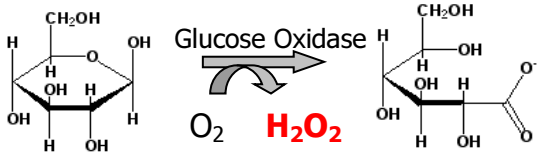
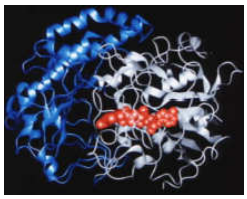
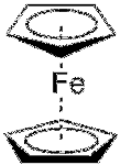
Welcome to Lecture 10

Last time: Glucose monitoring for diabetics



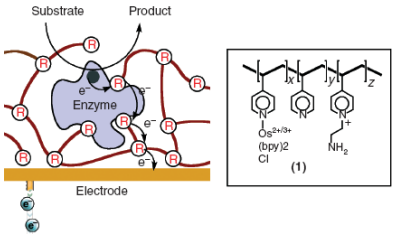


Mediators: replace natural substrate in co-enzyme regeneration reactions

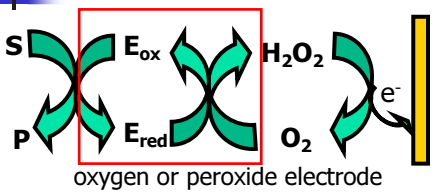


Ferrocene is one of the most widely used mediators in electrochemical biosensors

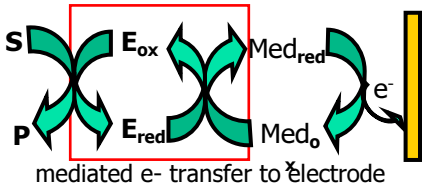
Enzyme wired electrodes:



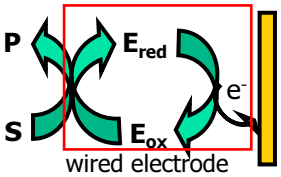
Three generations



oxygen or peroxide electrode

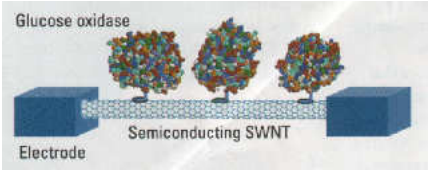


mediated e- transfer to electrode

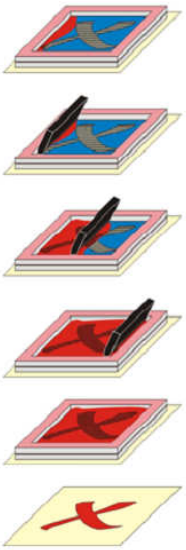


wired electrode

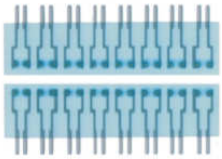
Testing Made **Small and Simple**



Screen printing technology



A modern production scale printer



Screen printed electrodes

Business models

Hardware or disposables?

Making money on Pheromatch…

Who is the customer – dating agencies or individuals?

Any more bright ideas ☺?

In fact, this business exists:

http://www.scientificmatch.com/html/index.php

Launched at the end of 2007 (Boston, MA)

ScientificMatch.com

why pay matchmaker prices when you can have chemistry?

our private, secure, personalized system will find **you** the most perfect matches possible.

Chances are increased that you'll love the natural body fragrance of your matches

ScientificMatch uses your DNA* to maximize the chances of finding chemistry—**actual, physical chemistry**—with your matches

1/2 price extended! Just 1,000 bucks for perfection ☺

Our February promotion was so successful, we want to keep it going--for a full year! Through Valentine's Day of '09, join us for **50% off.** Get a **lifetime membership** for only $995.

*Only a few genes are analyzed

Three component matching: chemistry, values, personality

Plan for today

- Many modern optical transducers exploit surface-specific phenomena, while largely ignoring what happens in the bulk of the sample, i.e. only events that occur at the surface of transducers are observed and measured
- There are two basic types:

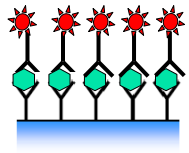
  (i) transducers that rely on the use of a particular label (e.g. fluorescence)

  (ii) label-free sensors (e.g. detection of mass change within a certain layer at the surface)
- Both types enable real-time detection of analyte binding within a surface confined volume

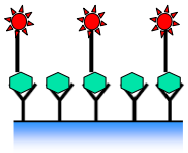
Today: SPR and QCM

What is the difference?

Indirect assay: labeling (e.g. fluorescence) is required to detect binding

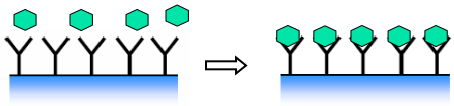


Sandwich assay



Competitive assay

Direct assay: the binding event is detected DIRECTLY – no labels used



Regardless of how the measurement is done, there are some important common characteristics

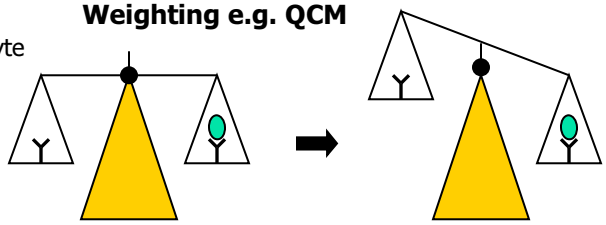
Label-free detection

How can we detect binding?

Ab

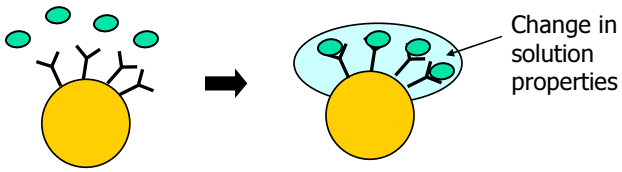
Analyte

Weighting e.g. QCM



Today we will discuss the technologies that enable to do just this

SPR and others



but first, more on the properties of light

Light beam at the interface

The velocity of light *v* depends only on the material in which it travels, and it is determined by the index of refraction, n

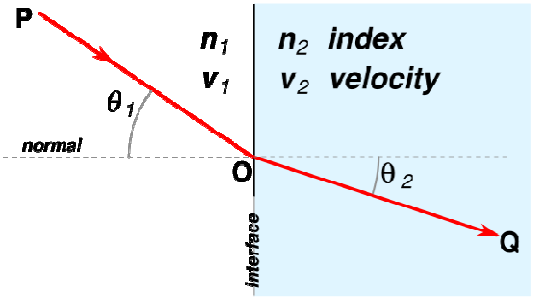
n = c/ *v*, where c is the speed of light in vacuum and v is the speed of light in material

For example, typical glass has a refractive index of 1.5; this means that light travels at 1/1.5 = 0.67 times of its speed in the vacuum

- When light is incident on an interface between two media of different refractive indices, a part of the beam will be transmitted into the 2nd media and a part will be reflected
- In addition to being partially reflected, the light is also refracted (i.e. "bent") at the interface

Refraction

Refraction is the bending of a wave when it enters a medium with a different n (travels with different speed)



The extent to which the light beam is bent is described by Snell's law of refraction

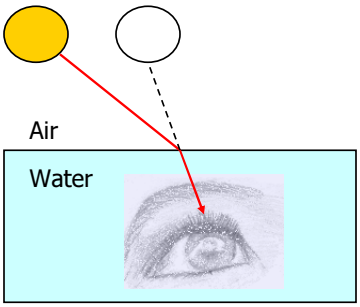
$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{v_1}{v_2} = \frac{n_2}{n_1}$$

or $n_1 \times \sin\theta_1 = n_2 \times \sin\theta_2$

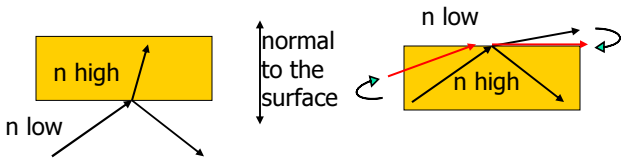
Note that in the case of θ1 = 0° i.e. light beam is perpendicular to the surface, θ2 = 0° regardless of the values of n1 and n2. Hence, light entering a medium **perpendicular to the surface is never bent**

Familiar phenomenon

Sunlight always appear to shine down steeply, even when the sun stands low



Total internal reflection



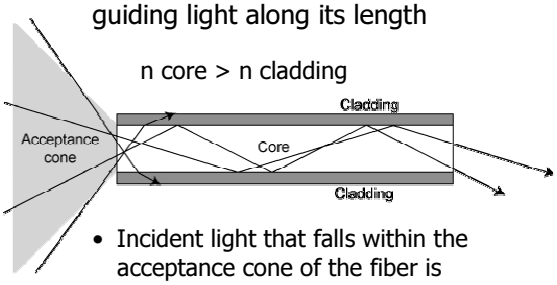
When light goes from low to high refractive index medium it is <u>bent towards</u> the normal to the interface

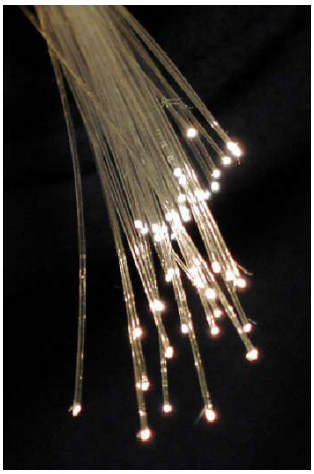
When light goes from high to low refractive index medium it is <u>bent away</u> from the normal to the interface

- Changing the incidence light angle, when going from a high to low refractive index media, changes the out-coming light until a critical angle (θc) is reached. At this point, the light leaves the high refractive medium at a tangent to the interface
- At angles above the critical angle all of the incoming light is reflected. This is called total internal reflection (TIR)

