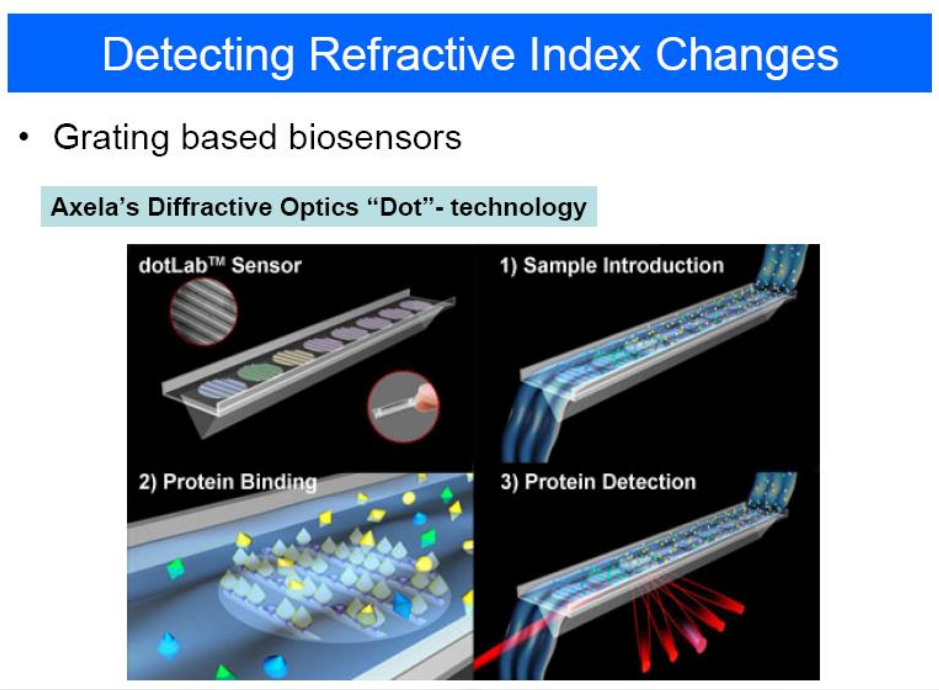
Refractive index based

• **MEAN**: Binding of molecules in solution to surface-immobilized receptors alters ***refractive index of medium near the surface***.

• **OUTPUT**: Δn monitored ***in real time to measure accurately the*** amount of bound analyte, its affinity for the receptor and ***association/dissociation kinetics of the*** interaction

• **ANALYTE**: from low-molecular-mass drugs to multiprotein complexes and bacteriophage

• **DETECTION LIMITS**: interaction affinities from mM to pM in strength



APPROACHES

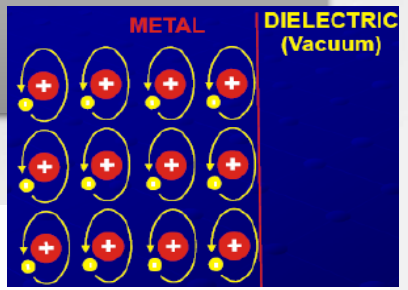
1. Surface Plasmon Resonance (based on Total Internal Reflection)
2. Interferometric devices
3. Photonic Crystal structures

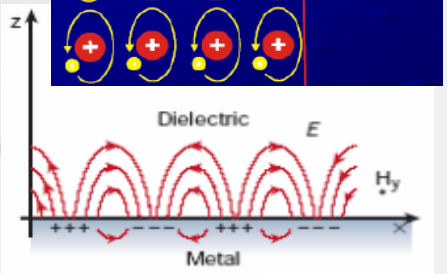
Surface Plasmon Resonance (SPR)

– Most sensitive technique: $\Delta n < 10^{-7}$.
– Detect changes in a thin layer adjacent to the sensor surface

What is surface plasmon?

Light adsorbed from evanescent wave by free electron of a thin metal layer.
→ Electron start to move in a collective oscillation
→ PLASMON: collective excitation of electrons at metal/dielectric interface.

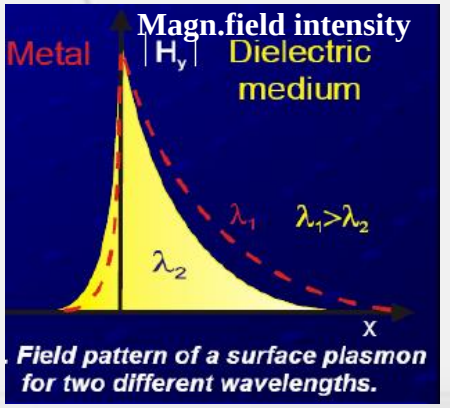




RESULT: induction of photon-plasmon electromagnetic waves (SPW = surface plasmon wave or surface plasmon-polariton) with the following properties:

- experience higher (and nonlinear) refractive index, cannot be directly coupled to free radiation
- Transverse magnetic (TM) in character: electric field perpendicular and magnetic field parallel to the interface
- localized at the metal-dielectric interface, evanescent in perpendicular direction
- propagate along the interface with a **propagation constant** $\beta = \frac{\omega}{c}\sqrt{\frac{\varepsilon_M \varepsilon_D}{\varepsilon_M + \varepsilon_D}}$

with ω angular frequency and ε_D and ε_M dielectric functions of the dielectric and metal. The real and imaginary parts of the propagation constant describe spatial periodicity and attenuation of an SPW in the direction of propagation, respectively.



Field pattern of a surface plasmon for two different wavelengths.

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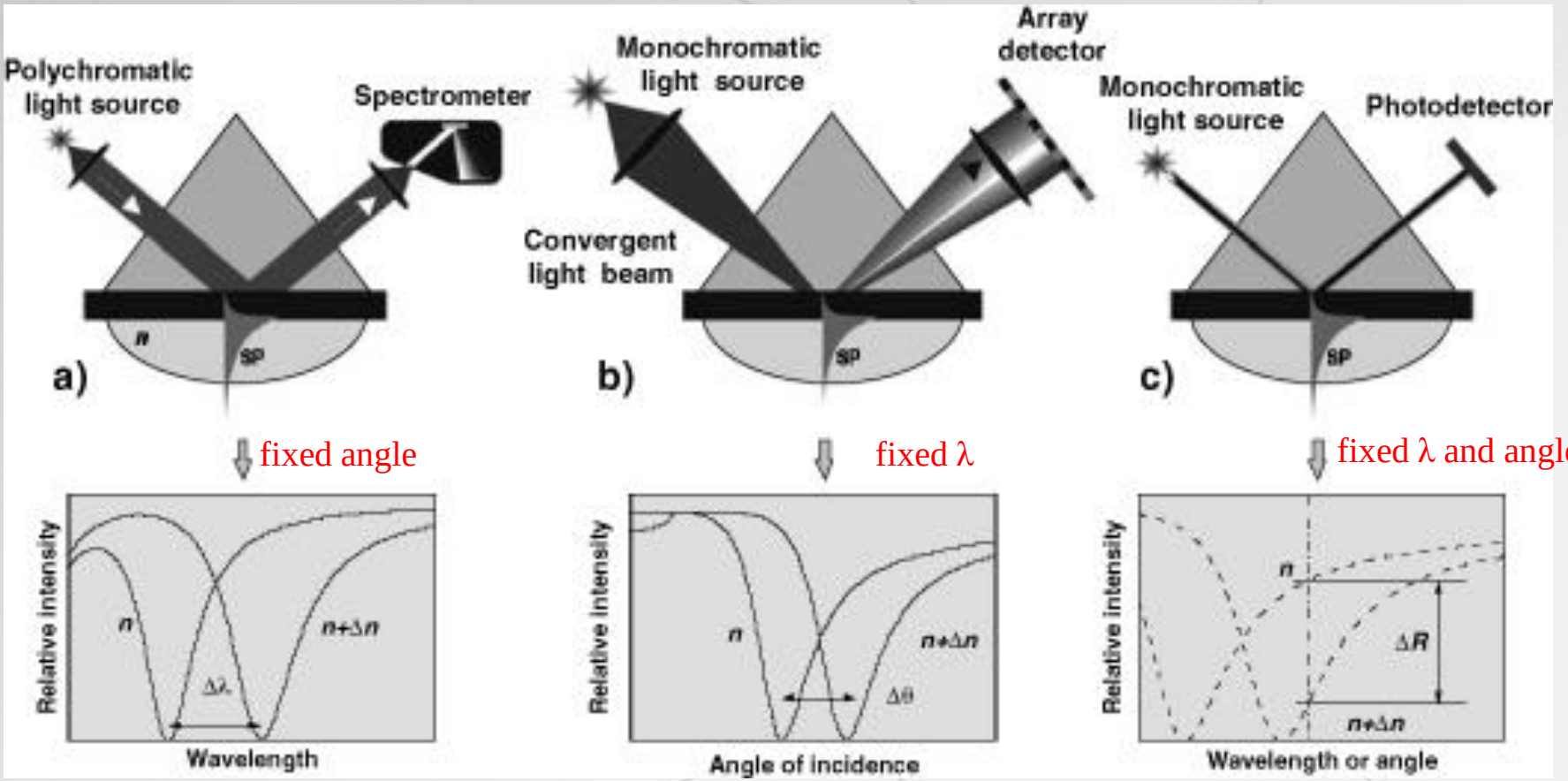
Optical Read-out / Modulation schemes