Optical fiber

An optical fiber is a glass or plastic "string" which is designed to keep light inside its "core" by total internal reflection, thus guiding light along its length





- Incident light that falls within the acceptance cone of the fiber is transmitted and the light outside of the acceptance cone is lost in the cladding
- However, not all the light energy in the fiber is confined to the core, some fraction of the energy travels in the cladding as an evanescent wave

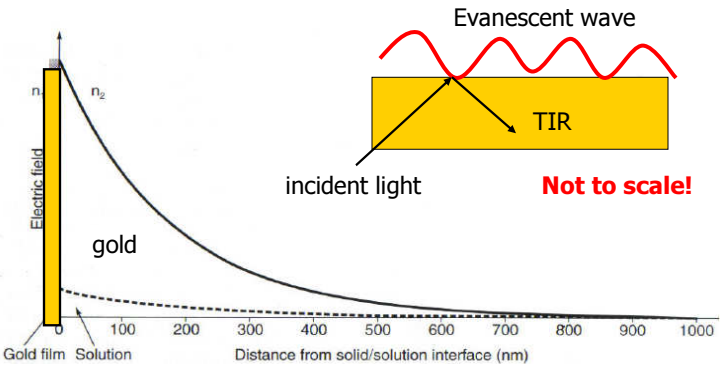
Evanescent wave

- In TIR the incident light is not totally reflected at the interface, some of its energy (the electromagnetic field component) still penetrates a short distance into the second medium with lower refractive index. There it forms an electromagnetic field that oscillates with the same frequency as the incident light, creating the so-called **evanescent wave**
- The evanescent wave can go back into the first medium as a reflected ray, and it's energy can also be absorbed or used to excite fluorescence just as the incident light
- The evanescent wave is confined to the interface, which makes it an **ideal tool to look at various interfacial phenomena…**

http://www.olympusmicro.com/primer/java/tirf/evaintensity/

Evanescent wave penetration

The main distinction of the evanescent wave is that it only penetrates a very short distance into the medium and then decays exponentially as it moves away from the surface



Evanescent electric field amplitude vs distance from the surface

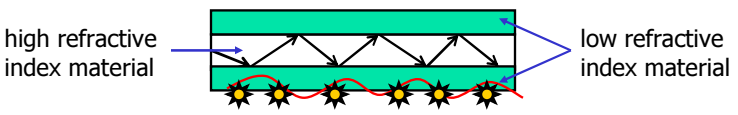
Applications in sensors

The confinement of the evanescent wave to the interface enables us to **exclusively** monitor events at or near the surface as the bulk of the sample was not even there

For example, in the case of TIR/evanescent wave fluorescence measurements the excitation (or emitted) light does not even have to get into the sample compartment – thus, much less non-specific signal (noise) is generated

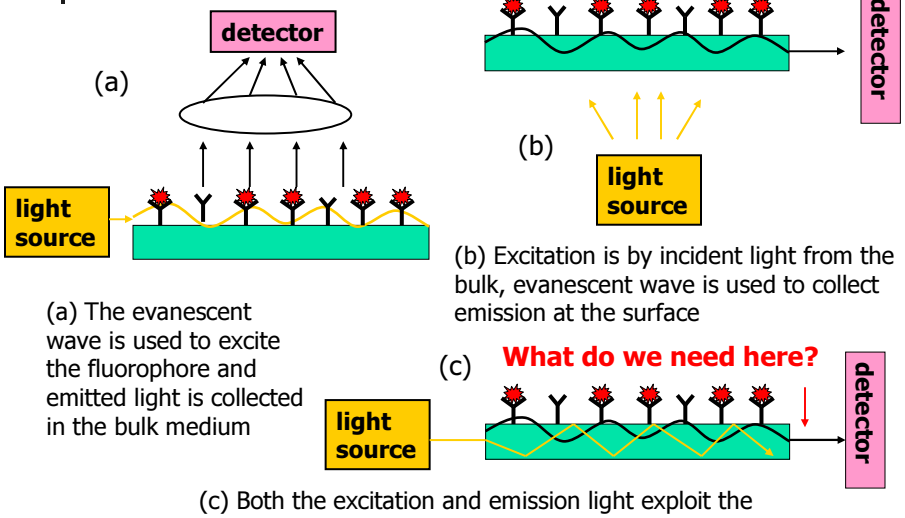
A simple planar waveguide:

Total internal reflection at $\theta > \theta c$



Evanescent wave excites fluorophores exclusively near the surface

Biosensor's configurations



(a) The evanescent wave is used to excite the fluorophore and emitted light is collected in the bulk medium

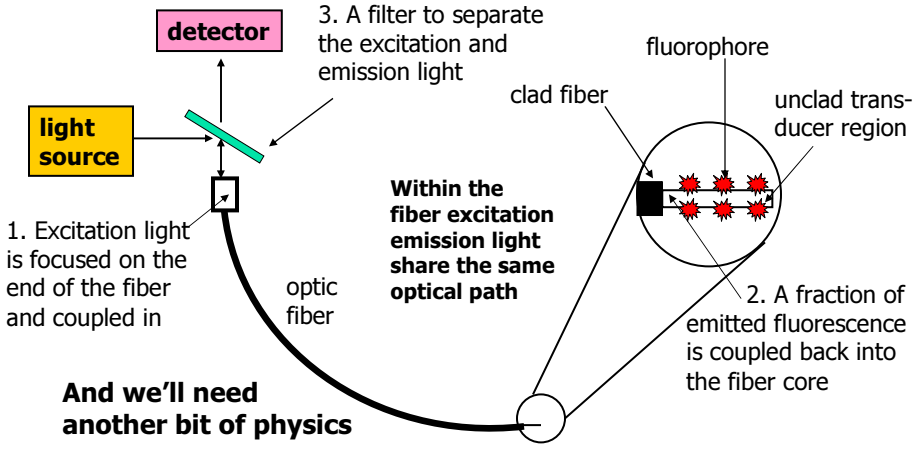
(b) Excitation is by incident light from the bulk, evanescent wave is used to collect emission at the surface

(c) **What do we need here?**

(c) Both the excitation and emission light exploit the evanescent wave; the light travels through a waveguide.

A fiber optic biosensor

Stripping the cladding from the end section of the fiber is one of the most common ways of using optical fiber as a transducer

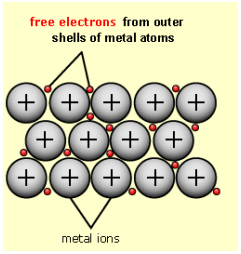


And we'll need another bit of physics

Plasma

- **Plasma** is a conductive assembly of charged or partially ionized* particles e.g. an ionized gas

Does this sound familiar?



Due to its unique properties plasma is considered to be a distinct state of matter (separate from gases) and, in fact, it is the most common form of matter in the Universe

*"Ionized" is a reference to the presence of free electrons, which are not bound to a particular atom or molecule

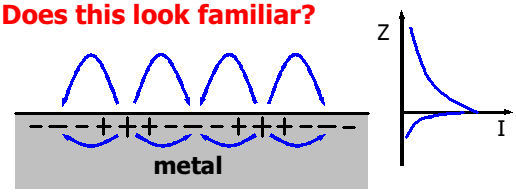
Plasmons

Just as photons are quantizations of light, **plasmons** are quasi-particles resulting from the quantization of plasma oscillations i.e. collective oscillations of the free electron gas density

Plasmons confined to the surface are called **surface plasmons**

Surface plasmons respond strongly to electromagnetic fields e.g. light in the visible range of the spectrum. The charge density oscillations of surface palsmons are called surface plasmon-polariton waves

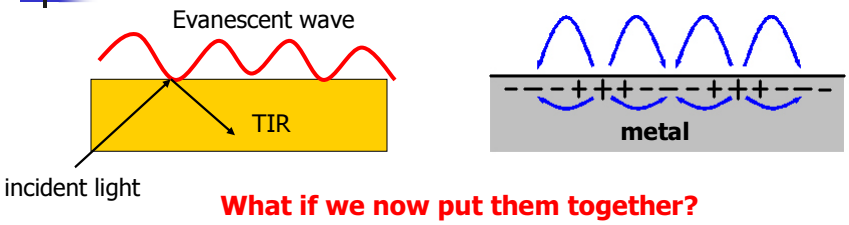
Does this look familiar?



Note the exponential dependence of the electromagnetic field intensity on the distance away from the interface

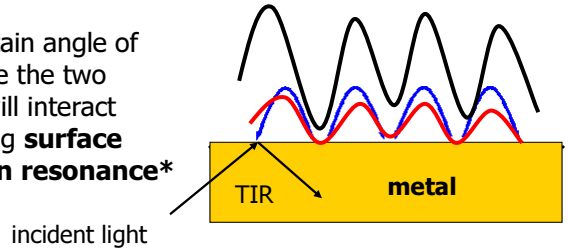
Schematic representation of an electron density wave propagating along a metal/dielectric interface

Surface plasmon resonance



What if we now put them together?

At a certain angle of incidence the two waves will interact producing **surface plasmon resonance***



*Resonance is a vibration of large amplitude in a mechanical or electrical system caused by a relatively small periodic stimulus

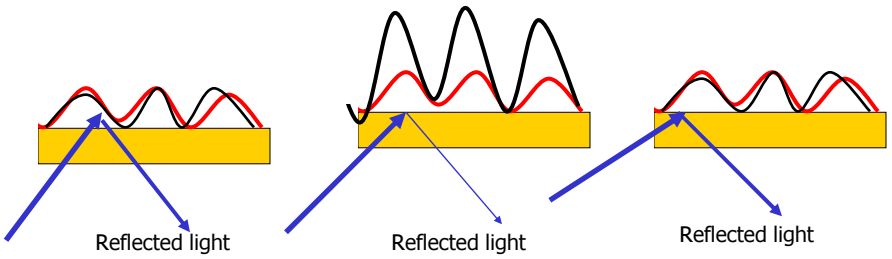
SPR sensing technology

- Surface plasmons can be efficiently excited with polarized* light in the visible range of the spectrum
- The SPR technology is based on the observation that under certain conditions, photons from incident light can excite surface plasmons on metallic slabs (e.g. gold), thereby transforming the energy of light (photons) into the energy of surface plasmons
- When a plasmon is excited, a photon disappears; this results in decreased intensity of reflected light. In essence, plasmons "highjack" the energy and less light is reflected as a result
- The energy transfer between the photons and plasmons depends on the angle of incidence light

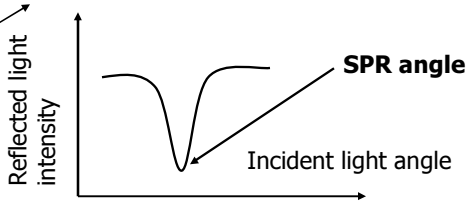
*A wave with electric field oscillating in the same plane

Surface plasmon resonance

At a certain (resonant) angle, when the energy transfer is maximal, a pronounced dip in the intensity of reflected light is observed - this angle is measured by a CCD chip



Plasmons "highjack" the energy of the incoming light and less light is reflected – a dip is observed at a resonant angle of incident light

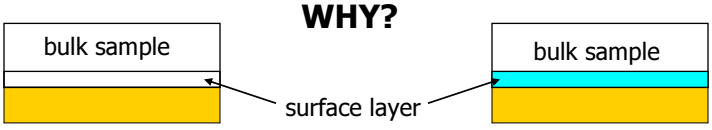


Measurements

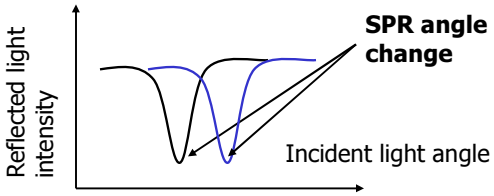
Why would we want to measure the resonant angle? Any ideas?

Actually, we do not… What we really want to measure is **the change** in the resonant angle

WHY?

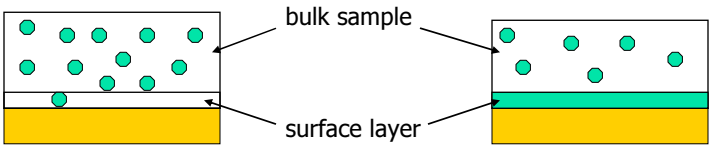


Because if we see a change in the resonant angle, we know that the refractive index at the surface has changed

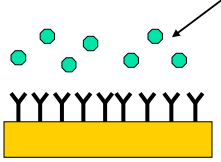


Analyte binding

Binding of analytes e.g. proteins results in a resonant angle shift – the more bound, the larger the shift

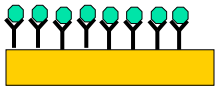


Change in refraction index



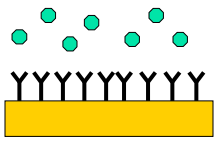
at RT and ~600nm n for H_2O – 1.333

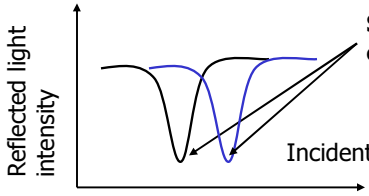
For protein solution 0.1g/ml – Δn=0.0188



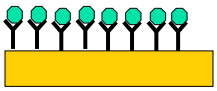
Surface plasmon resonance

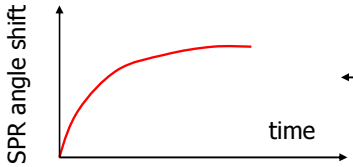
Thus, SPR is an optical method for measuring the refractive index of very thin layers of material adsorbed on a metal





For biosensors this is caused by the binding of analytes



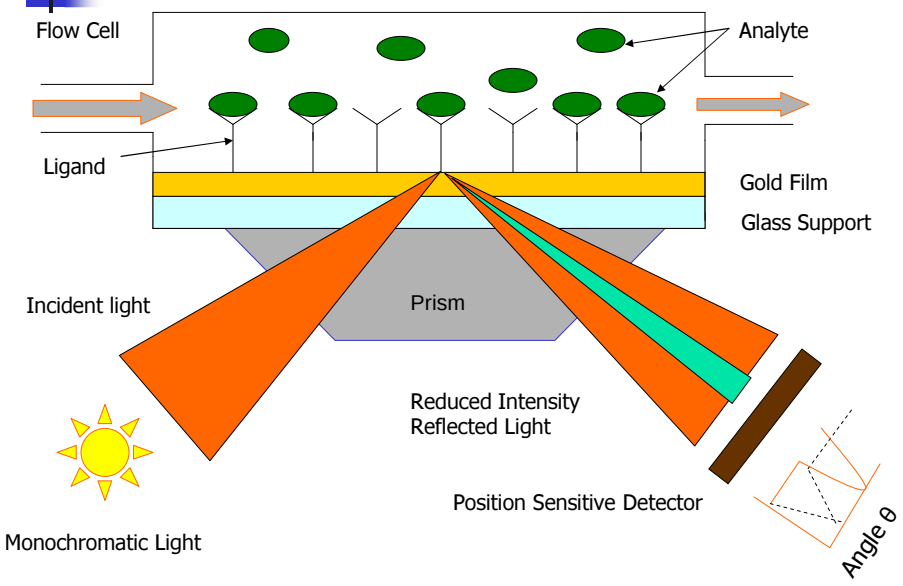


With SPR we can also follow the kinetics of analytes binding/desorption **in real time**

SPR measurements

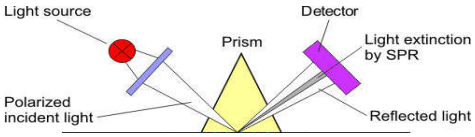
- For a certain angle of incidence, much of the illumination energy is absorbed in a surface plasmon wave traveling along the gold surface
- This resonance angle strongly depends upon the refractive index of the material immediately adjacent to the metal (gold) surface; determination of this angle provides a sensitive measurement of refractive index near the surface
- Because many biological and chemical substances have higher RI than water, the presence of tiny quantities of such substances on the surface can be detected and quantified in real time
- To make the sensor specific for the substance of interest an appropriate bioreceptor is attached to the surface – the result is a **SPR biosensor**

Let's build an instrument



The working principle

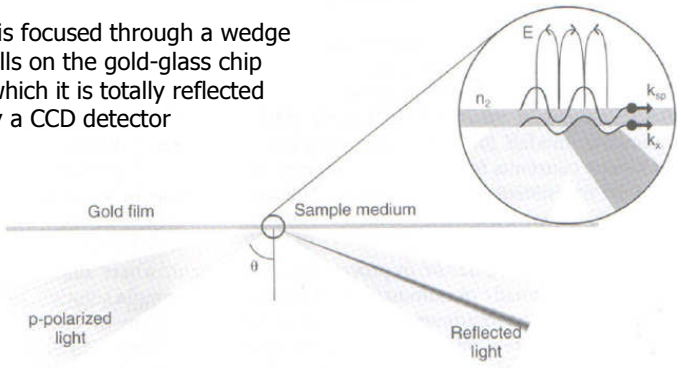
Summary



The light beam is focused through a wedge shaped prism falls on the gold-glass chip interface from which it is totally reflected and analyzed by a CCD detector

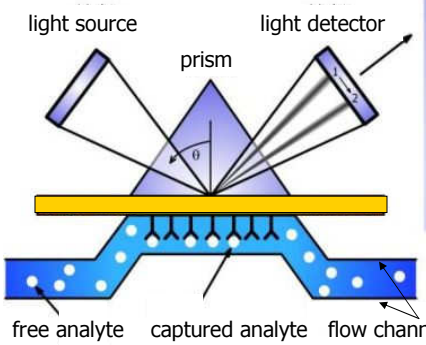
At certain θ a coupling between the incident light and the surface plasmons occurs

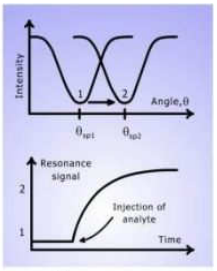
Because the intensity of the field decays exponentially as a function of distance from the interface, it is only sensitive to changes that occur at the biosensor's surface e.g. binding



The working principle

Binding of the target analyte to the sensor results in the increase in refraction index of the medium <u>at the surface</u> and, hence, the increase in the angle at which the resonance is observed

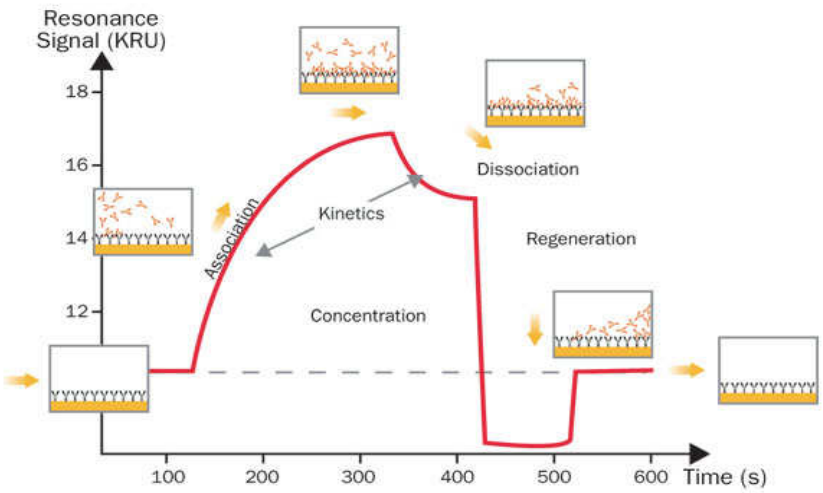




Sensorgram

By continuously monitoring the difference, the binding event can be observed in real time