WAVELENGTH MODULATION **ANGULAR MODULATION** **INTENSITY MODULATION**

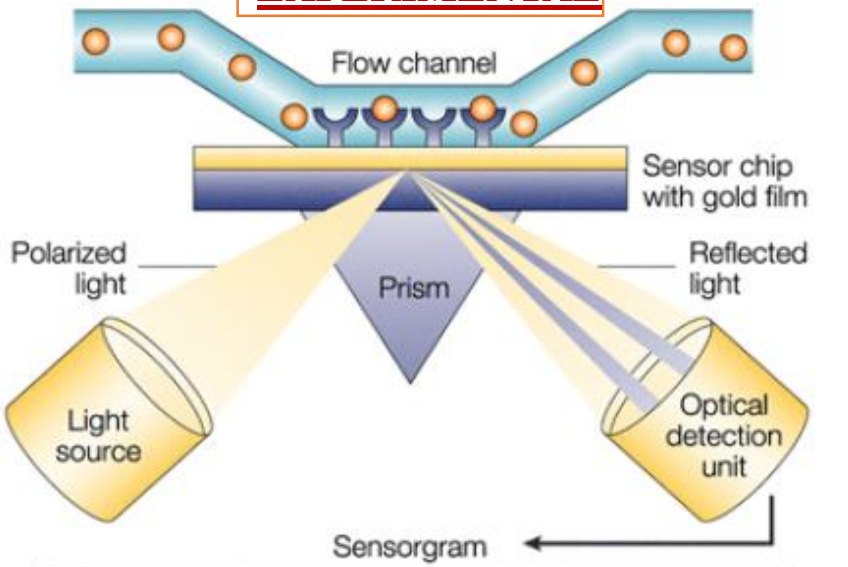


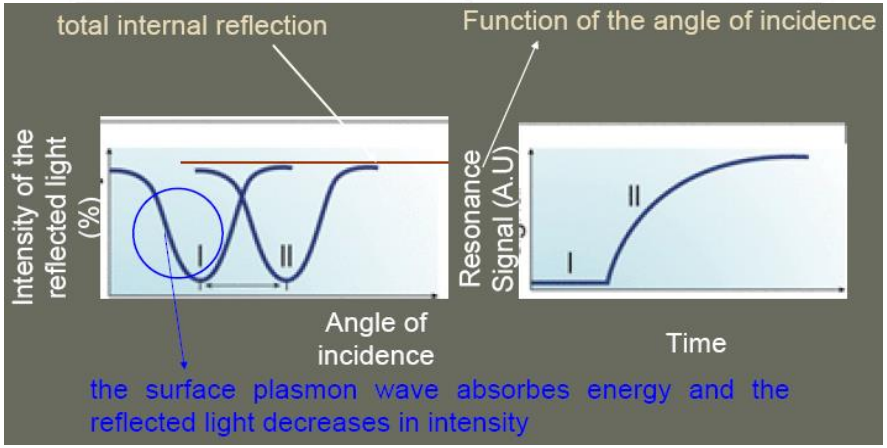
PHASE MODULATION

POLARIZATION MODULATION

Data processing for SPR

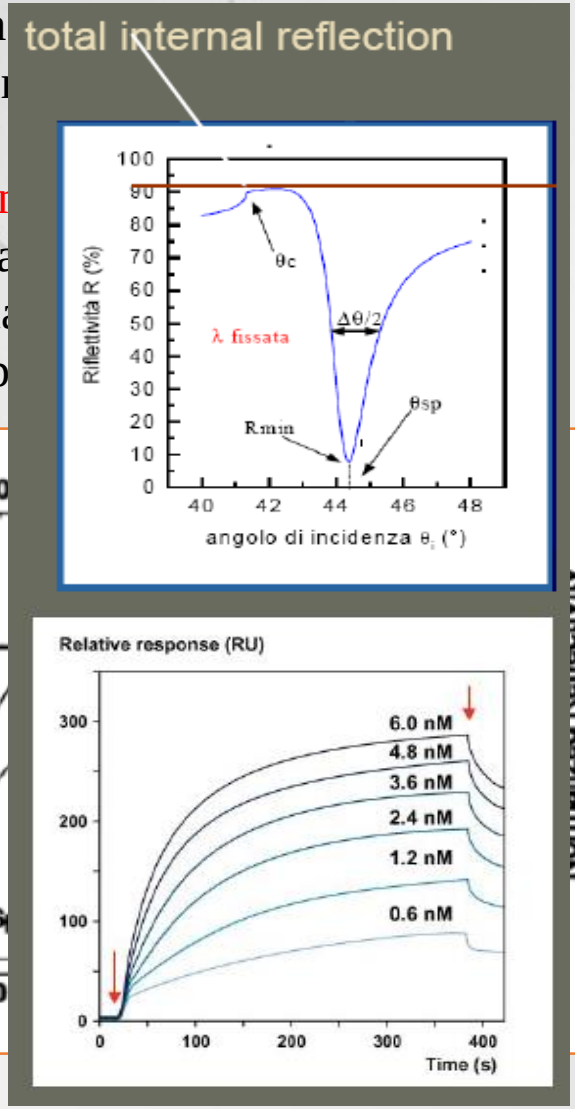
EXPERIMENTAL

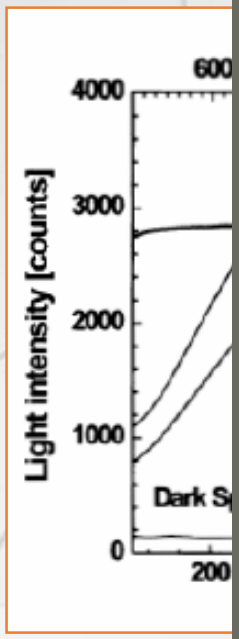




1. Signal normalization
- subtractin
- normalizi

2. Finding r
- direct mea
- polynomia
- centroid p





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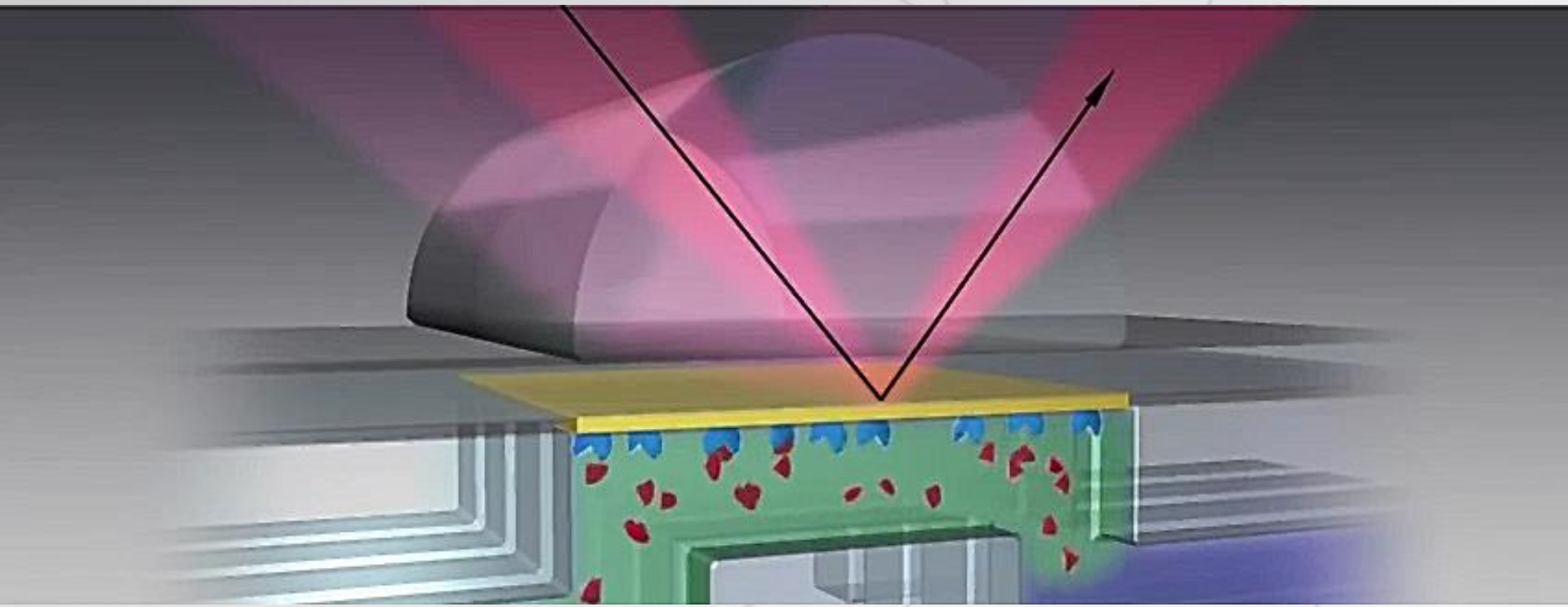
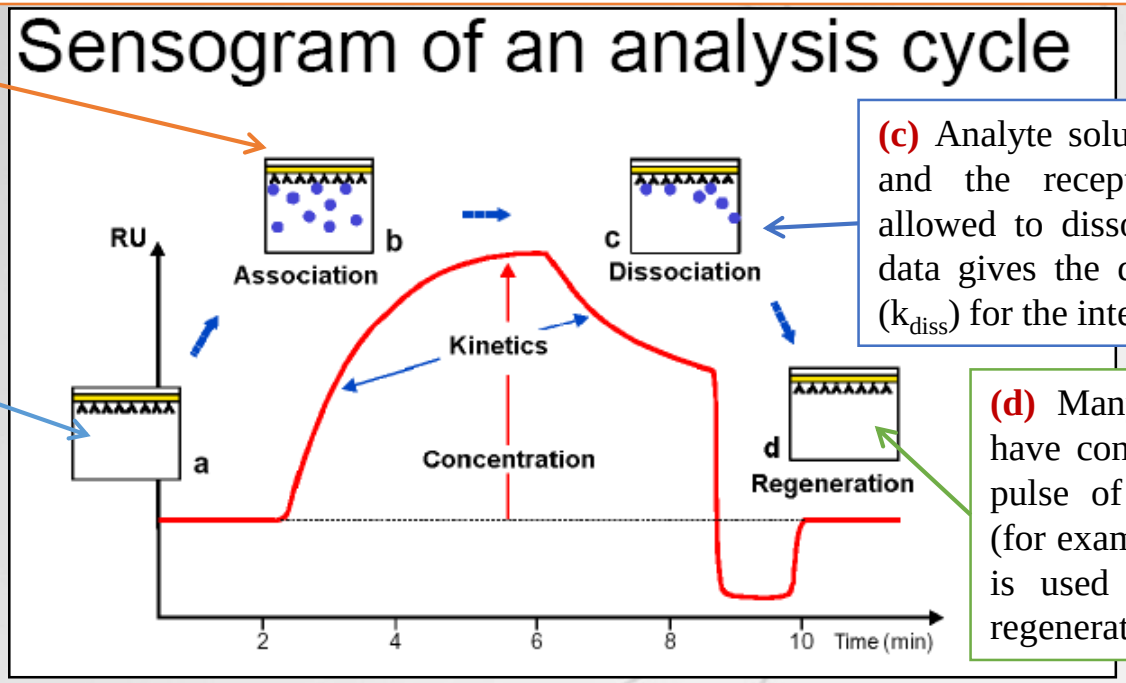
SPR



Measurement cycle / Sensorgrams

(b) Analyte solution passed over receptor → n increases → increase in resonance signal.

- Analysis of binding curve gives observed association rate (k_{obs}).
- If analyte concentration known, then association rate constant of interaction (k_{ass}) can be determined.
- The response level at equilibrium is related to the concentration of active analyte in the sample.



(a) Buffer contacted with receptor, *e.g.* through microfluidic flow cell.

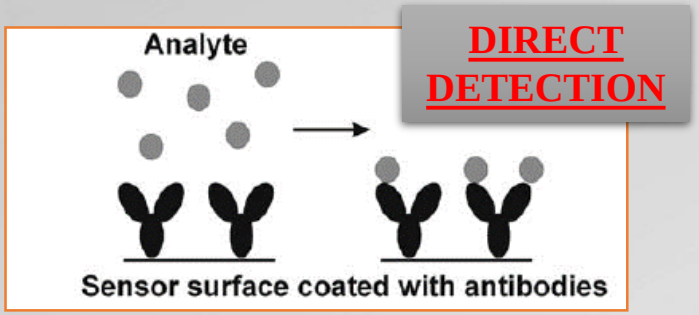
(c) Analyte solution replaced by buffer, and the receptor–analyte complex is allowed to dissociate. Analysis of these data gives the dissociation rate constant (k_{diss}) for the interaction.

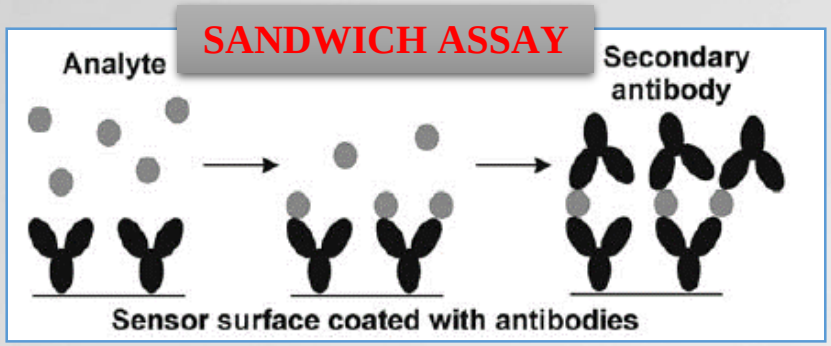
(d) Many complexes in biology have considerable half-lives, so a pulse of a regeneration solution (for example, high salt or low pH) is used to disrupt binding and regenerate the free receptor.

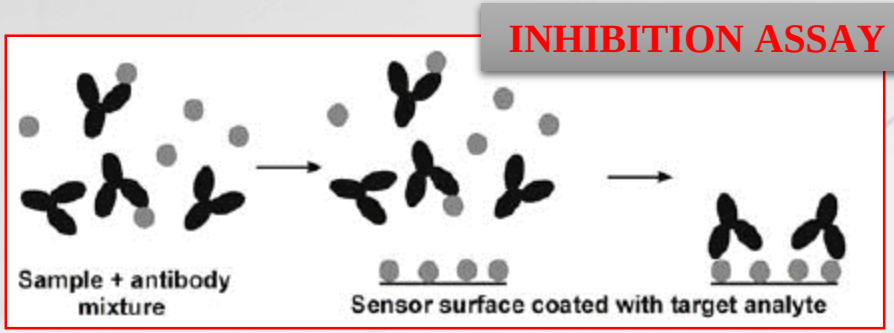
- Entire binding cycle normally repeated several times at varying concentrations of analyte to generate a robust data set for global fitting to an appropriate binding algorithm.
- The affinity of interaction can be calculated from ratio of rate constants (KD = k_{diss}/k_{ass}) or by linear/nonlinear fitting of response at equilibrium at varying concentrations of analyte.
- In addition to determining interaction affinities and kinetics, thermodynamic analysis of biomol. interaction also possible.

SPR biosensing / Formats

- Various measurement formats to ensure that binding event produces measurable sensor response.
- Most frequently used are:







- **In direct detection format**, analyte in a sample interacts with a biomolecular recognition element (antibody) immobilized on the sensor surface, Fig. 6. The resulting refractive index change is directly proportional to the concentration of analyte.

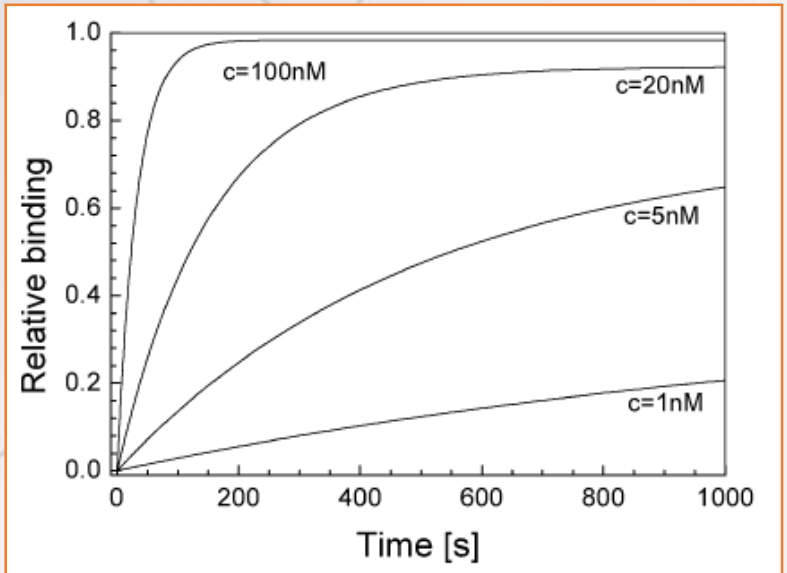
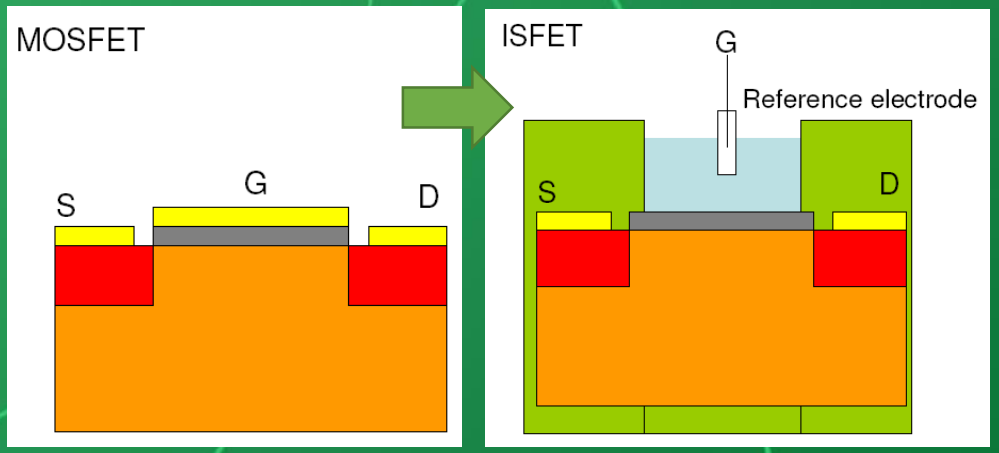


Fig. 7 Direct detection. Binding between antibody and analyte calculated for four different concentrations of analyte, $k_a=3\times10^5$ mol^{-1} L s^{-1}; $k_d=5\times10^{-4}$ s^{-1}

ISFET



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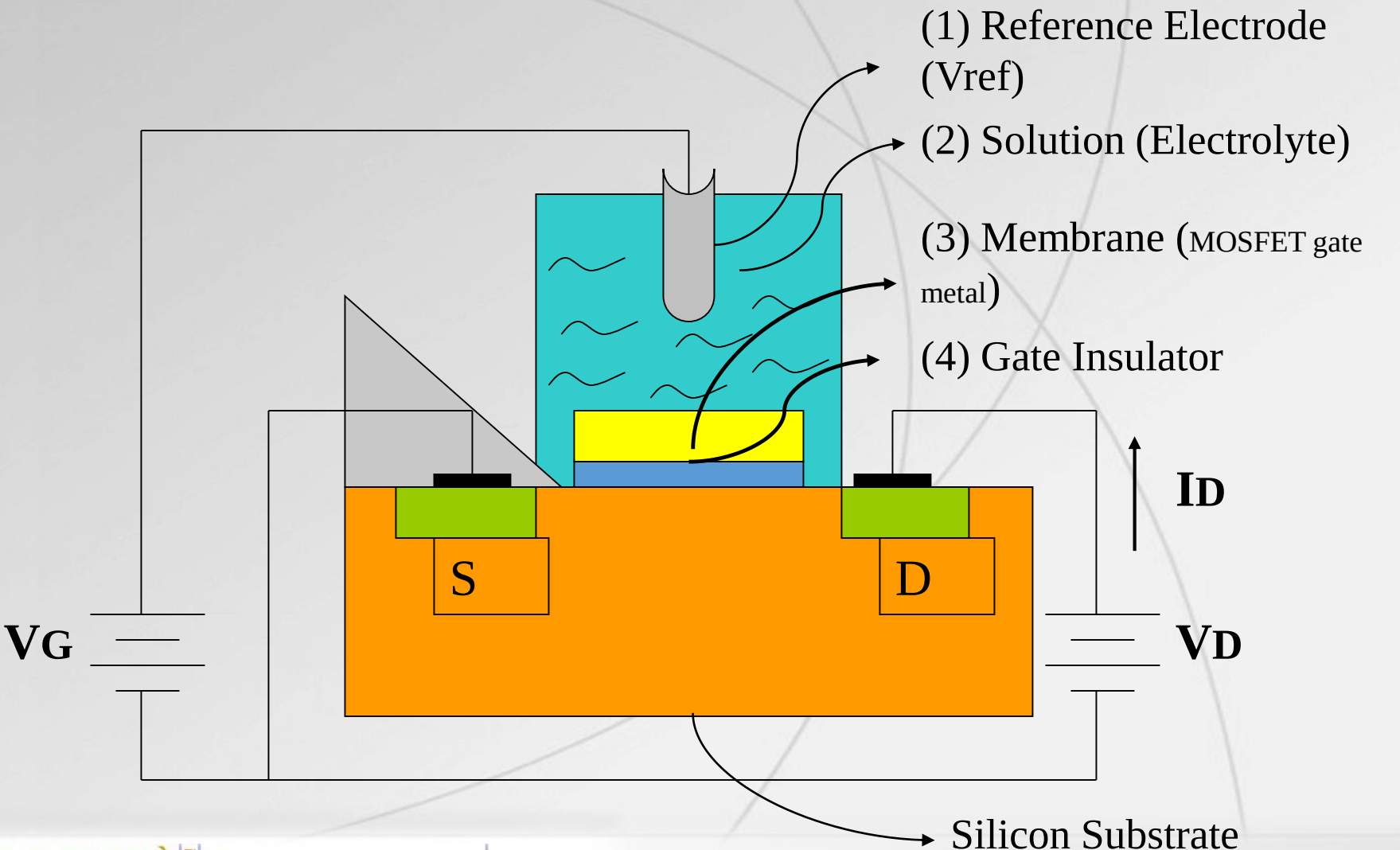
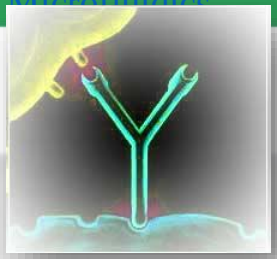
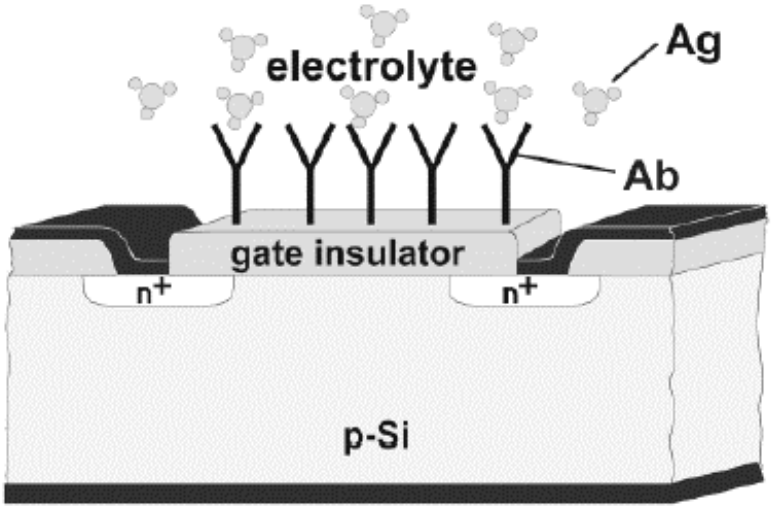
ISFET/CHEMFET sensors



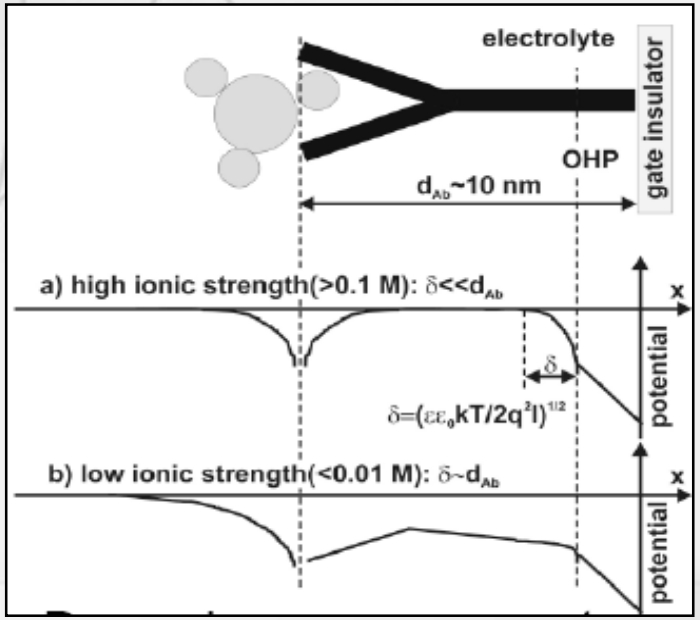
ImmunoFET



Measures surface charge



- Concept by Schenck (1978).
- Gate modified by immobilising antibodies or antigens (often in a membrane).
- DIRECT DETECTION CONCEPT. Since a FET basically represents a surface-charge measuring device, and since antibodies and antigens (or more generally, proteins) are mostly electrically charged molecules, the formation of an antibody–antigen complex on the gate leads to a detectable change in the charge distribution and thus, directly modulates the drain current.