The actual experiment



And the instrument

http://www.biacore.com/

Similar laboratory instruments are now sold by other companies too:

- Leica Microsystems
- MWC Technologies
- IBIS Technologies
- Toyobo Co., Ltd

Developed and commercialized by Biacore (Pharmacia, Sweden) in the 1990s

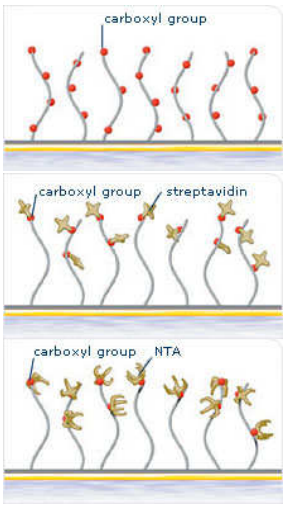
In 2006 Biacore was acquired by GE Healthcare for $390M

Sensor chips

Autosampler – suitable for HTS

Classic biacore chips use Dextran but thiol on gold SAMs can be used too

Biacore sensor chips



Surface coverage and sensitivity

Typically, the sensitivity depends on both the transducer and the biosensor characteristics

Consider a binding event, where receptor R bind analyte L

$[R] + [L] \rightleftharpoons [RL]$ and hence $\frac{[RL]}{[R][L]} = K$

Let's use equation $[Rt] = [R] + [RL]$ to substitute R (t - total)

$$K = \frac{[RL]}{[R][L]} = \frac{[RL]}{([Rt]-[RL])[L]} = \frac{[RL]}{[Rt][L]-[RL][L]}$$

then, $K\,[Rt]\,[L] = K\,[RL]\,[L] + [RL] = [RL]\,(1 + K[L])$

For reactions on a surface, let's introduce surface coverage, Γ

$$\frac{\Gamma}{\Gamma_{max}} = \frac{[RL]}{[Rt]} = \frac{K[L]}{1+K[L]} \quad \text{or} \quad \Gamma = \frac{\Gamma_{max}\,K[L]}{1+K[L]}$$

What does this mean?

A transducer would generate a signal proportional to the number or surface density of analyte at the surface e.g. a mass of analyte (direct sensing) or concentration of label (indirect sensing)

Let's work out the expression for min detectable coverage

$$\Gamma_{min} = \frac{\Gamma_{max}\, K[L_{min}]}{1+K[L_{min}]}$$

solving for Lmin, and assuming Γmax >> Γmin, we obtain

$$[L_{min}] = \frac{\Gamma_{min}}{K\,(\Gamma_{max} - \Gamma_{min})} \sim \frac{\Gamma_{min}}{K\ \Gamma_{max}}$$

Hence, the minimum detectable coverage (i.e. sensitivity) is better for receptors with high binding constants (K) and high surface coverage Γ

Selectivity, on the other hand, would depend on the properties of the receptor and the nature of the sample

SPR: key features

- No labeling requirements and real-time analysis are two key features of SPR technology
- Compared to some other label-free interaction technologies SPRs have higher throughput
- Optical configuration and sensor device are simple i.e. no optical gratings and no moving parts
- Gold provides an excellent surface for ligand immobilization; other metals or semiconductors can be used too
- Real-time monitoring makes it possible to extract detailed information about binding events, including the association and dissociation rate constants, and Kd. Very useful for e.g. pharma applications

Pharma: lead optimization

Drug + receptor =

D-R complex

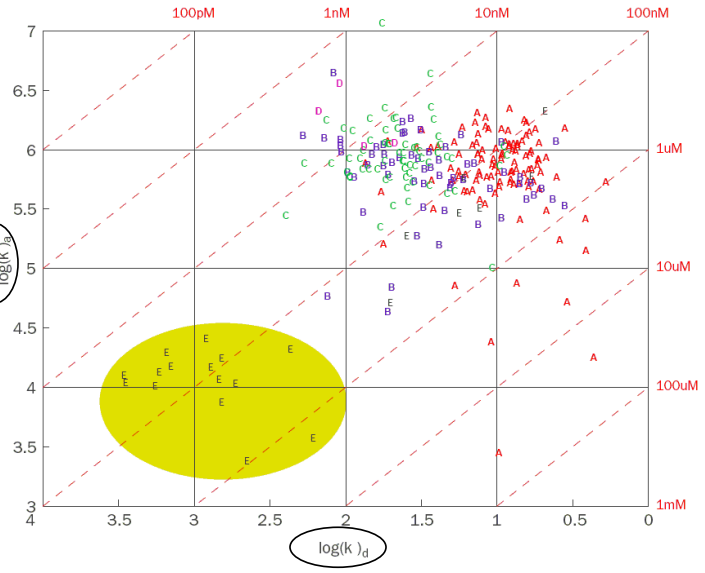
$$K = \frac{[D\text{-}R]}{[D][R]} = \frac{ka}{kd}$$

where ka and kd are rate constants

$$[D] + [R] \xrightarrow{ka} [D\text{-}R]$$

$$[D\text{-}R] \xrightarrow{kd} [D] + [R]$$

Compounds are grouped according to ka and kd

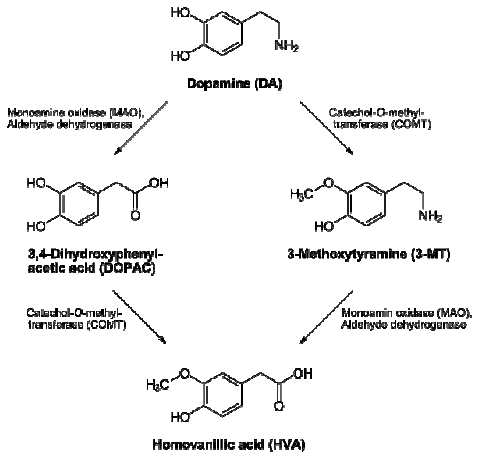


Lead optimization

- Drug under study is a T-Cell activation blocker for rheumatoid arthritis
- A library was screened and results are grouped (A-E) depending upon kinetic characteristics of the compounds tested
- A-D group showed high affinity, while group E showed lower affinity
- If analyzed by other method compounds in group E would be discarded
- However, SPR analysis revealed that group E compounds have slow dissociation kinetics (yellow shaded region) – a VERY useful feature – and this compounds were further optimized to improve affinity

Dopamine sensing

DA is an important neurotransmitter in CNS implicated in disorders such as the Parkinson's disease; concentrations in biological samples ranges from 10^{-9} to 10^{-5}M

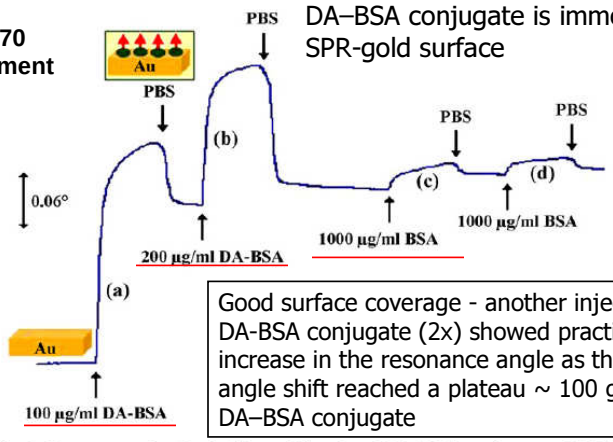


- Metabolites of DA e.g. DOPAC are present in body fluids in high concentrations and cross-reactivity can be a problem
- Also other metabolites e.g. uric acid and ascorbate have similar redox potential and interfere with electrochemical detection

SPR dopamine biosensor

SPR 670 instrument

DA–BSA conjugate is immobilized on SPR-gold surface

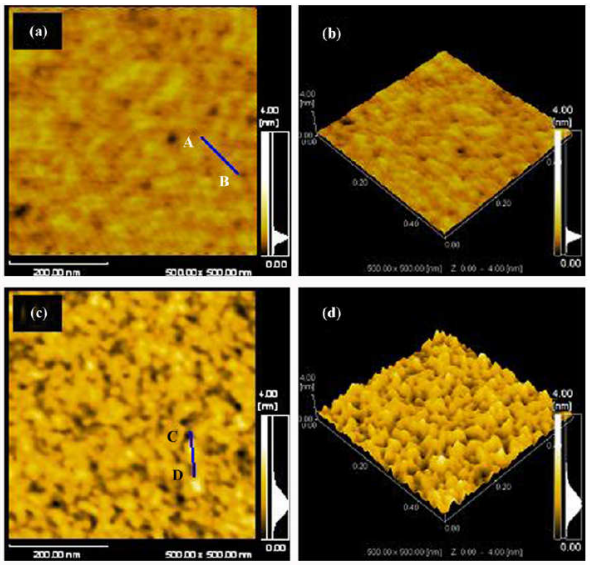


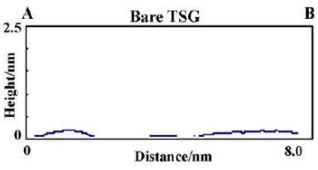
Good surface coverage - another injection of DA-BSA conjugate (2x) showed practically no increase in the resonance angle as the SPR angle shift reached a plateau ~ 100 g/ml of DA–BSA conjugate

Fig. 1. Sensorgram for physical immobilization of DA–BSA conjugate and BSA onto an SPR-gold surface. Carrier solution: PBS, flow speed 20 µl/min, flow duration: 10 min.

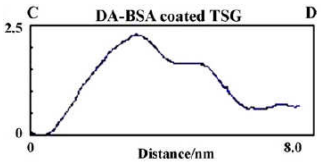
Biosensors and Bioelectronics 23 (2007) 421–427

AF micrographs of bare surface (a and b) and DA–BSA coated surface (c and d) in 2D (a and c) and 3D (b and d) views, respectively.
Scanning area: 500 nm×500 nm; the height profiles are shown at the right





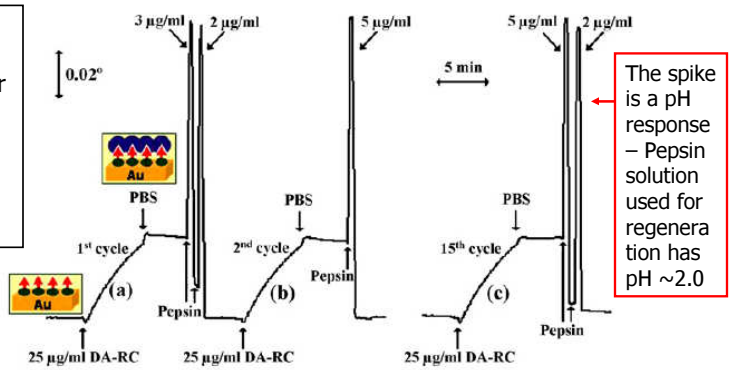
The AF micrographs clearly indicate the stable, complete and homogeneous immobilization of the DA–BSA conjugate over the gold surface



Selectivity, sensitivity and…

Reproducibility: the sensor is regenerated by washing with Pepsin solution to dissociate tertiary DA-RC-BSA complex

Dopamine receptor (DA-RC) is used for detection to attain good selectivity and to enhance sensitivity

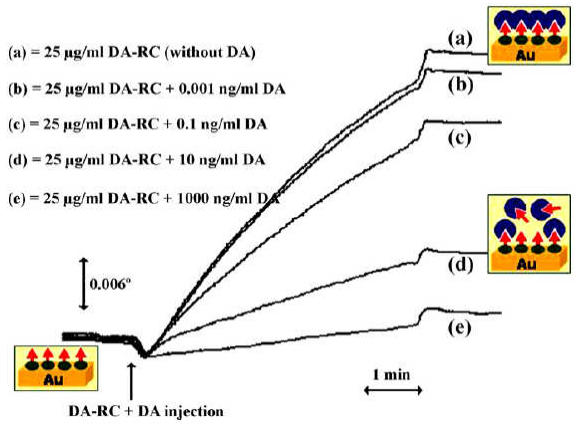


The spike is a pH response – Pepsin solution used for regeneration has pH ~2.0

Sensorgrams for the binding interaction of DA–BSA conjugate with dopamine receptor - DARC. (a) 1st Cycle, (b) 2nd cycle and (c) 15th cycle

Sensor response to DA

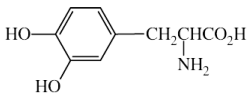
Overplayed sensograms at different [DA]

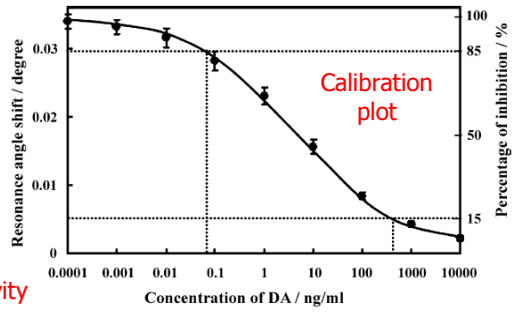


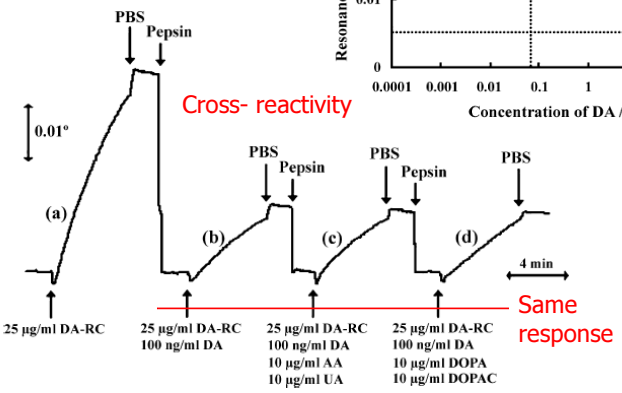
Carrier solution: PBS, flow speed: 40l/min, flow duration: 5 min

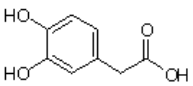
Selectivity

3-(3,4 dihydroxyphenyl)-alanine, L-DOPA









3,4 dihydroxyphenyl acetic acid, DOPAC

AA -ascorbic acid;
UA- uric acid

Defense applications

Fabrication of a novel immunosensor using functionalized self-assembled monolayer for trace level detection of TNT by surface plasmon resonance

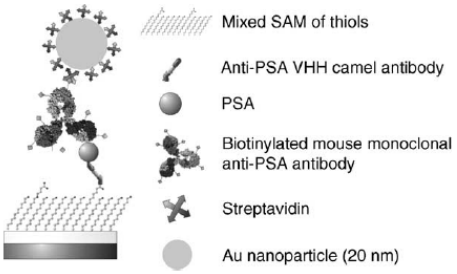
- TNT is a well-known explosive compound used in the preparation of landmines for military and terrorist activities
- Contamination of soil and ground water with TNT is of the major concern because of its biological persistence, toxicity and mutagenicity.
- Thus, detection of TNT is of tremendous importance in wide areas including landmine detection, environmental monitoring and homeland security

Talanta 72 (2007) 554–560

Early detection cancer

Prostate-specific antigen (PSA) – a biomarker present in cancer patients' serum

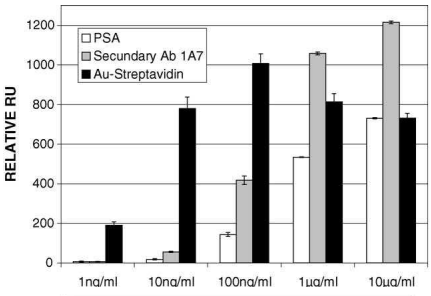
PSA sandwich assay in SPR biosensor

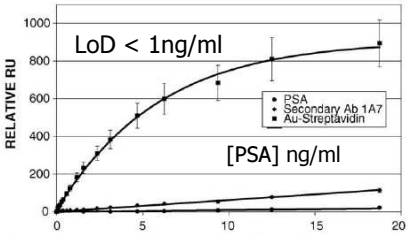


There is no effective cure for the advanced stage metastatic prostate cancer - early detection is critical

Biosensors and Bioelectronics 21 (2005) 483–490

Biacore 2000





TNT Biosensor

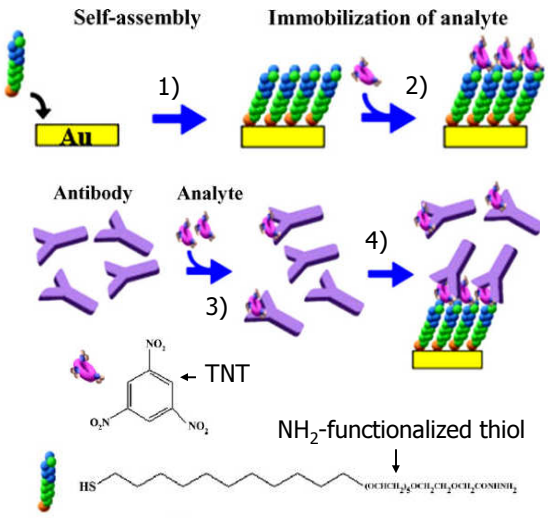
Indirect competitive immunoassay:

1) Self-assembled layer of PEG-NH_2 on gold surface

2) Immobilize TNP-derivative over the PEG-NH_2 SAM surface (amide coupling) with TNT "sticking out" to allow for binding with a TNT-specific antibody

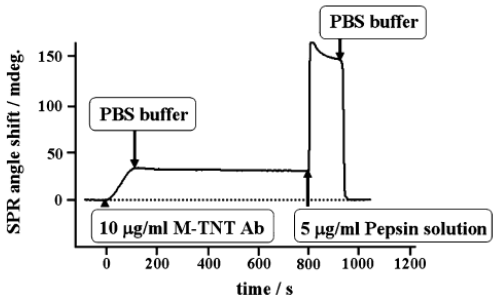
3) TNT is mixed with a fixed concentration of antibody (M-TNT Ab) and are allowed to incubate for 10 min at RT

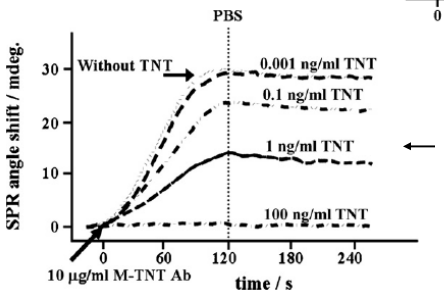
4) TNT in the sample binds to the Abs, thus reducing the amount of Abs that can bind to the immobilized TNT



Biosensor's response

SPR response for the flow of 10g/ml M-TNT Ab over TNT immobilized PEG-NH_2 SAM surface followed by elution with pepsin solution