- In principle, under ideal conditions, ImmunoFET theoretically capable of measuring the concentration of immunomolecules with a very low detection limit and a wide concentration range of 10^{-7}–10^{-11} M with about a 10 mV response signal.
- Ideal conditions that are required in this coherence are: a truly capacitive interface at which the immunological binding sites can be immobilised; a nearly complete antibody coverage; highly charged antigens and a very low ionic strength.

Analyst, 2002, 127, 1137–1151

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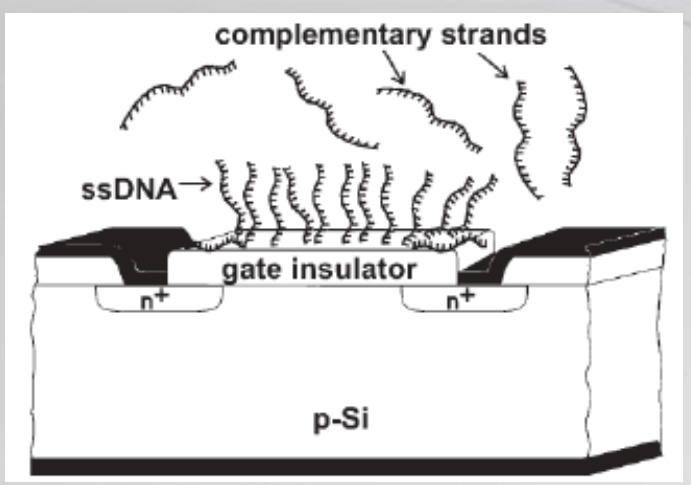
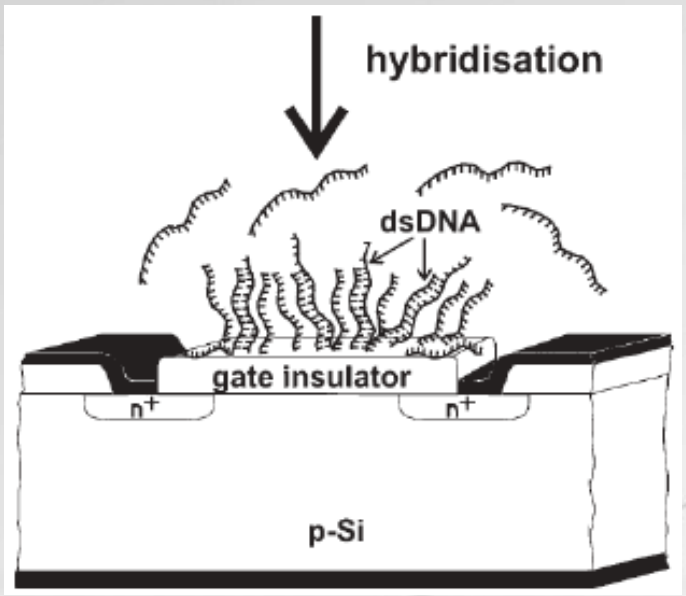
GenFET





Label-free detection of DNA hybridization

- well-defined sequences of ssDNA onto a transducer (poly(dT)).
- complementary DNA added to the buffered electrolyte.
- denaturisation was performed by immersing the sensor in boiling, deionised water for 0.5 h.

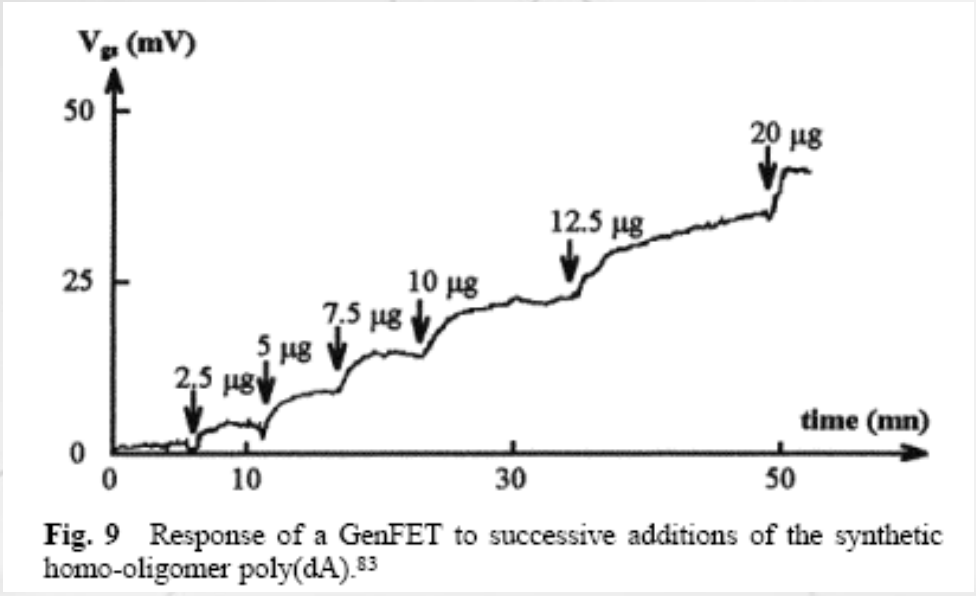
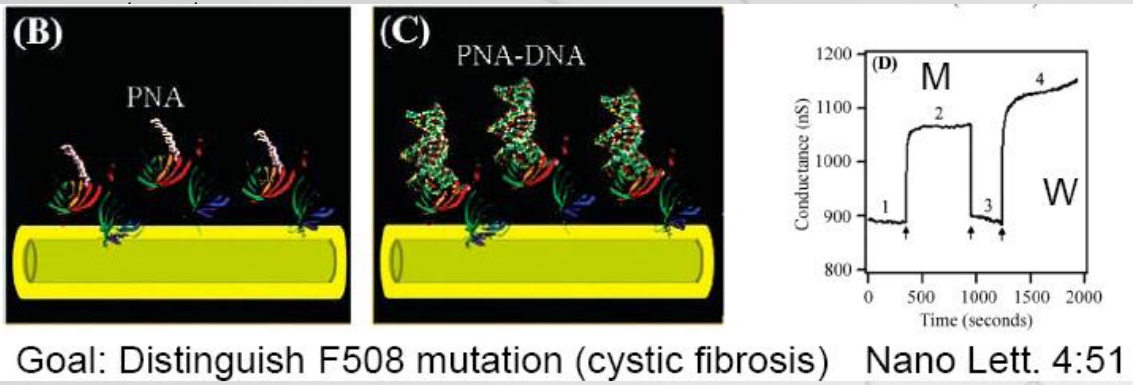


Fig. 9 Response of a GenFET to successive additions of the synthetic homo-oligomer poly(dA).[83]

Electrical detection using nanowires

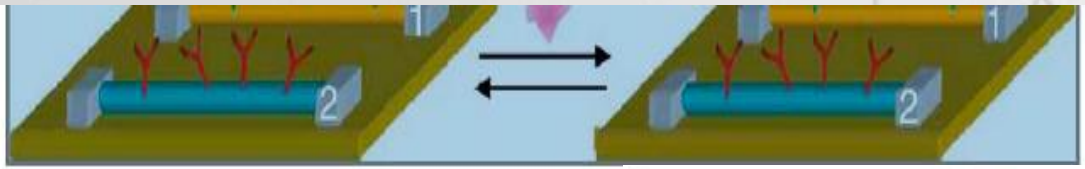
Large surface area to volume ratio → whatever happens at the surface will have a huge impact on the current through th

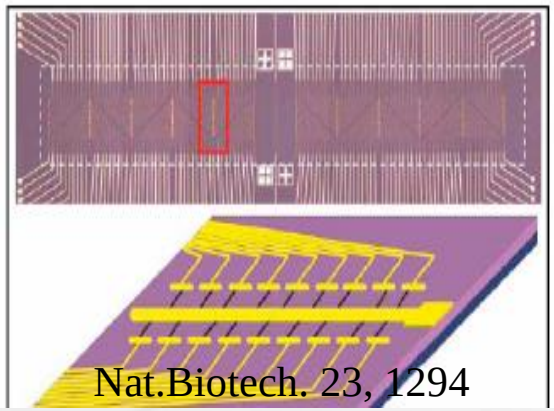
DNA



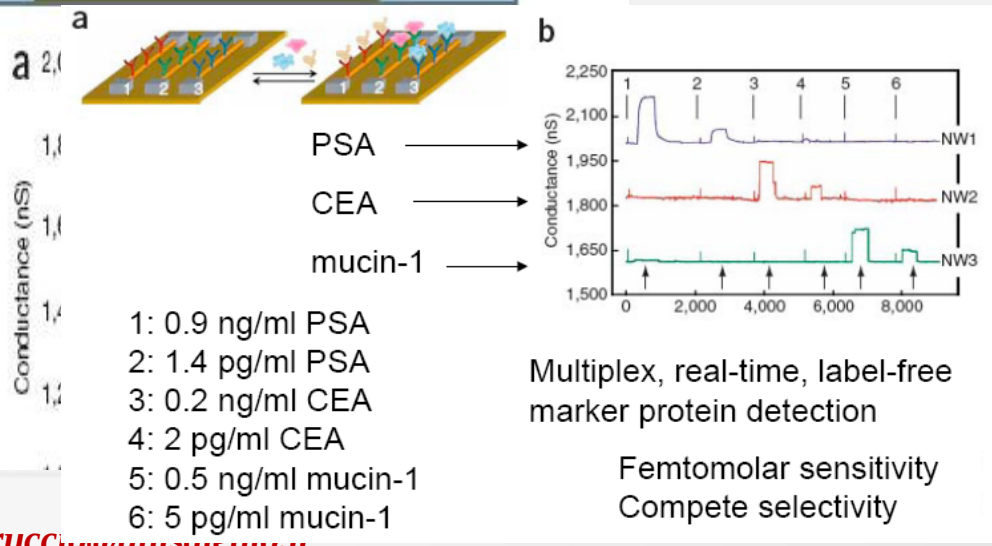
Goal: Distinguish F508 mutation (cystic fibrosis) Nano Lett. 4:51

Proteins - PSA





Nat.Biotech. 23, 1294

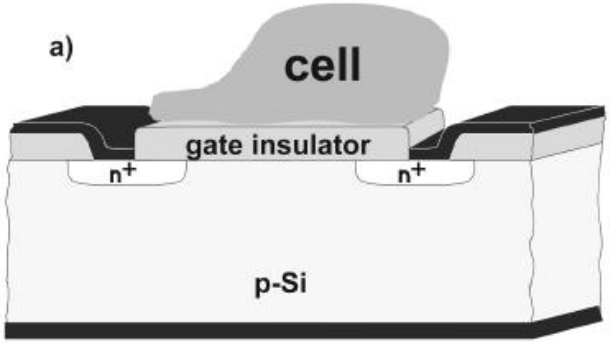


1: 0.9 ng/ml PSA
2: 1.4 pg/ml PSA
3: 0.2 ng/ml CEA
4: 2 pg/ml CEA
5: 0.5 ng/ml mucin-1
6: 5 pg/ml mucin-1

Multiplex, real-time, label-free marker protein detection

Femtomolar sensitivity
Compete selectivity

Cell-based BioFET



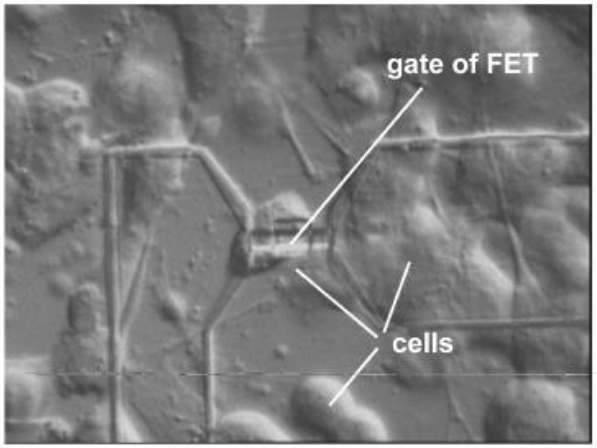
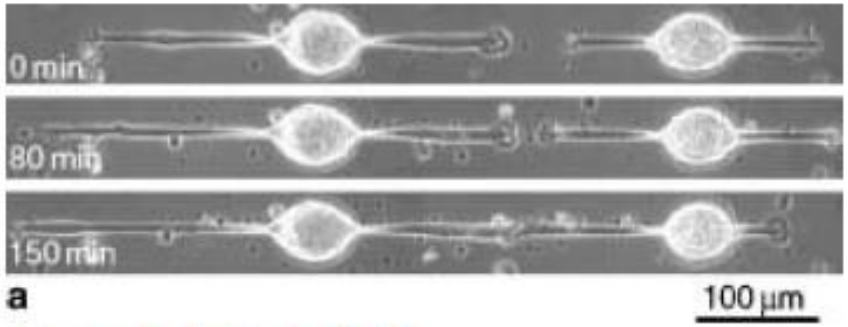
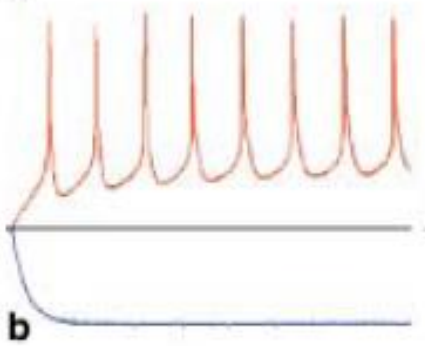
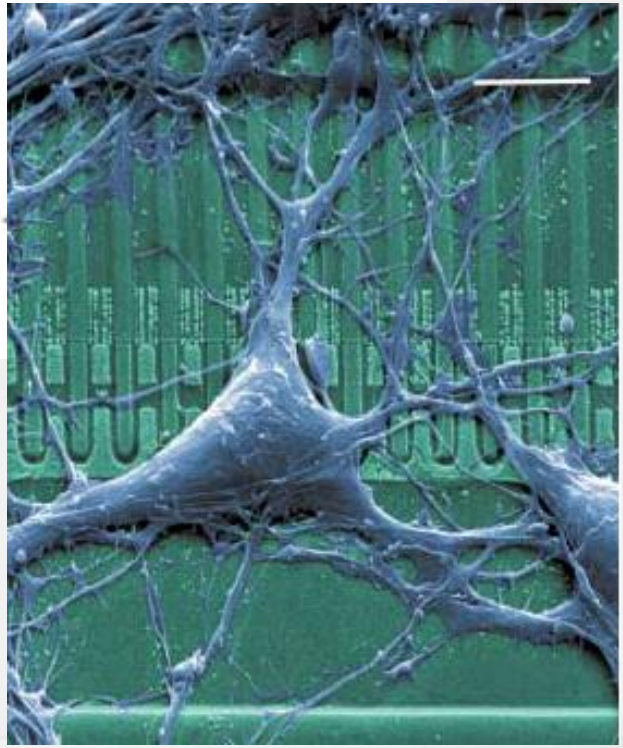


Fig. 10 'Cell–transistor' hybrid: (a) schematic set-up; (b) videomicroscopic photo of genetically modified HEK 293 cells on an ISFET array (unpublished photo, with kind permission of H. Ecken). In the centre of the photo one cell completely covers the sensitive gate area of the ISFET.