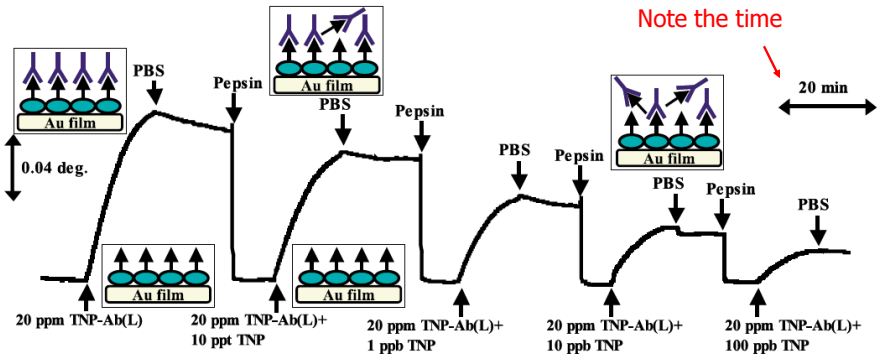


SPR response for the immunoreaction between M-TNT Ab and TNT immobilized PEG-NH_2 SAM surface in the absence and in the presence of TNT

The signal goes down ☺

Performance

Consecutive injection of TNT samples

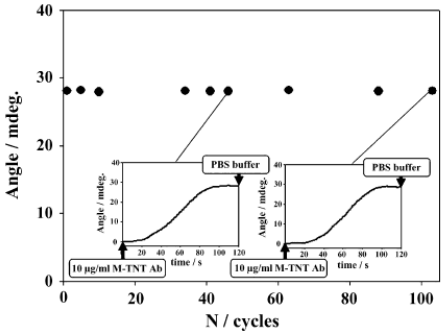


SPR response of to TNT solutions of various concentrations

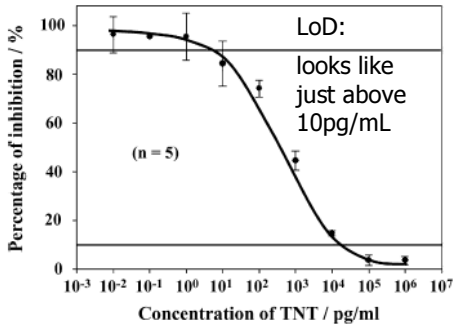
Biosensors and Bioelectronics 20 (2005) 1750–1756

Sensitivity and stability

Biosensor's stability and reproducibility of the response during multiple detection cycles



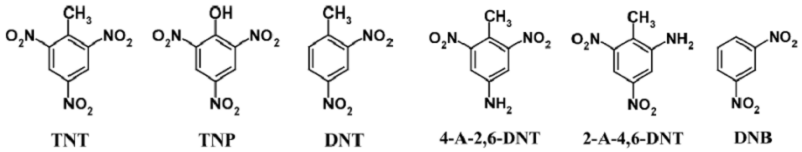
Calibration curve and sensitivity of TNT detection



Several reports show 100+ analysis/regeneration; in a couple of cases 500-1,500 surface regeneration cycles were reported

Selectivity

Cross-reactivity of nitroaromatic derivatives in SPR TNT assay



TNT TNP DNT 4-A-2,6-DNT 2-A-4,6-DNT DNB

Analyte	IC50 (ng/ml)	Cross-reactivity, %
TNT	0.4	100
2,4-DNT	65	0.61
1,3-DNB	48	0.83
2A-4,6-DNT	115	0.35
4A-2,6-DNT	470	0.09
TNP	140	0.29

It's just a matter of finding the right Ab

Very cool sensor but it is too big to take on a field trip ☹

Is there anything we can do about it? **After the break**

Size matters

The pharmaceutical industry is more than happy to use bulky lab instruments as long as they work well

However, for many other applications (e.g. defense, environment, food safety, point-of-care diagnostics), it would be MUCH more useful to have a small and/or low cost instrument

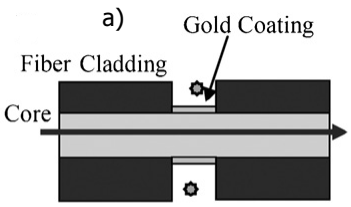
It is also desirable to have a portable muli-channel SPR so that several surveillance targets can be dealt with at once e.g.

- Various biological and chemical agents
- A number of environmental pollutants
- Several pathogenic microorganisms at once

Can SPR be miniaturized?

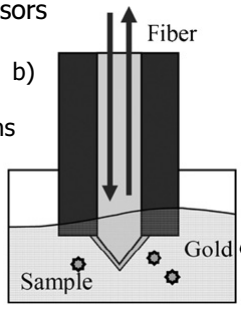
Miniaturization

Much attention is now focused on developing integrated portable and reusable low cost SPR biosensors

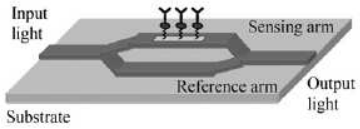


Optical fiber SPRs

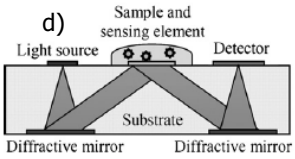
Two fiber-SPR configurations (a) cladding-removed fiber-SPR and (b) micro-prism fiber-SPR



Complex optical functions can be incorporated into a single substrate



Planer SPR biosensors:



(c) waveguide SPR device and (d) diffractive optical coupling elements

Hoa et al (2007) Biosensors & Bioelectronics, 23 151-160

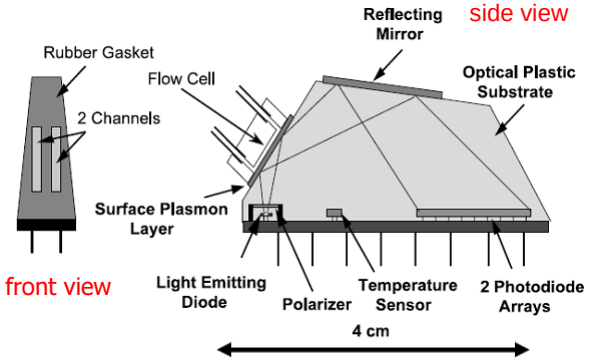
Spreeta by TI

- Spreeta is a miniature, low cost, surface plasmon resonance transducer designed for biosensing applications
- Spreeta is tailored for integration into peripheral technologies that, when combined, form a complete biosensing system
- The Spreeta SPR sensing components developed by Texas Instruments are unique in that the all of the optoelectronic components needed to perform an SPR measurement are incorporated into a rugged molded plastic chip the size of a fingertip

http://www.sensata.com/products/sensors/spreeta.htm

Spreeta in more detail

- A light emitting diode (LED) with a wavelength of 830 nm shines light on the gold-coated glass slide
- After being reflected from the slide surface and a mirror on top of the sensor, the light beam reaches two photodiode arrays (at the bottom)



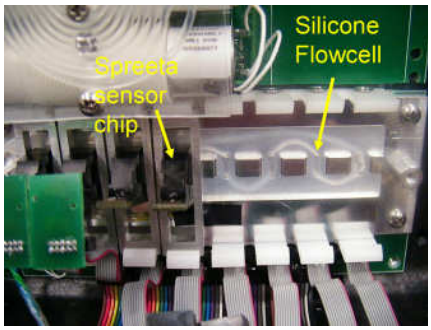
- A flow cell (6 µL) consists of a 6 mm thick Teflon block with inlets and outlets for each channel and a 0.4 mm thick silicone rubber gasket with two side-by-side laser-cut chambers (1/16 mm each) that generates flow cells for the two sensor channels
- The response is digitized by a 12-bit analog to digital (A/D) converter

Spreeta chips in the SPIRIT

- Portable 24-analyte SPR based on TIs' Spreeta sensing chips
- Build as clamshell enclosure the instrument weigh 3 kg measuring 28 cm×22 cm×13 cm
- Multichip design allows for a total detection of 24 areas that can be simultaneously monitored by SPR
- The instrument reports refractive index (RI) values every second, with a typical noise level of 1–3×10^{-6} RI units

Biosensors and Bioelectronics 22 (2007) 2268-2275

Inside the SPIRIT system



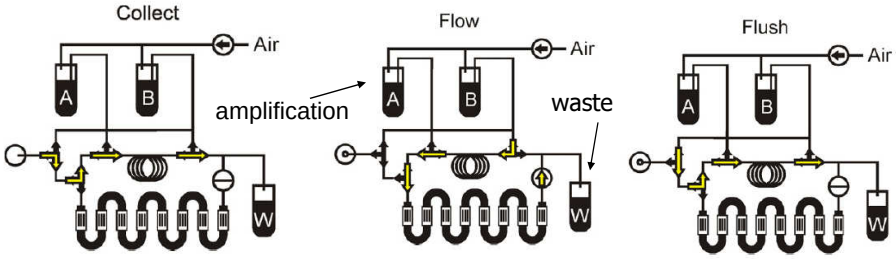
Inside the cell - liquid sample flows over each of the sensor chips in series

Each chip's surface has three sensing areas, each coated with bioreceptors for simultaneous detection of multiple analytes (up to 24 different targets)

- The fluidics assembly is built around 8 three-channel Spreeta 2000 devices fastened into a cast silicone flow-cell
- The flow-cell design allows for easy removal and replacement of individual sensors; the Spreeta sensors slide into mounting brackets on the flow-cell sealing the sensor surface onto the cell's silicone
- The flow-cell is held by an aluminum backing plate equipped with thermoelectric heater/cooler for T control (heat sink attached)

Schematic SPIRIT's fluidics

Three-way valves (arrows) and a peristaltic pump switch control the fluid flow



- In collection, fluid flows from the sample port into the injection loop
- In analysis, the sample flows from the loop into the serpentine flow-cell
- In the flush step, buffer (B) is automatically injected into the sample loop, cleaning the system

In addition, the valves can switch to flow amplifying reagent (e.g. secondary Ab) over the sensors or to wash the sensor surfaces prevent cross-contamination

Analysis sequence

(i) A sample is injected into the sensor's sample port through the injection loop (any excess goes into the waste reservoir, marked W on the previous slide)

(ii) The sample flows over the sensors - when the injection loop filled up, the valves are switched so that fluid flow reverses and the content of the injection loop flow across the sensor

(iii) Binding of analytes to the receptors on the sensor surface is determined and the concentrations are calculated from the corresponding calibration curves

(iv) Wash or regeneration - the flush step is identical to the injection step, except that the flow is directed from the buffer reservoir (B) instead of the sample port

An A/D converter and a microcomputer are used to digitize the outputs and control SPIRIT – that's all ☺

Let's see what it can do

Model studies to demonstrate the potential in biomedical and defense applications

Small molecules: 2,4-Dinitrophenol-L-lysine as a representative small molecule (DNP is used as a model because it is similar in structure and size to the explosive molecule trinitrotoluene (TNT))

Proteins: The bacterial toxin SEB (MW 28,000) and ricin A chain (MW 32,000)

Virus: Norwalk virus is a small (27 nm) highly communicable virus that causes gastrointestinal illness

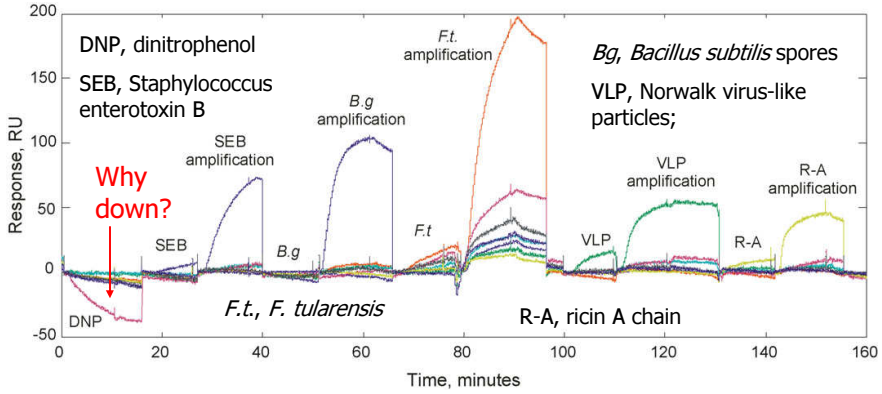
Bacteria: Francisella tularensis LVS, (irradiated), the micro-organism that causes tularemia

Spores: Bacillus subtilis (instead of anthax spores ☺)

Biosensors and Bioelectronics 22 (2007) 2268–2275

Simultaneous analysis

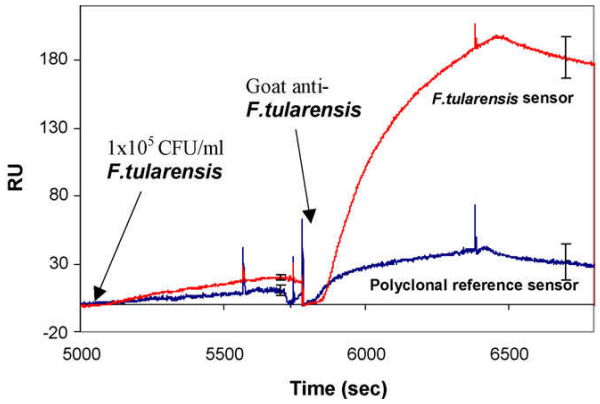
Simultaneous detection of six analyte - overlaid plots of RI traces from each of the 8 three-channel sensors



- High overlay is with secondary Abs to amplify the signal
- The sensor specific for each analyte responds as this analyte is introduced

Amplification

Detection of 1×10^5 CFU/ml F. tularensis



The average response of three sensor amplified goat anti-F. tularensis vs normal goat serum surfaces

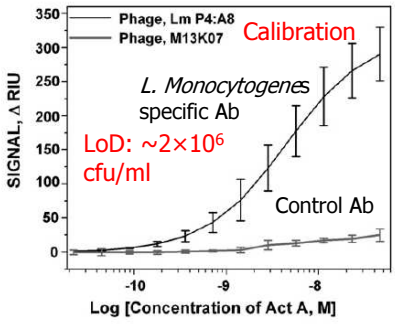
Food safety

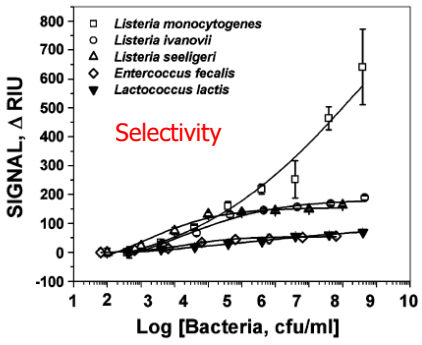
We talked about E coli 1057, remember?

- Food-borne diseases cause an estimated 76 million illnesses, accounting for 325,000 hospitalizations and more than 5,000 deaths in the United States each year
- The economic impact of these four pathogens in the United States is estimated to cost up to US$ 5.4 billion annually for foodborne cases
- Conventional culturing on selective media take days; detection using polymerase chain reaction (PCR) and ELISA also take considerable time, e.g. 6-8 hours
- Inexpensive, rapid and portable Spreeta-based sensor would be really great…

Spreeta in food safety

SPR biosensor for the detection of *L. monocytogenes,* a Gram-positive aerobic bacterium responsible for a number of food poisoning outbreaks



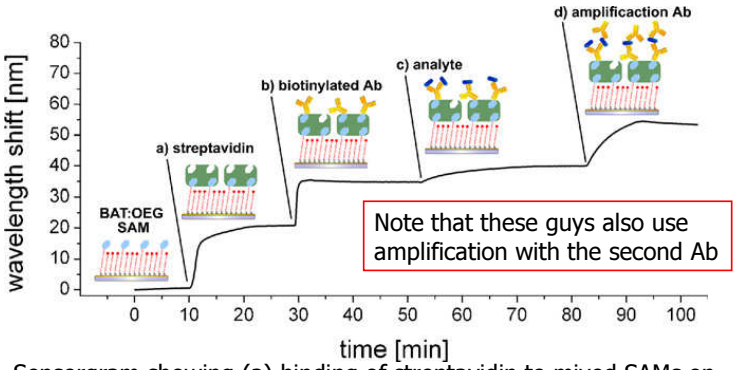


A phage-displayed antibody was used. Remember?

Biosensors and Bioelectronics 23 (2007) 248–252

Or better the whole lot at once

Quantitative and simultaneous detection of four foodborne bacterial pathogens with a an 8-channel SPR biosensor

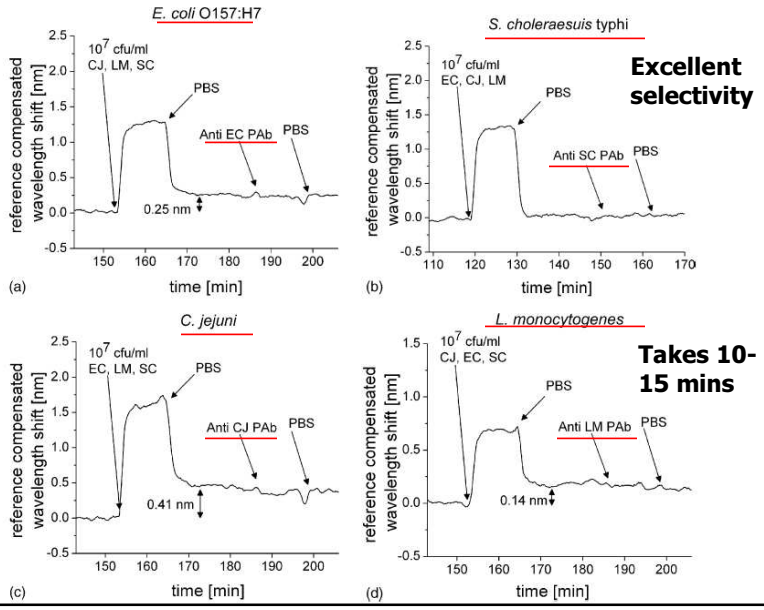


Sensorgram showing (a) binding of streptavidin to mixed SAMs on Au, (b) binding of biotinylated PAb to streptavidin, (c) direct detection of an analyte, and (d) secondary amplification using PAb

Biosensors and Bioelectronics 22 (2006) 752–758

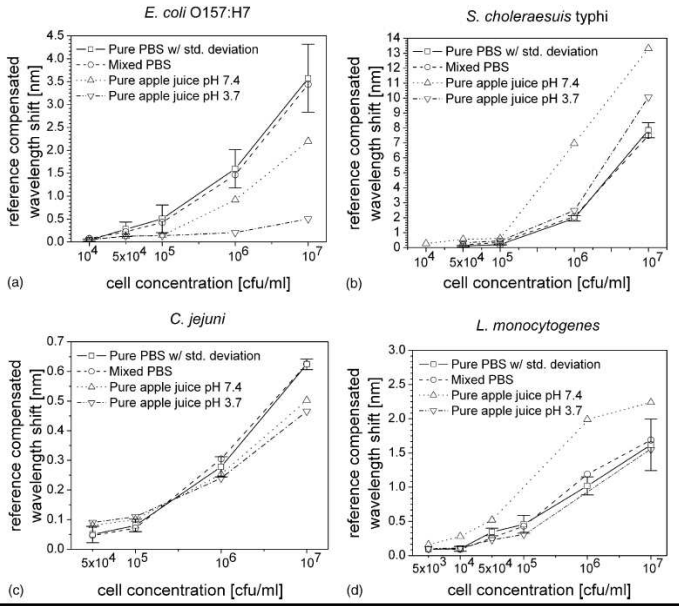
Simultaneous detection of Escherichia coli O157:H7, Salmonella choleraesuis, Listeria monocytogenes, and Campylobacter jejuni