Biosensors in Lecce

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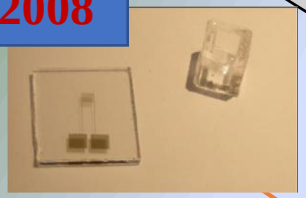
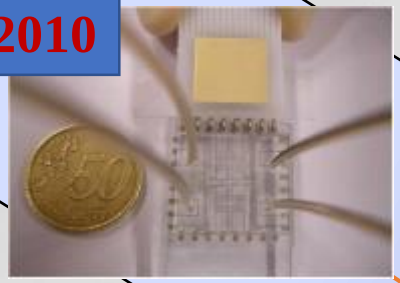
EIS Biosensors & biochips

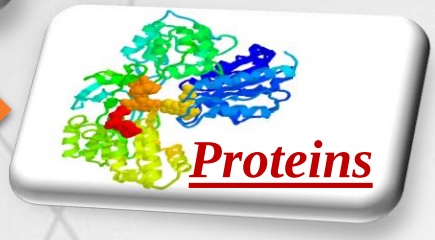
2008



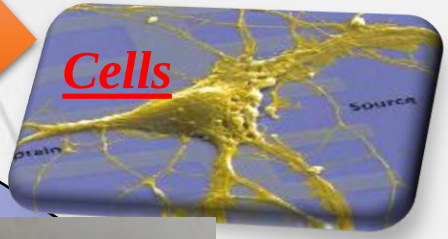
DNA

2010





Proteins



Cells

2013

Diagnostics

Food
Environment

2018

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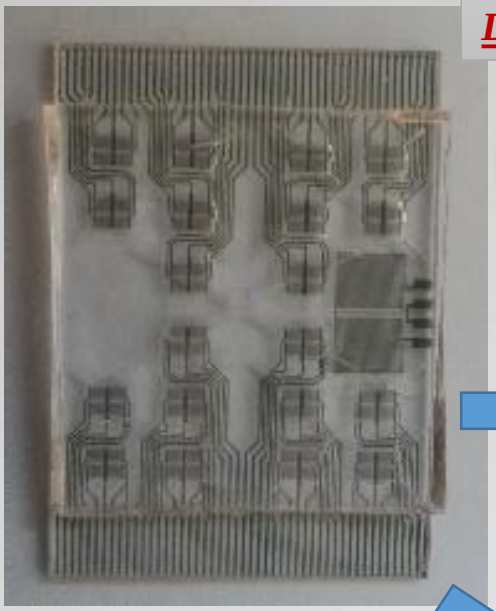
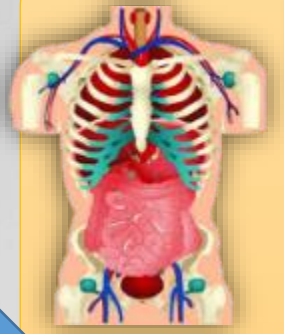
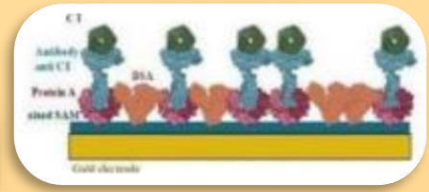
Multipurpose applications



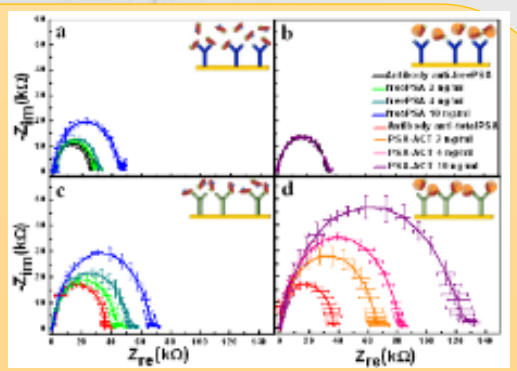
MEDICAL DIAGNOSTICS



1. Immunoassay: PSA & PDAC

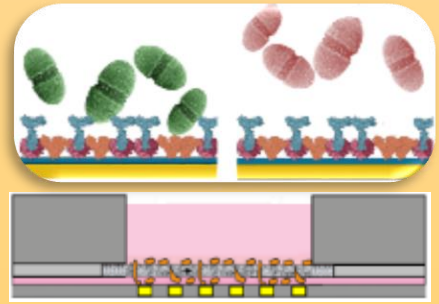


M. S. Chiriacò et al.,
Lab on a Chip 13, 730 (2013).
Analyst, 138, 5404 (2013)



2. Cytotoxicity & Migration assays

Primiceri et al., Biosensors & Bioelectronics 25, 2711–2716 (2010)
Lab on a chip, 11, 4081, (2011)



FOOD / ENVIRONMENT

Allergens, toxins, pathogens, contamination

M. S. Chiriacò et al.,
Talanta 2015, 142, 57-63.
E. Primiceri et al., Analytical Methods, 2016, 8, 3055-3060.

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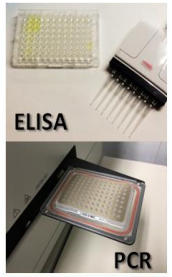
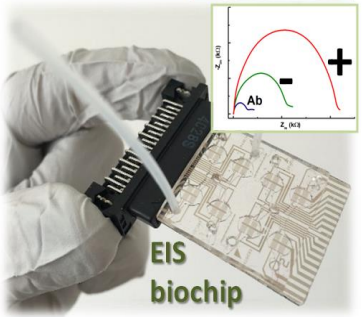
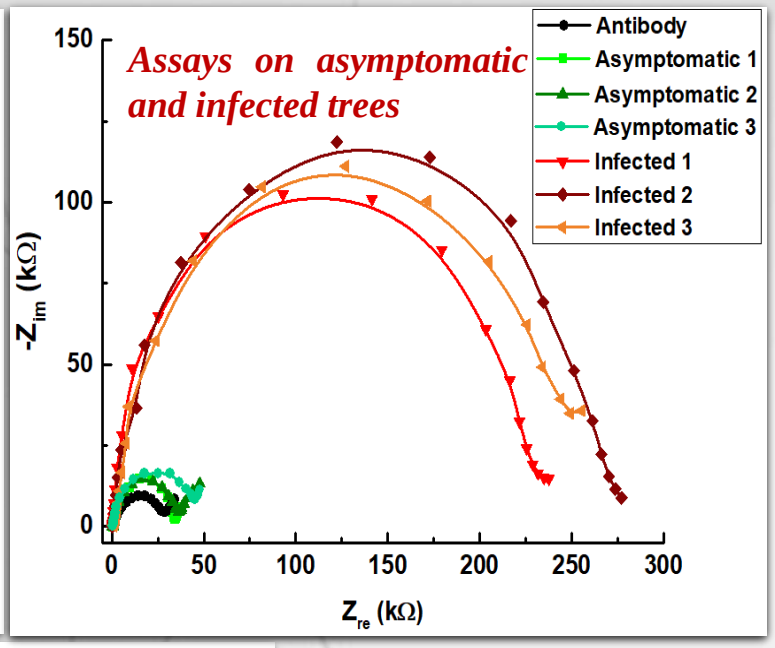
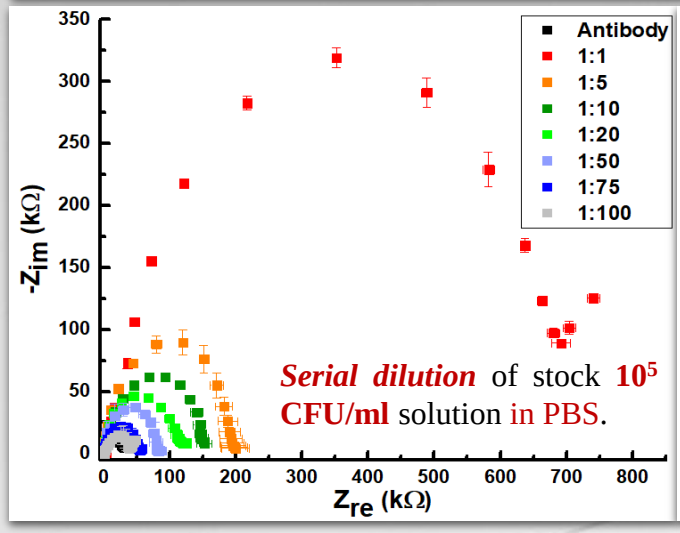
Appl. 2: Environmental threats - Xylella

Xylella fastidiosa spiked samples

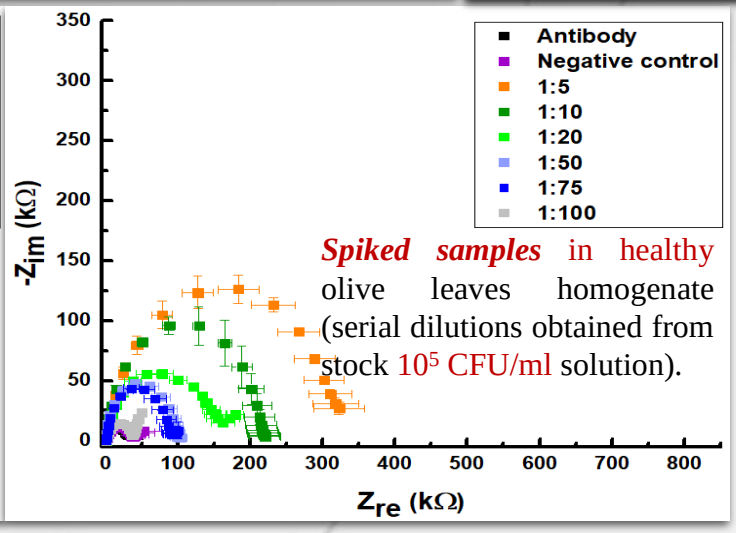








Serial dilution of stock **10^5 CFU/ml** solution in PBS.



Spiked samples in healthy olive leaves homogenate (serial dilutions obtained from stock 10^5 CFU/ml solution).

Sample dilution	ELISA	PCR	LOC
1:1	+	+	+
1:5	+	+	+
1:10	+	+	+
1:20	-	+	+
1:50	-	+	+
1:75	-	+	+
1:100	-	+	-
1:1000	-	+	-

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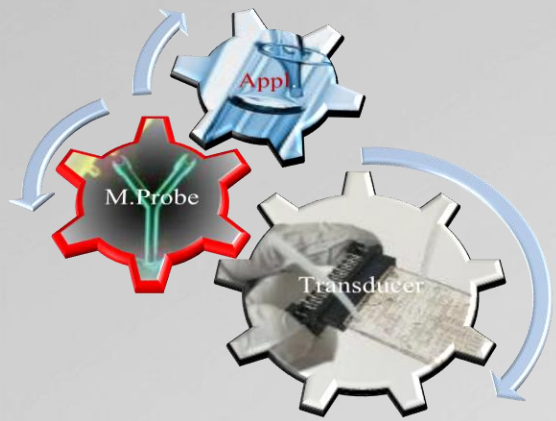
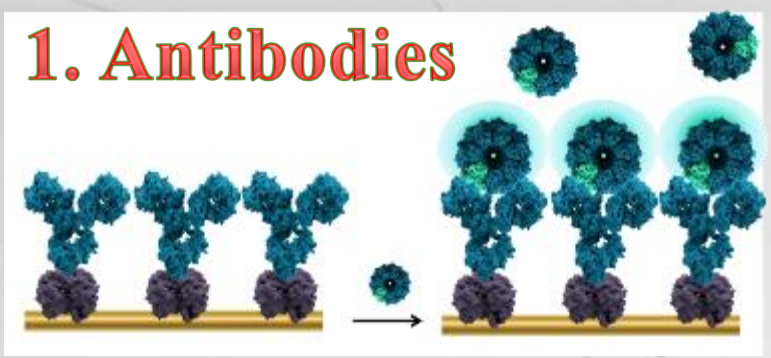
M.S. Chiriacò et al., Scient. Reports, 2018, in press

The sensing element

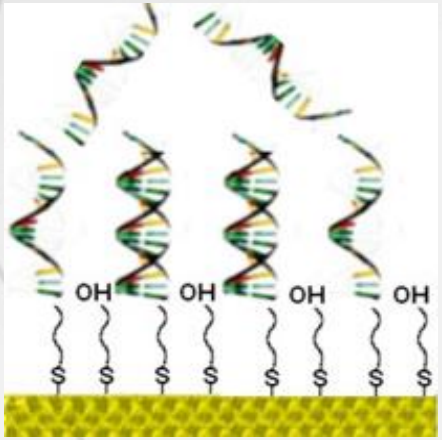


1. Antibodies

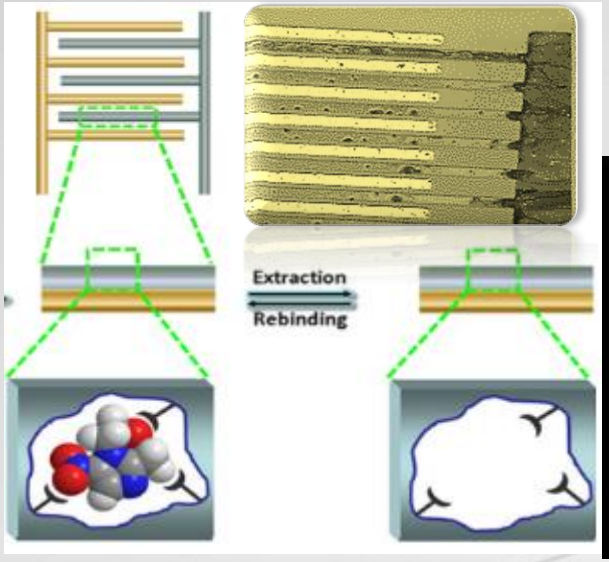


2. Aptamers

3. DNA



4. MIP



in collaboration with Prof. C.Malitesta

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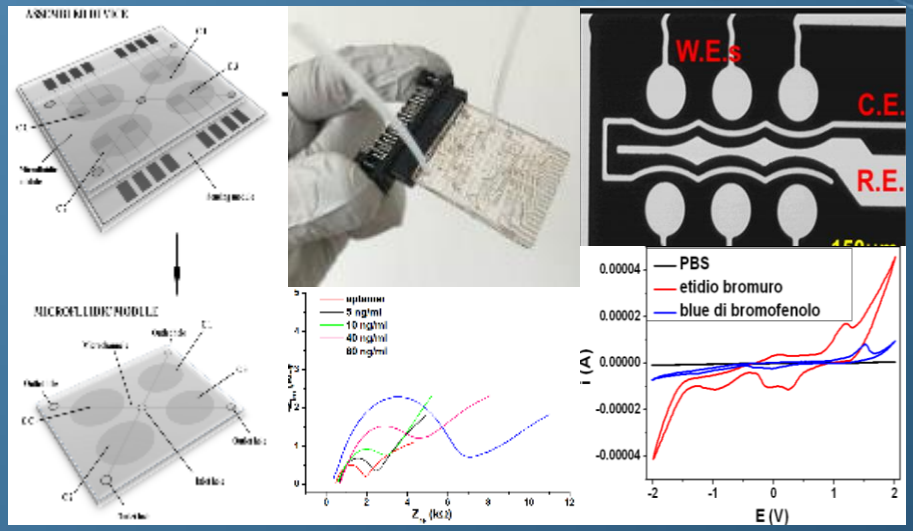
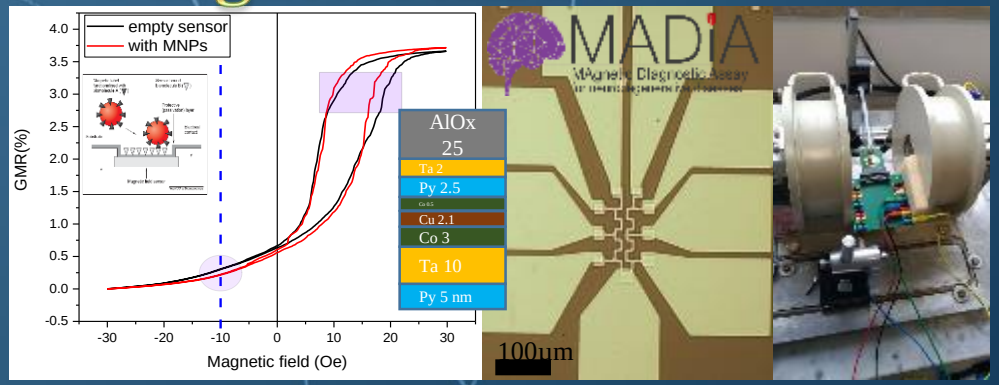
The transducer / sensor

EIS & EC

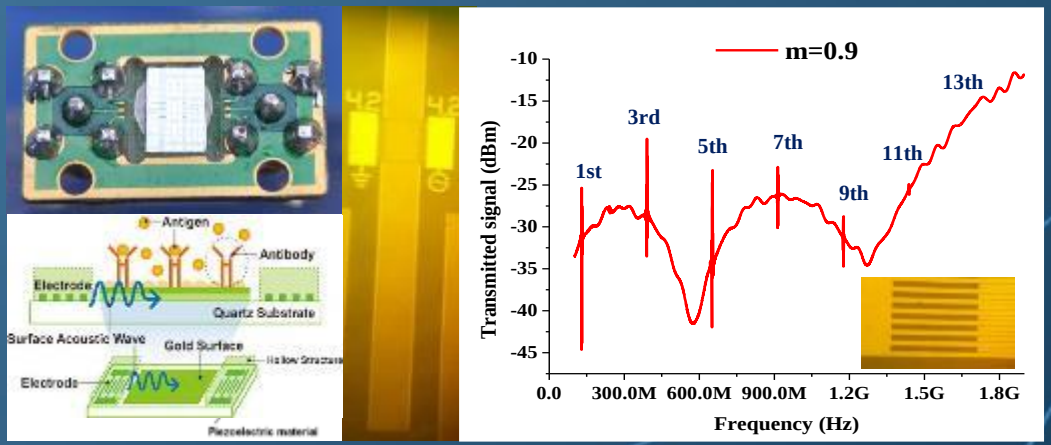


Magnetoresistive



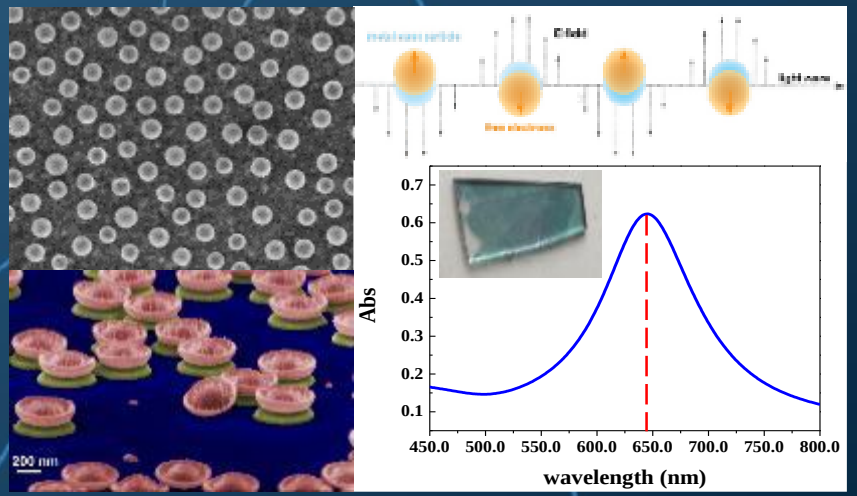
In collaboration with G.Reiss, A. Dediu

SAW



In collaboration with A. Passaseo, V. Tasco

SPR



In collaboration with R. Rella, M.G. Manera

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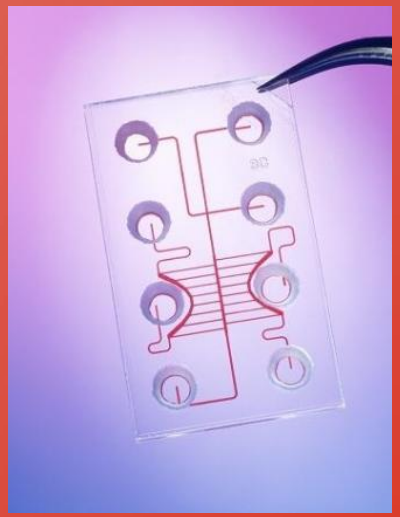
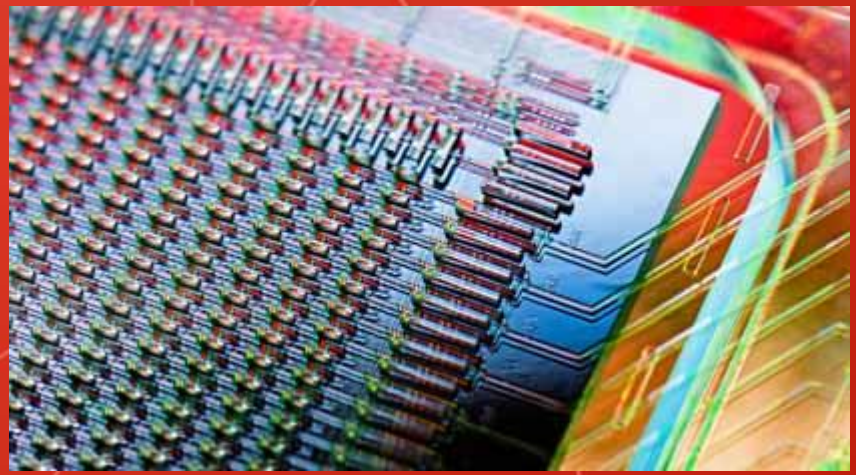
Microfluidics





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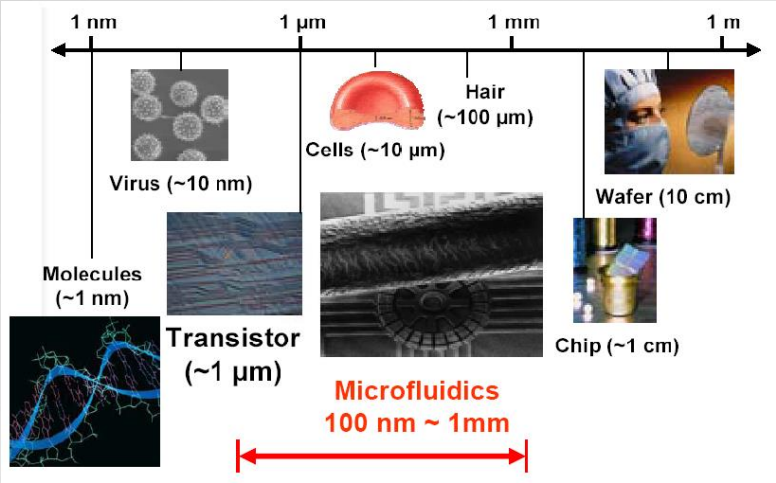
What is microfluidics

Science/technologies for handling minute amounts of fluids in miniaturized systems

- **In microelectronics** emphasis on **reducing sizes**
- **in microfluidics** on making **more complex systems** with more sophisticated fluid-handling capabilities.
- **Same components** as larger scale systems: channels, chambers, reactors and active components (pumps, valves, mixers, filters, separators, etc.) on the size scale of a human hair or smaller
- **Different flows behavior** than at large-scale familiar from everyday life.

One dimension of flow is in µm.

Fluid flow in microchannels. Typical range is in nano- and microliters



A natural µ-fluidic device

The cardio-vascular system:

~~Main vein / artheries **diameter**: 10 – 30 mm~~

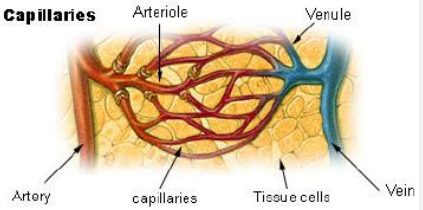
Capillar **diameter**: 5 – 10 µm

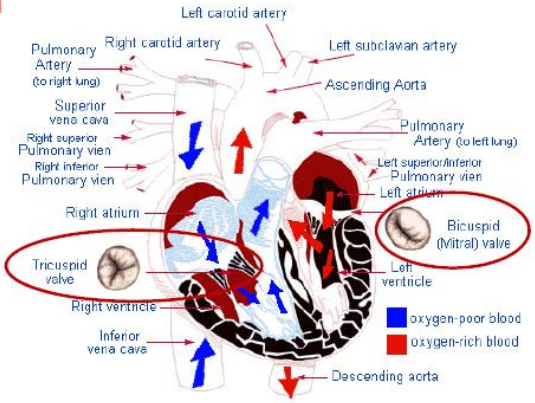
Blood **speed**: 1 – 10 mm/s

Heart (a **pump** system): ΔP

$\Delta P = \eta Q$ (Poiseville law)

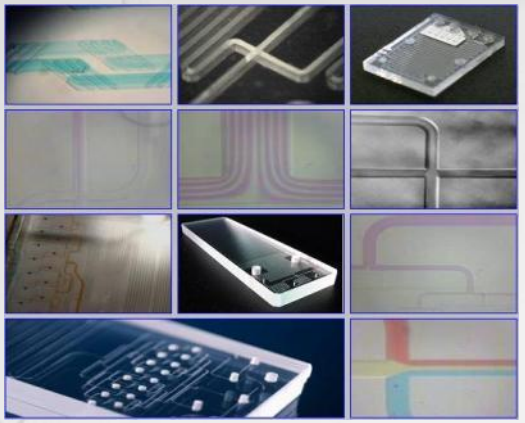
Heart and vein **valves**





What is microfluidics

- **New approaches to** synthesize, purify, and rapidly screen chemicals, biologicals, and materials using integrated, massively parallel miniaturized platforms.
- **Miniaturization of** laboratory equipment. Goal is to have a laboratory on a few square centimeters: **LAB-ON-A-CHIP**



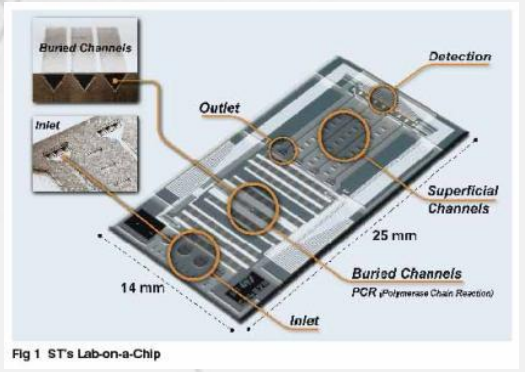
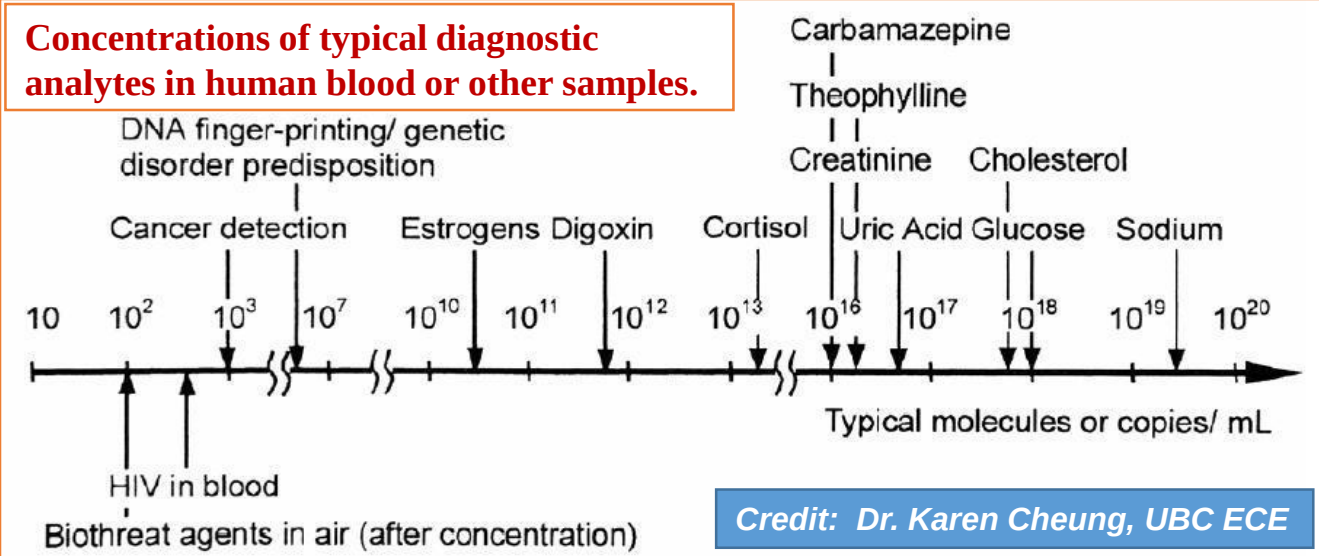


Fig 1 ST's Lab-on-a-Chip

Concentrations of typical diagnostic analytes in human blood or other samples.



Credit: Dr. Karen Cheung, UBC ECE

1 molecule in 1 μL = 1.6×10^{-18} M
1 molecule in 1 nL = 1.6×10^{-15} M
1 molecule in 1 pL = 1.6×10^{-12} M

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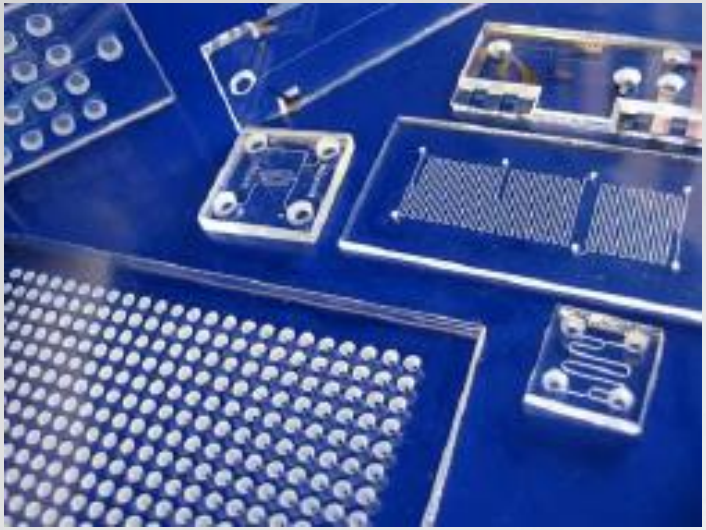
Glass vs Plastic Microfluidics

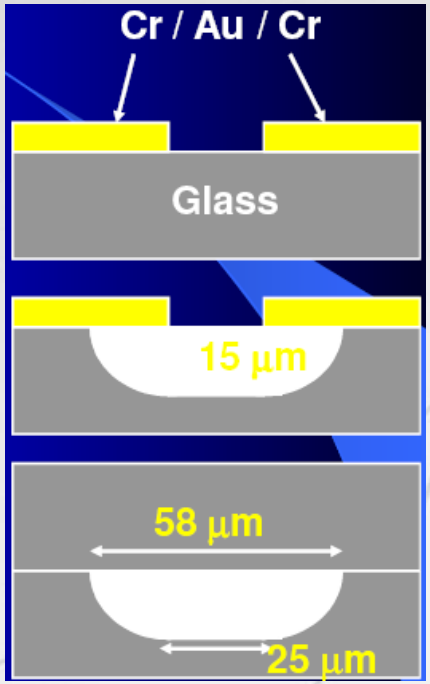
Glass

– Cheap
– Chemically inert
– Optically clear
– Hydrophilic
– Highly temperature resistant
– Known & accepted qualities



Fabrication

- Cr/Au/Cr mask
- HF/HNO_3 etchant
- drilled access holes
- bonded at 440° C



Plastics/Polymers

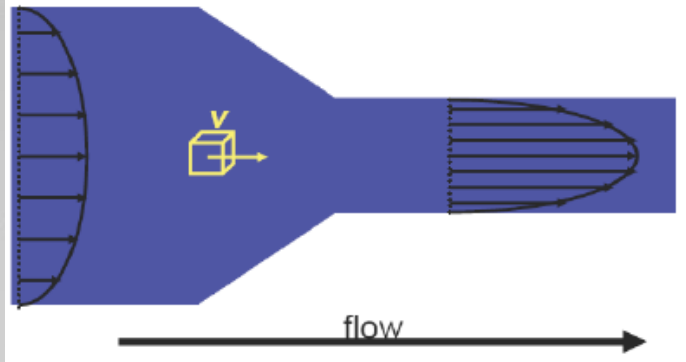
Advantages:

- low cost (disposable systems)
- versatility of material properties (mechanical, optical, thermal stability, biocompatibility, surface energy, ...)
- ease of fabrication (routine access to clean room environment not required)
- ease of sealing
- more accessible to chemists/biologists

Disadvantages:

- More care to control surface energy
- Incompatible with organic solvents
- Incompatible with high temperatures

Navier-Stokes Equation



- Velocity depends on space and time $\boldsymbol{v} = \boldsymbol{v}(\boldsymbol{x}(t),t)$
- Consider Newtons law for a infinitesimal volume V:

$$\mathbf{F} = m \cdot \mathbf{a} \Rightarrow \frac{\boldsymbol{F}}{V} = \boldsymbol{f_a} = \frac{m}{V} \cdot \boldsymbol{a} = \rho \cdot \frac{d\boldsymbol{v}}{dt} = \rho\left(\frac{\partial \boldsymbol{v}}{\partial t} + \boldsymbol{v} \cdot \boldsymbol{\nabla v}\right)$$

For incompressible Newtonian fluids:

$$\boldsymbol{f_a} = \rho\left[\frac{\partial}{\partial t}\boldsymbol{v} + (\boldsymbol{v} \cdot \boldsymbol{\nabla})\boldsymbol{v}\right] = \boldsymbol{f_{pressure}} + \boldsymbol{f_{friction}} + \boldsymbol{f_{volume}}$$

Navier-Stokes Equ.
(central relationship of fluid dynamics)

left hand side

- Change in momentum (Newton)
  - due to change of velocity over time at a given location
  - due to acceleration of fluid e.g. when moving into smaller flow channel cross sections (also in stationary cases)

right hand side

- Forces acting on fluid
- Pressure gradient
- Friction forces
- Volume forces


Navier (1785 – 1836)


Stokes (1819 – 1903)

Regimes

$$Re = \frac{\rho U l}{\mu} = \frac{U l}{\nu}$$

Laminar flow: fluid flows in parallel layers

< Reynolds Number (2100) <

Turbulent flow: rough flow, non parallel

$$\mathrm{Re} = \frac{\rho U L}{\mu} \approx \frac{\text{Forze d'inerzia}}{\text{Forze viscose}}$$

$\rho(H_2O) = 1\ g\ cm^{-3}$

$\mu(H20) = 10^{-2}\ g\ cm^{-1}s^{-1}$

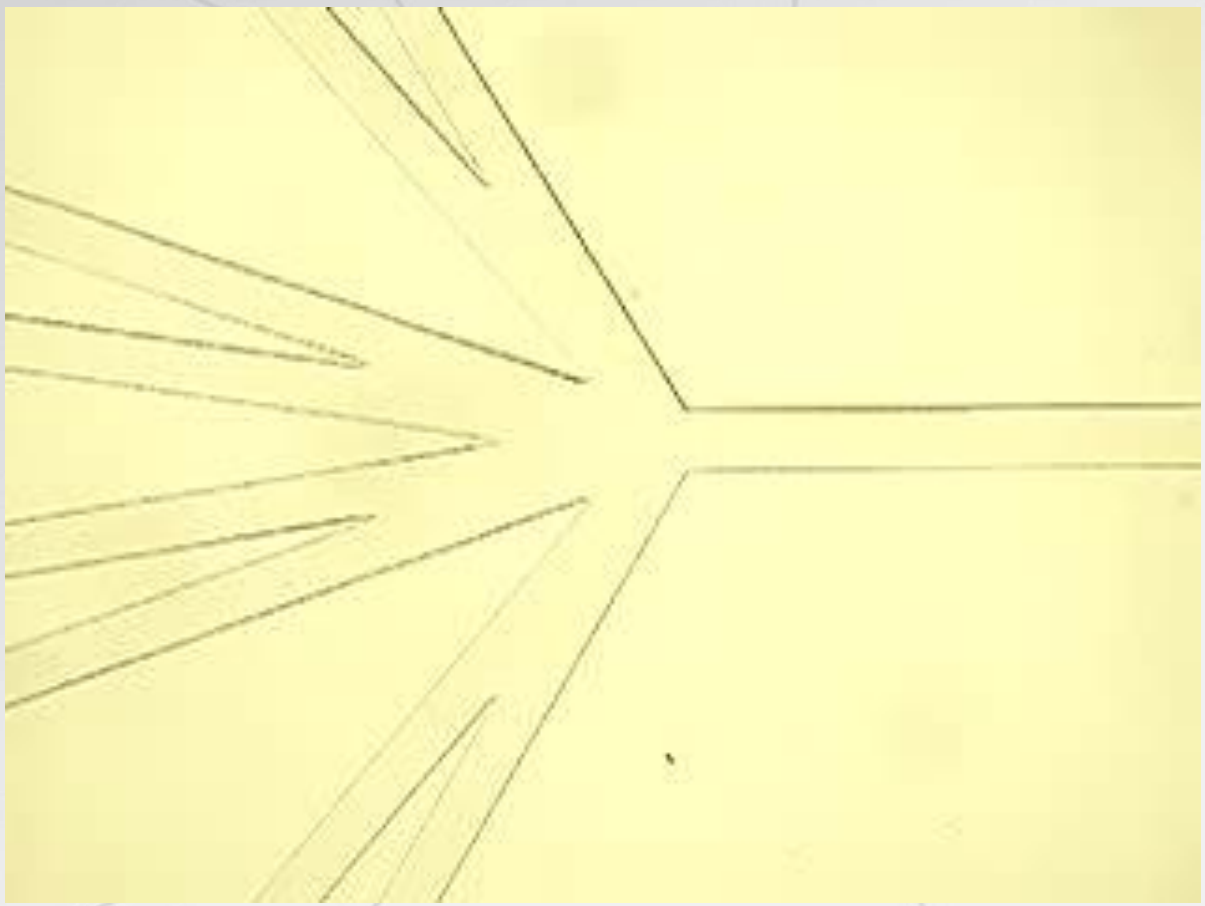
Michael Phelps; Re≈10^4

Poecilia Ret.; Re≈10^2

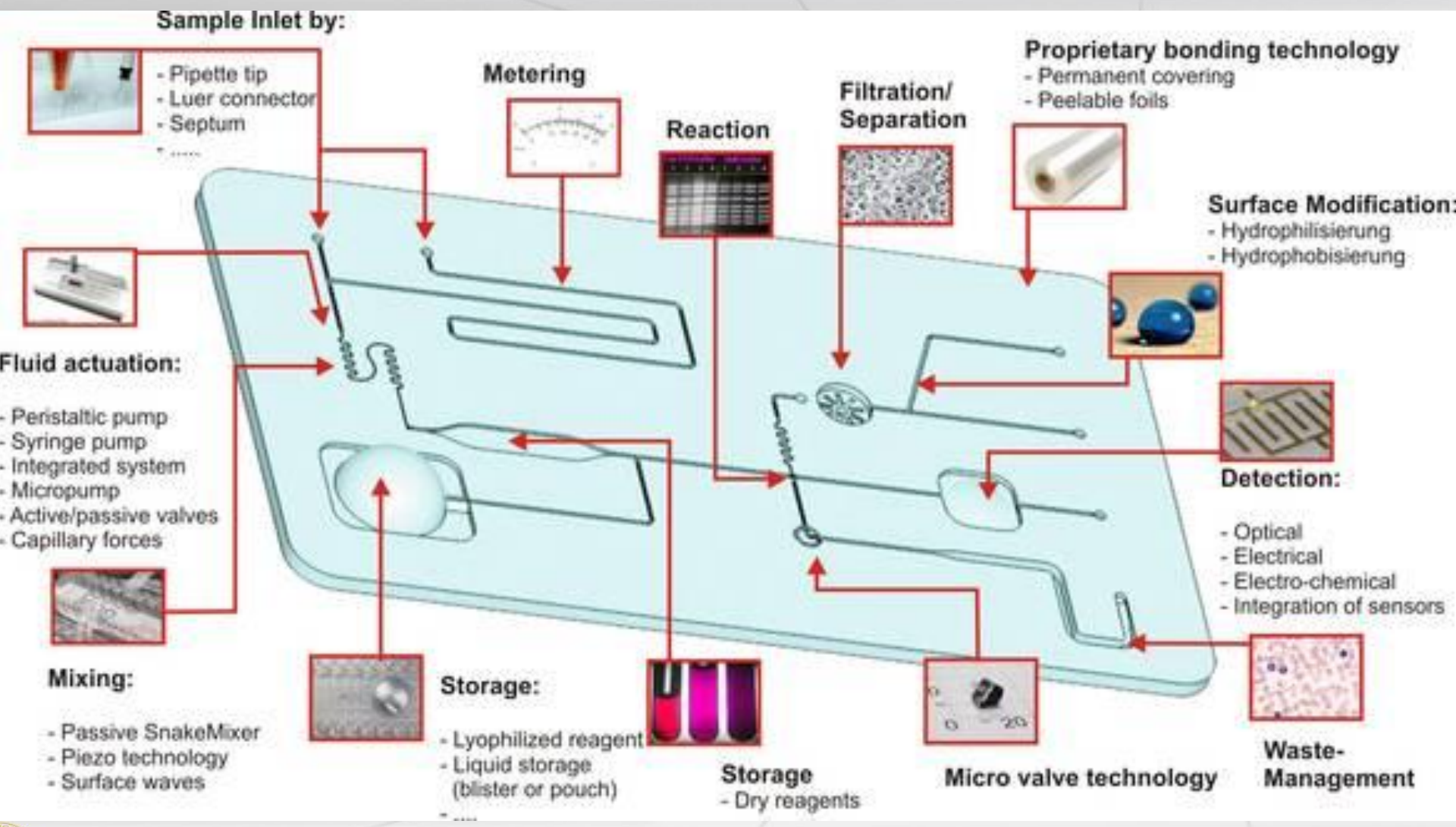
E.M. Purcell
Life at low Reynolds number
American Journal of Physics
45(1), 1977, 3-11.

What is the regime in a μ-fluidic device?

Effects of the micro-domain: laminar flow

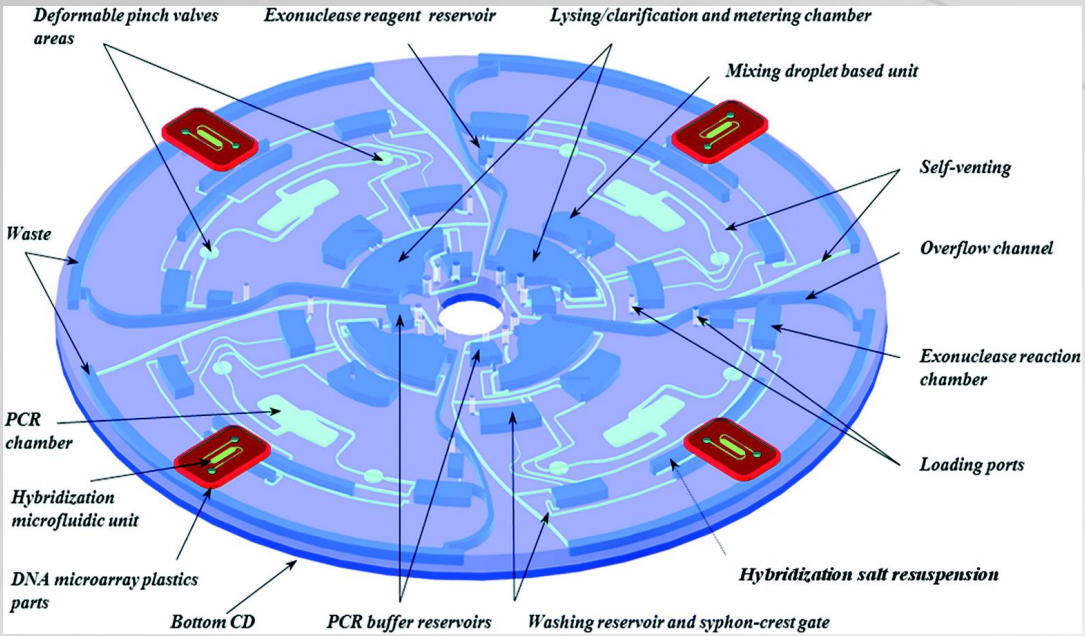


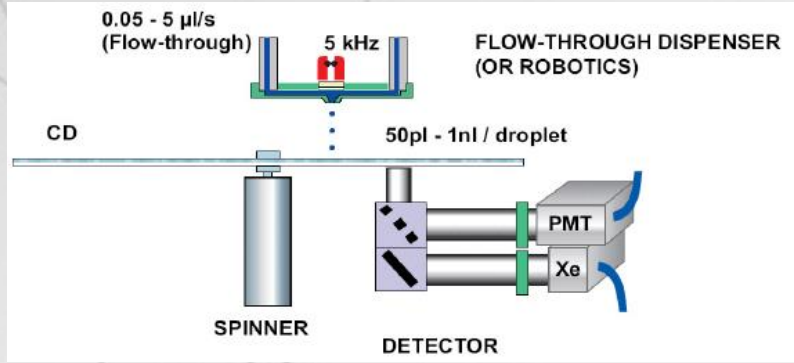
Microfluidic components

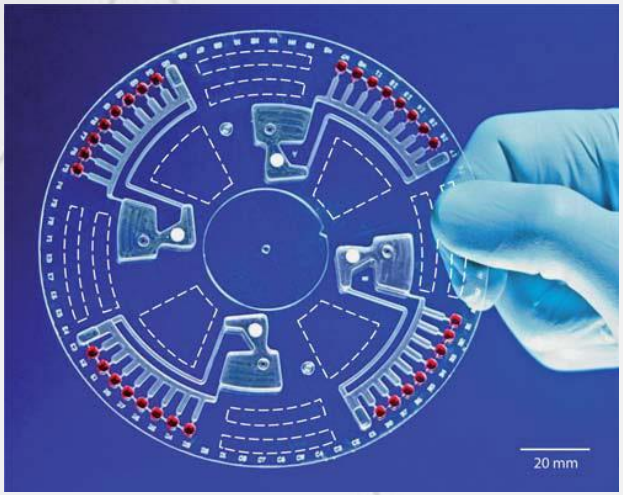


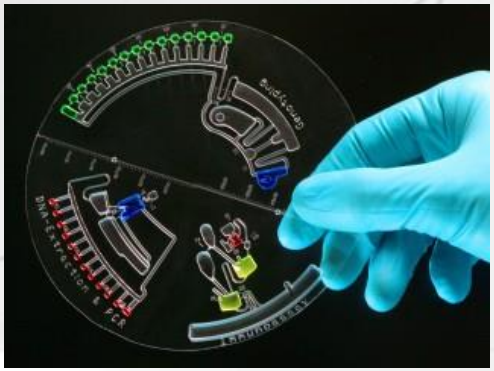
Centrifugal pumping (lab on a disc)

Spinning the CD creates the liquid flows: $F \approx m\omega^2 r$









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Droplet Microfluidics

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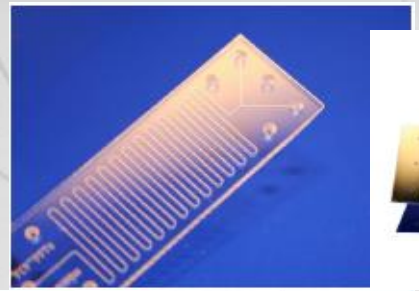
Chemical Research on chip

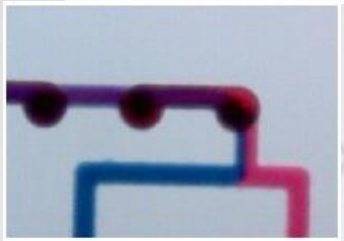
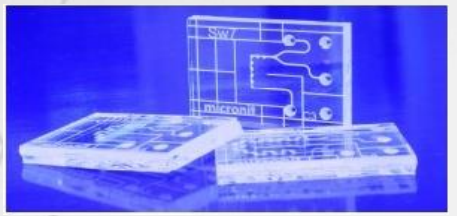
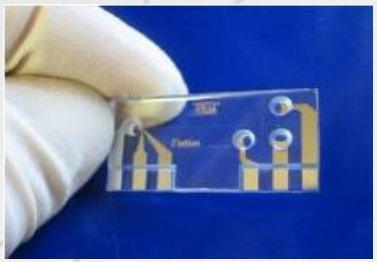
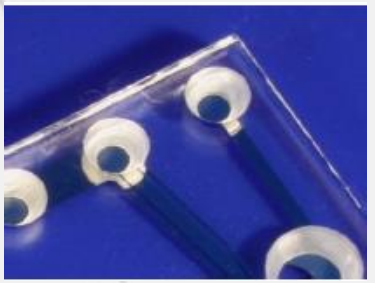
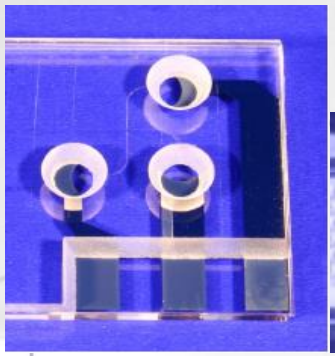
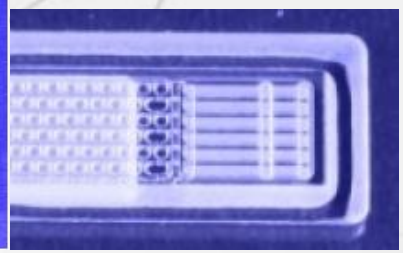
Microreactor
– High surface to volume ratio creates excellent mass and heat transfer possibilities
– High process yield and selectivity

Micromixer
– Very short mixing times (milliseconds)
  • Reaction kinetics research
– Ideally mixed conditions
  • No gradients

Detection
– Electrodes integrated in glass chips
– Electro chemical detection, temperature sensing

Temperature control
– Heaters
– Coolers
– Heat exchangers





Swirl mixing principle





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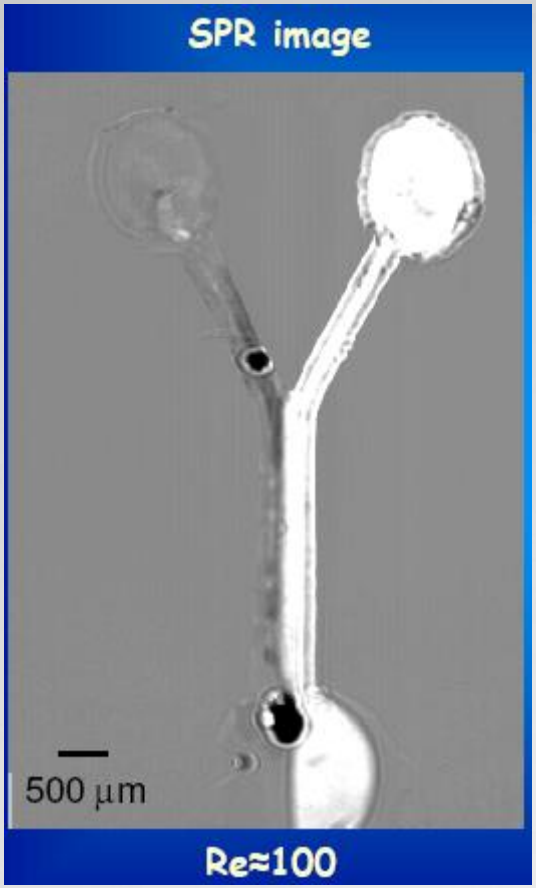
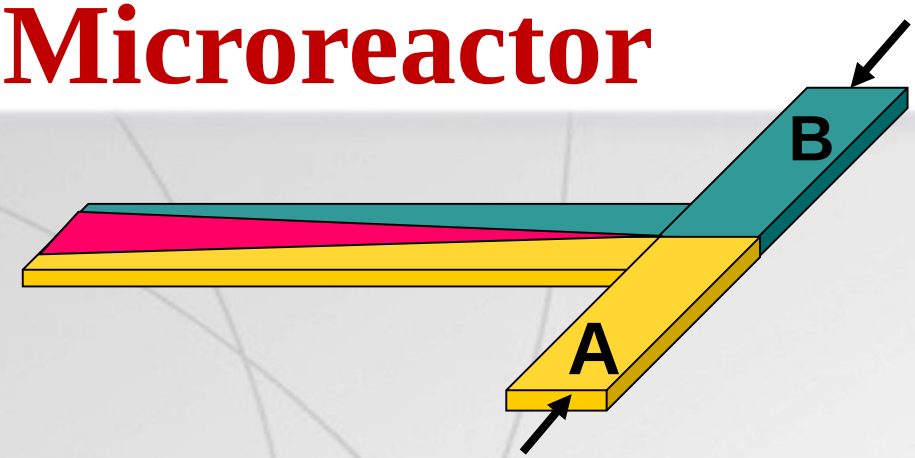
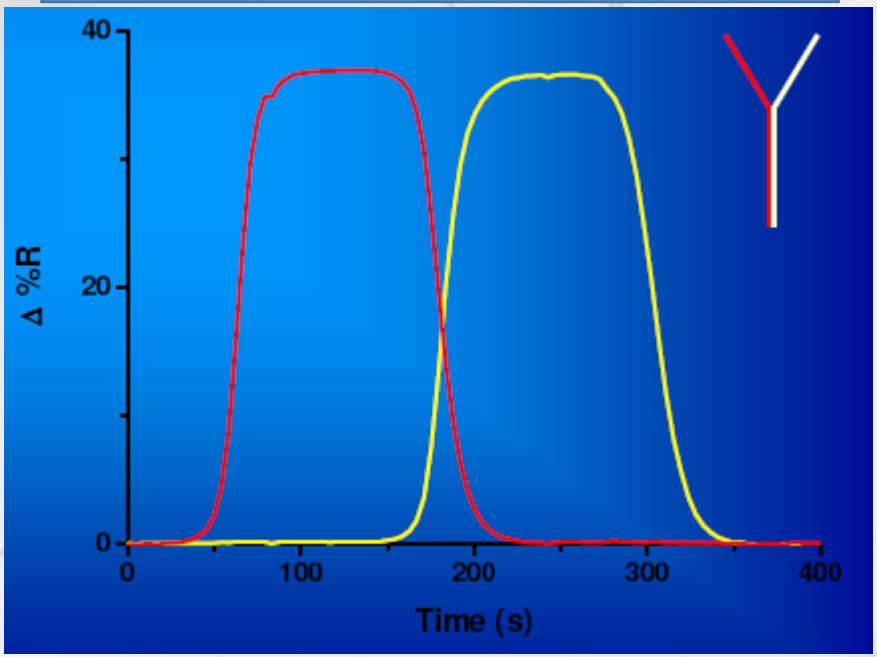
Mixing in T-Microreactor





Mixing is difficult in microsystems



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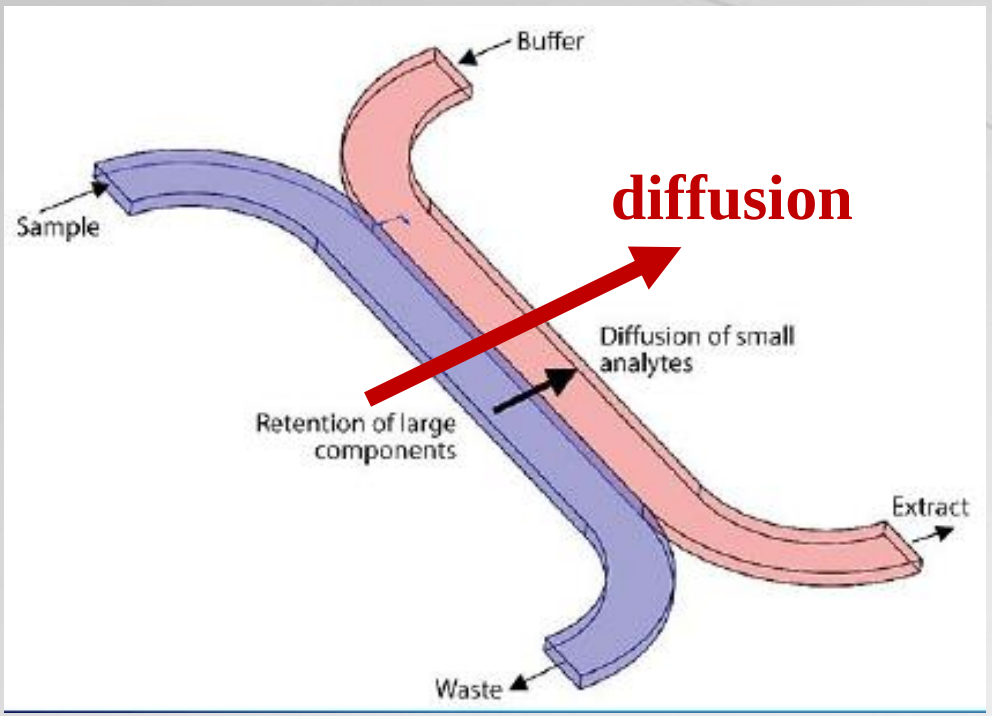
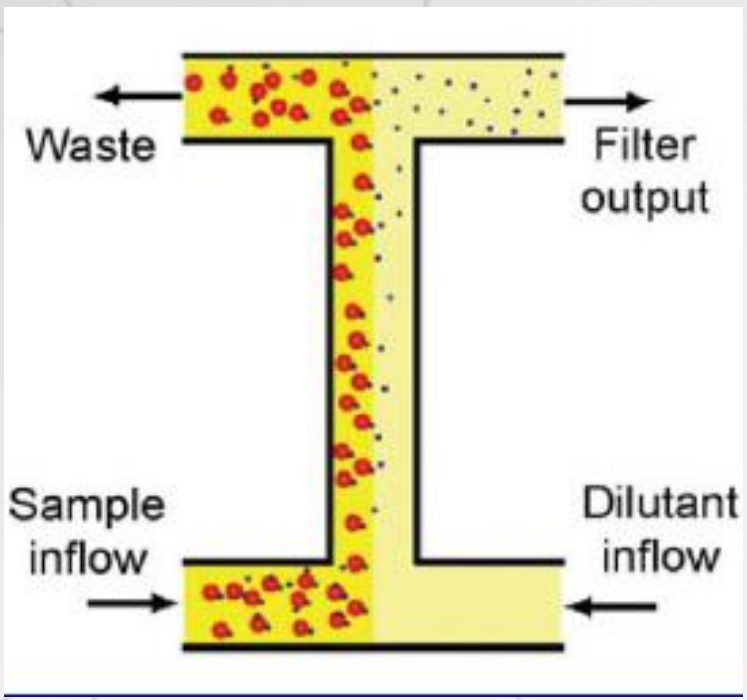
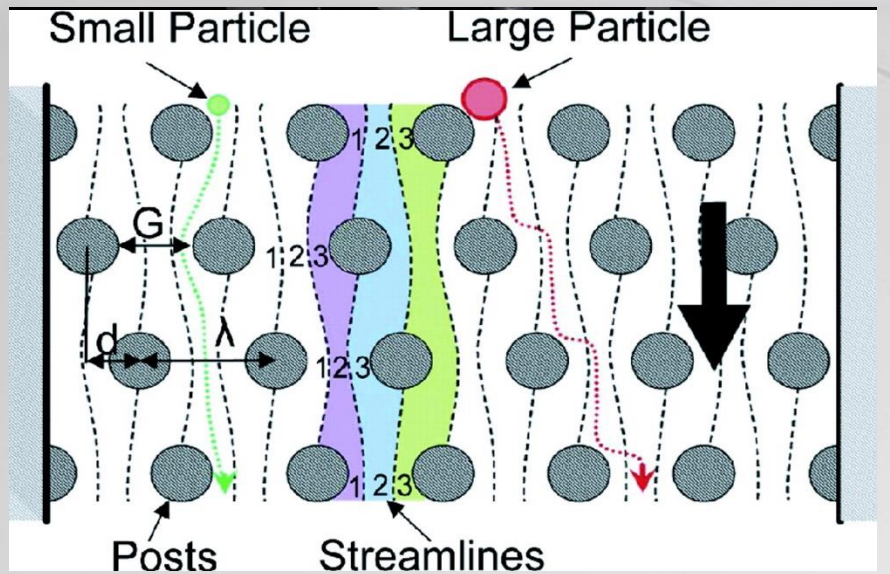
H-Filter

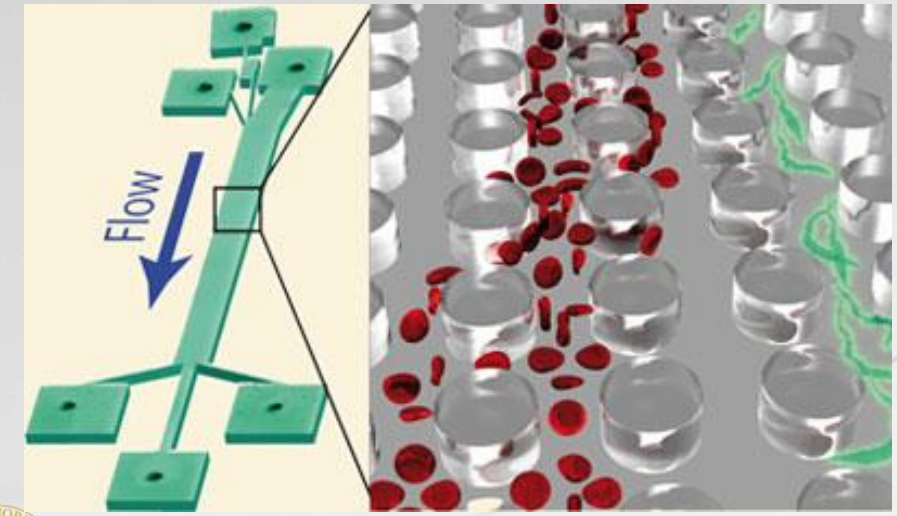


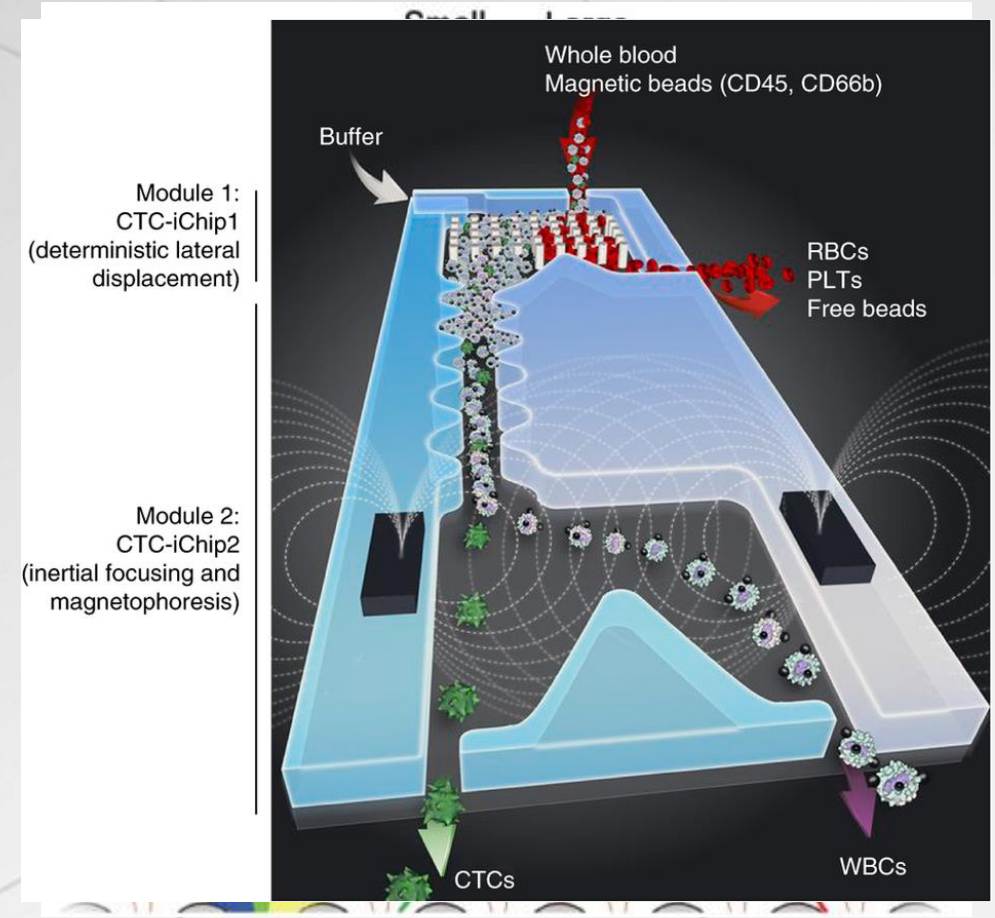


P. Yager et al. Nature, 442, 2006, 412.

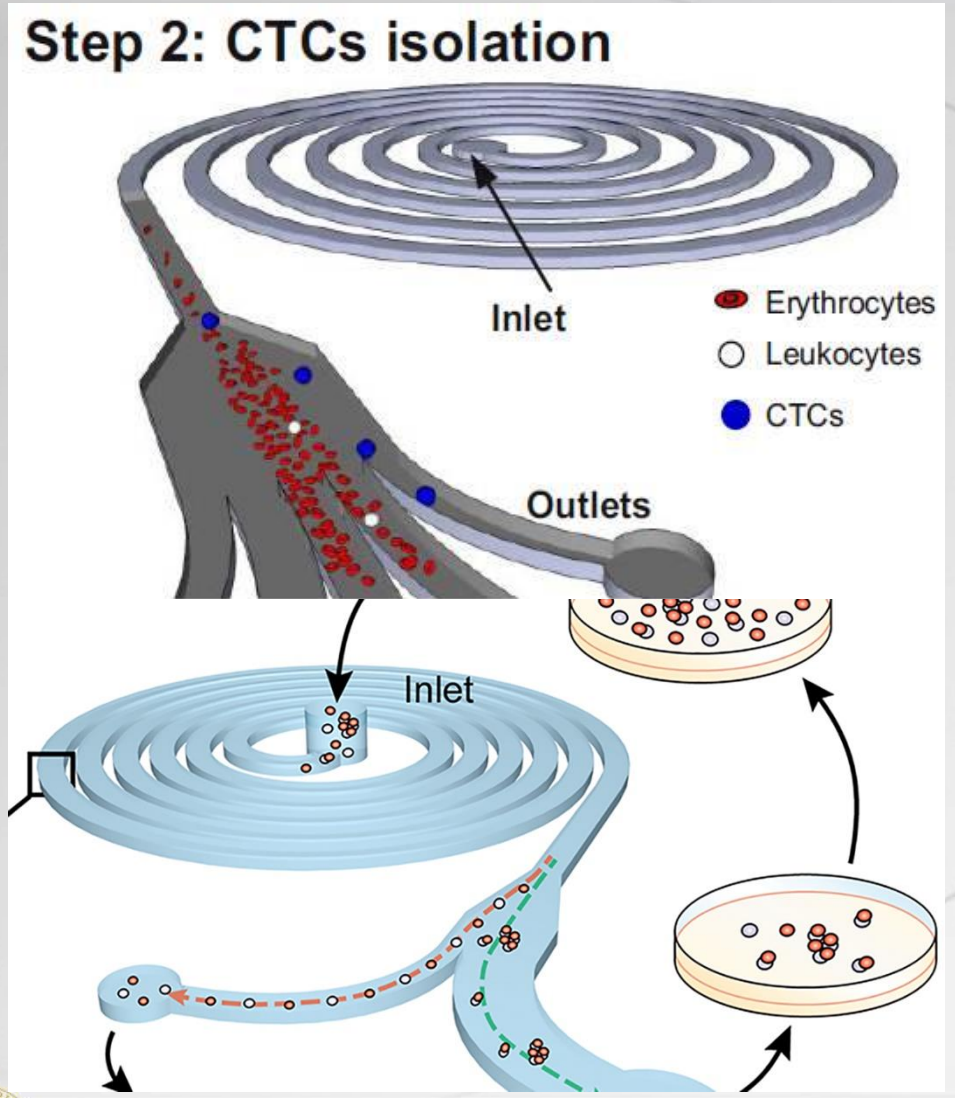
Deterministic lateral displacement

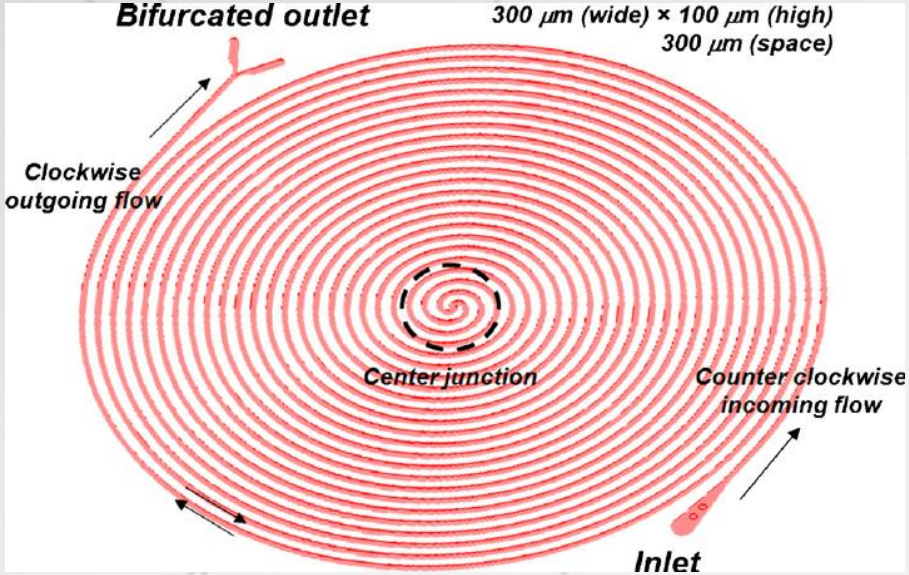






Centrifugal separation





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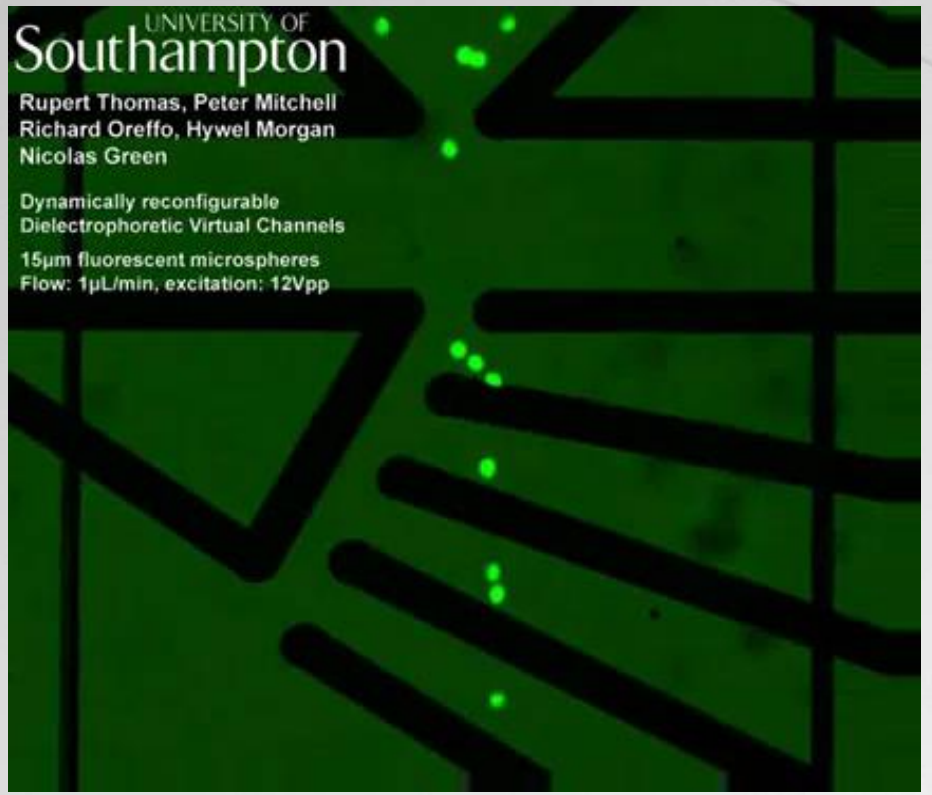
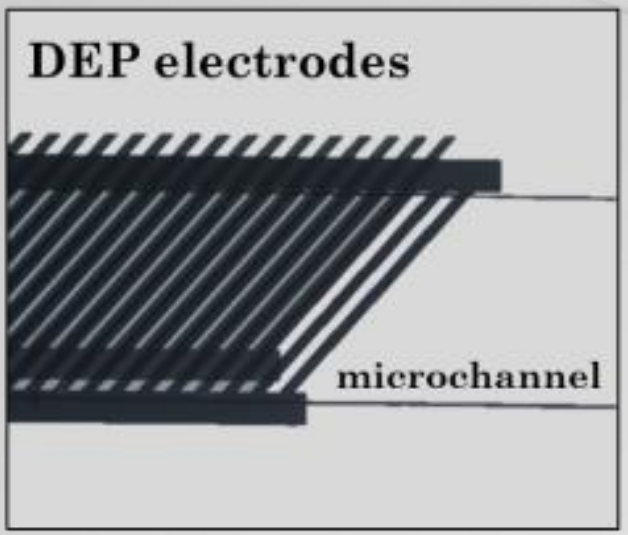
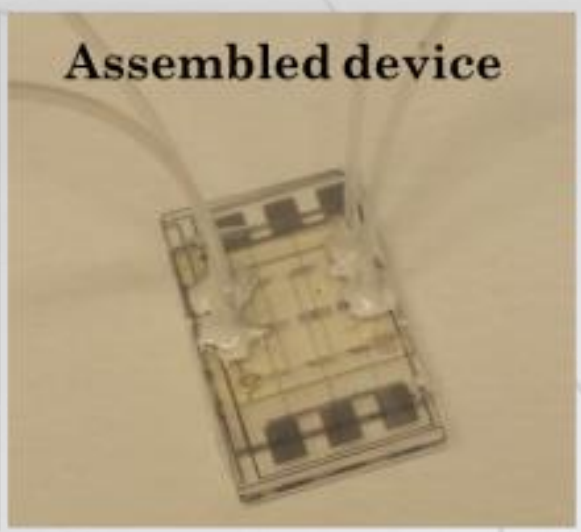
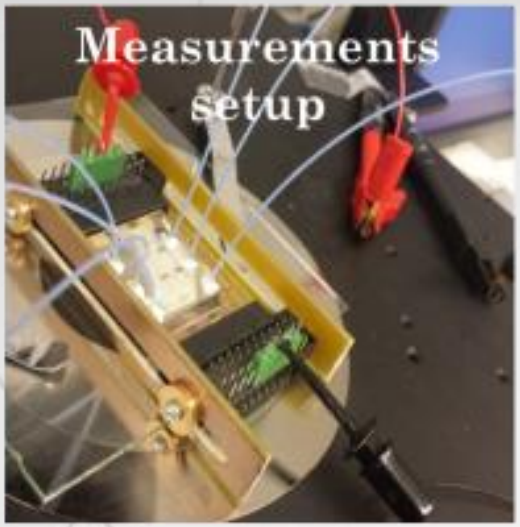
DEP separation

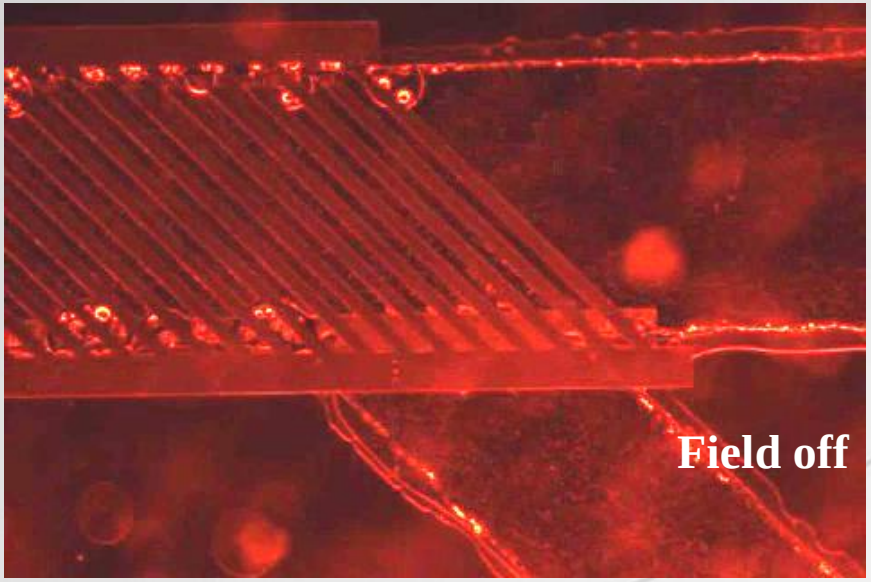


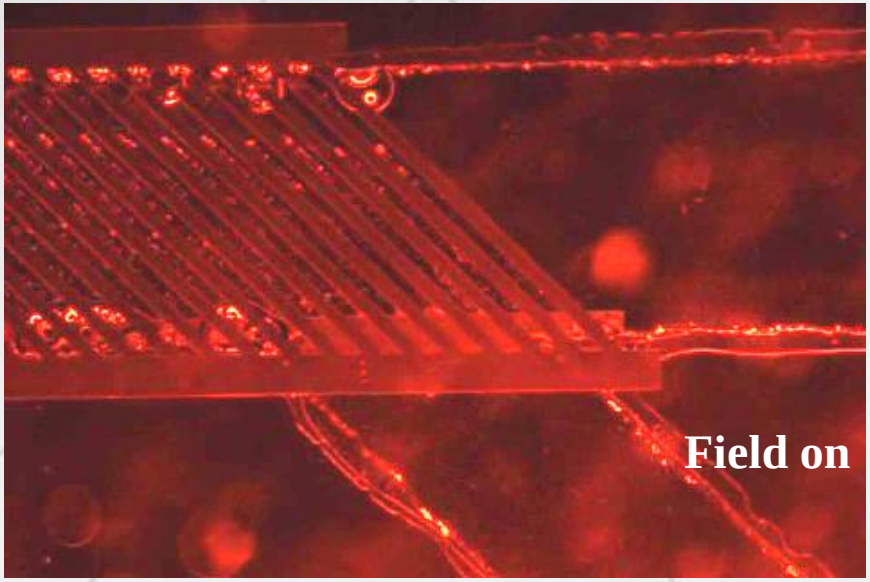
DEP devices for sorting (in Lecce)











In collaboration with V. Arima and F. Calabi

Rinovatis Project

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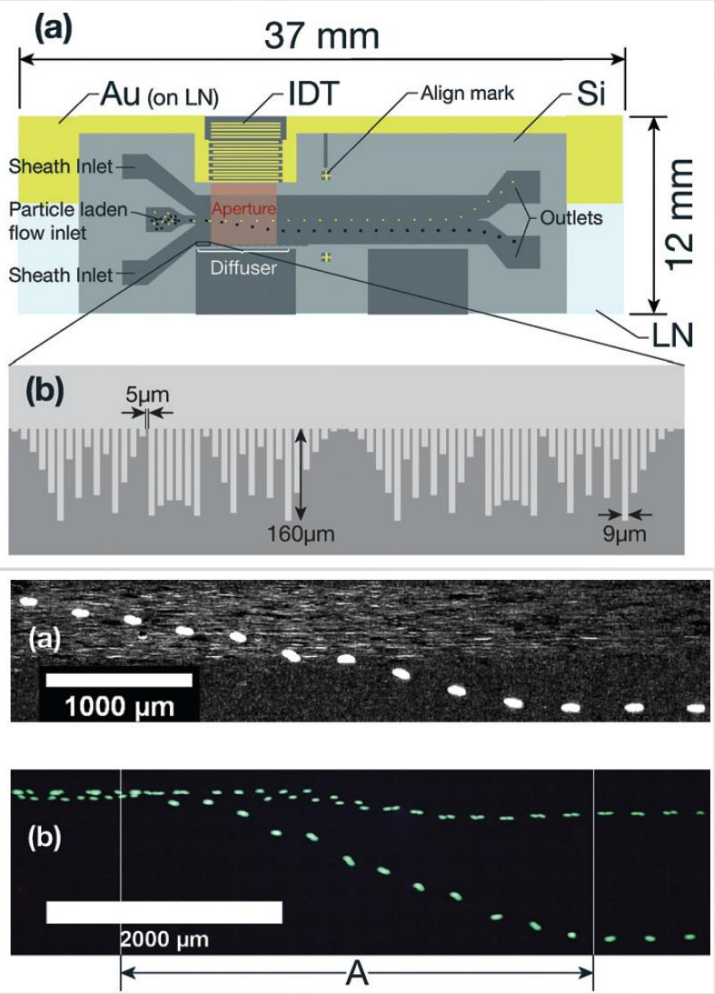
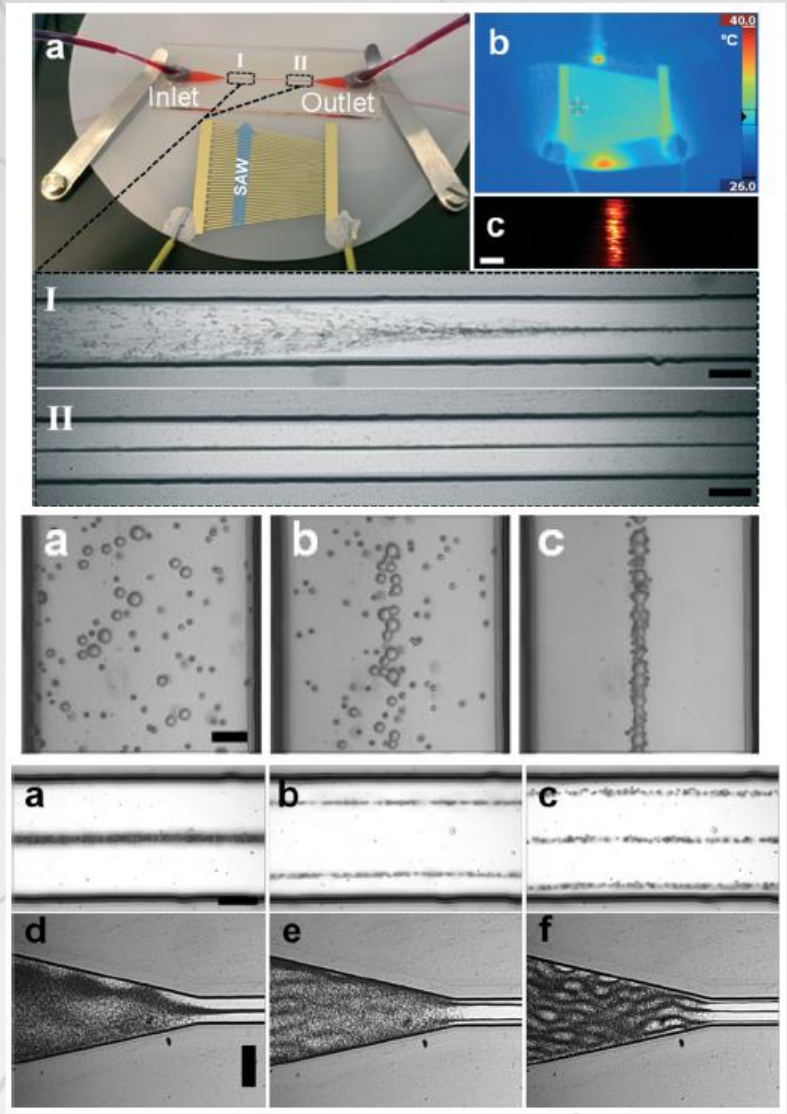
SAW Separation



SAW microfluidic sorting device able to displace and separate particles of different diameter in aqueous suspension.

Lab Chip, 2015, 15, 43



Lab Chip, 2014, 14, 4277

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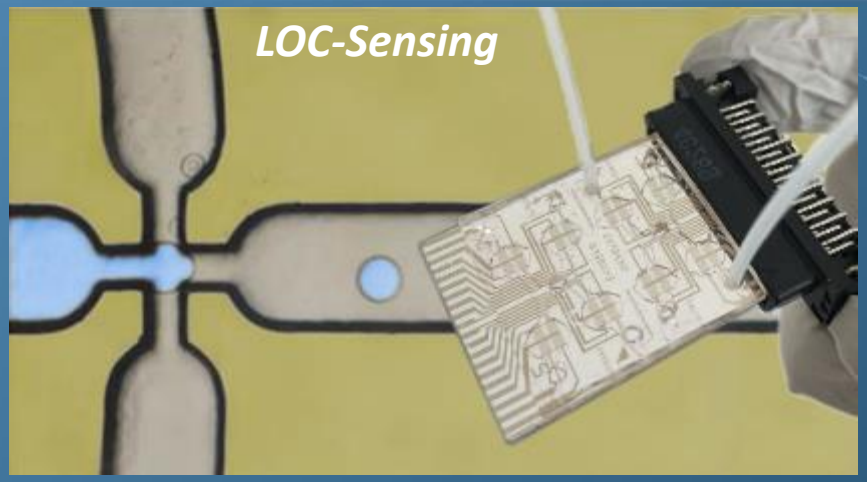
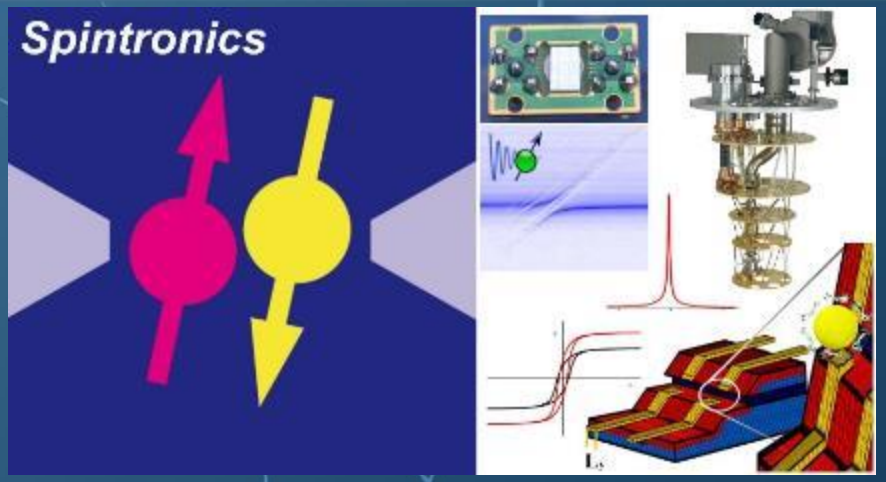
Acknowledgments

Research group





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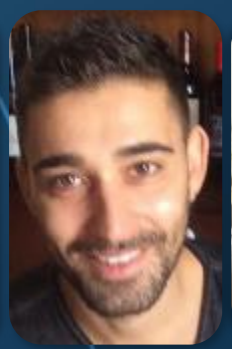

Angelo Leo

Fausto Sirsi

Past members: I. C. Lekshmi, S. Kumar, S. Karmakar, Z. Ameer, M. Scigliuzzo

Open for visitors and joint activities

Thanks for your attention

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MADiA MAgnetic DIagnostic Assay for neurodegenerative diseases