In all four cases no signal on addition of Ab to the target bug (red lines)



What happens in apple juice ☹

Also, only one bug is added to the apple juice, not all four



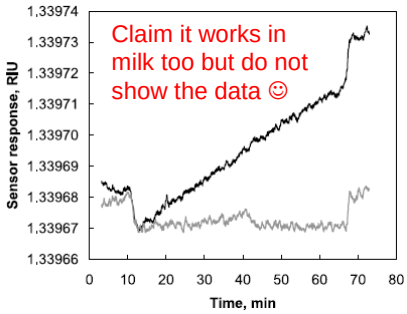
Adjusting pH of the juice seems to help with detection

Detection of toxins

Detection of Staphylococcus aureus enterotoxin B (SEB)

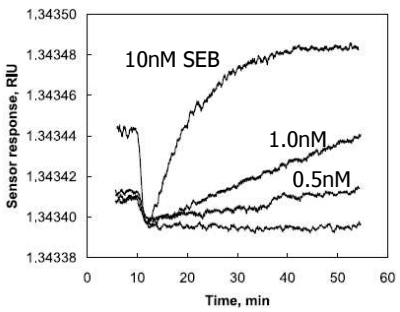
The food industry is interested in detection of SEB in milk and food samples, while antiterrorist monitoring programs are interested in detection of BW agents in aerosols and other matrices

Direct detection of 1 nM SEB in seawater



Spreeta sensor

Detection of SEB in urine



Biosensors and Bioelectronics 17 (2002) 573-584

SPR: conclusions

- Great direct assay platform - versatility and the commercial availability of instruments and chips
- Instrumentation range from highly sensitive and automated bench top SPRs to inexpensive (but still pretty good) hand-held devices
- There are applications in all major industry sectors: health care, defense, environment, food industry, etc
- Same problems as with other sensors – "dirty", real life samples can be tough to handle i.e. significant loss of performance as compared to model mixtures

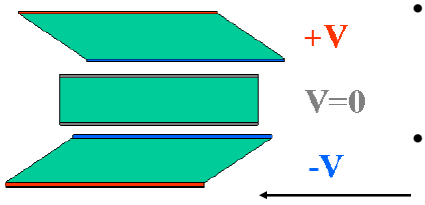
Quartz crystal microbalance

- QCM is a mass sensing device with the ability to measure very small mass changes (~1pg/mm^2) on a quartz crystal resonator in real-time.
- The physical basis of QCM operation originates in the converse piezoelectric effect, where the application of an electric field across a piezoelectric material induces a deformation of the material.
- The oscillation of this electric field leads to deformation of the crystal resulting in an acoustic wave propagating through the crystal.
- QCM measures the change in oscillating frequency of the crystal when it is altered as a result of analyte adsorption to the surface

Piezoelectric effect

In 1880 Jacques and Pierre Curie discovered that mechanical stress applied to the surface of some acentric materials resulted in an electrical potential across the crystal; the magnitude of this potential was proportional to the applied stress

The physical displacement of the atoms under applied mechanical stress leads to the change in net dipole moment and as a result the generation of charge in the crystal

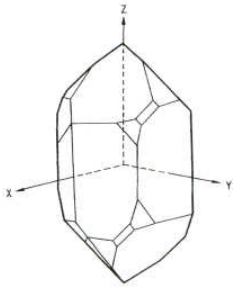


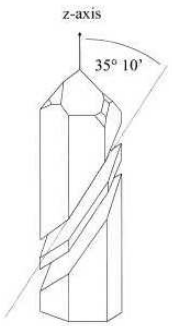
- Conversely, the application of a voltage across these crystals results in a corresponding mechanical strain
- The opposite polarity produces an identical strain, but in the opposite direction

AT cut quartz crystals

The mode of oscillation is determined by a cut-angle with respect to crystal orientation

For QCM the most commonly used are quartz AT cut α-crystals, made by slicing the quartz rod at an angle of 35°10' with respect to the optical axis



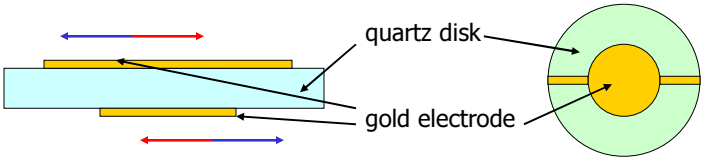


The advantages of AT crystals are (1) shear displacement is perpendicular to the applied field and (2) nearly zero frequency drift with temperature around RT

Quartz resonator

QCMs use a thin AT disk sliced from a single quartz crystal, which is sandwiched between gold electrodes that are vapor deposited on either side of the disk

When an alternating electric field is applied over the electrodes, the quartz crystal stars to oscillate



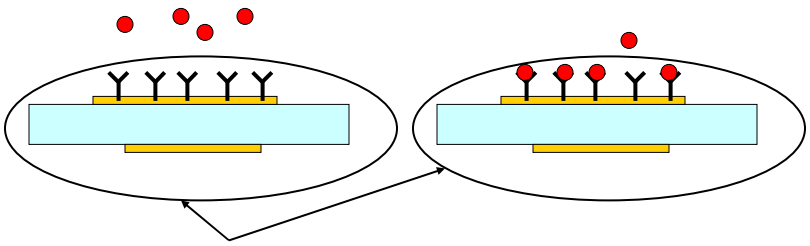
The result of this vibrational motion in the crystal is the formation of an acoustic wave that propagates across the crystal, reflecting back into the crystal at the surface

QCM Detection

In QCM a resonant oscillation can be established when the acoustic wavelength is equal to twice the combined thickness of the crystal and the electrodes

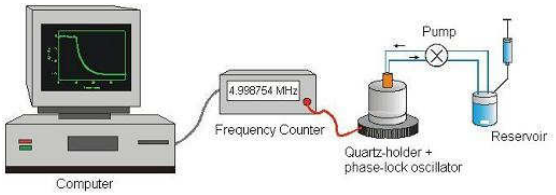
However, it changes with a mass change at the interface

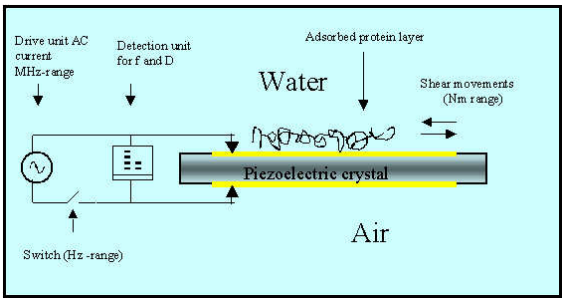
The change is resonant frequency is linearly related to the mass accumulation on the surface $\Delta f = C\Delta m$

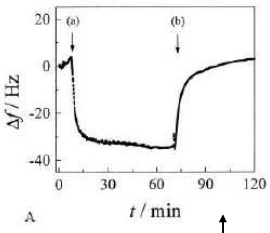


mass change ⟶ frequency change

QCM measurements







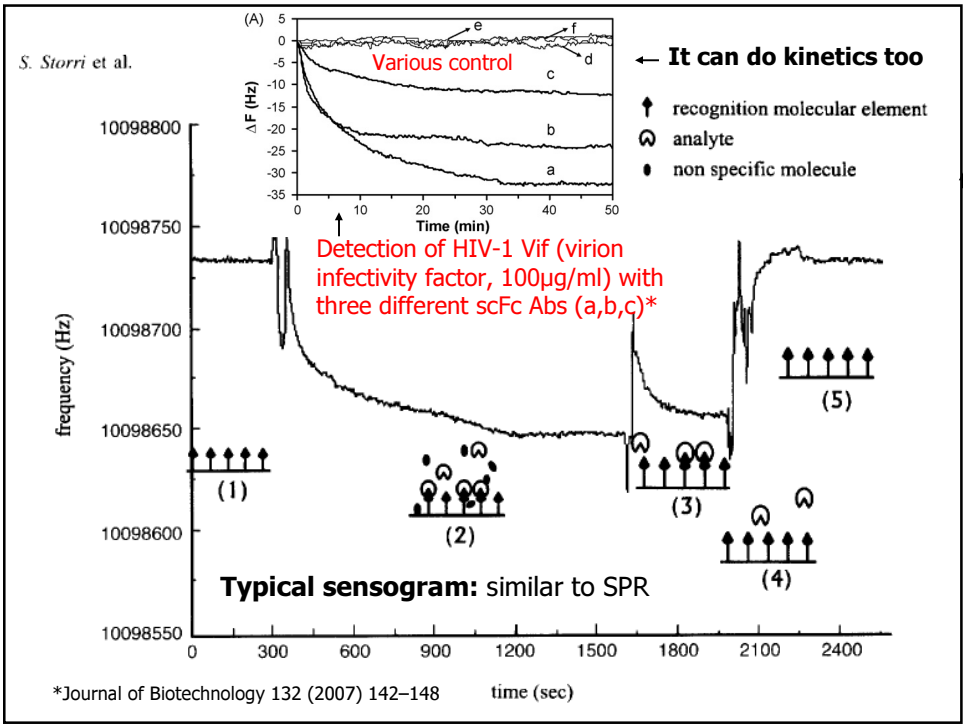
Time-dependent decrease in the resonance frequency of a 5 MHz quartz upon (a) addition of protein and (b) protein degrading enzyme, pronase E

Commercial QCM

KSV-QCM500 FEATURES AND SPECIFICATIONS

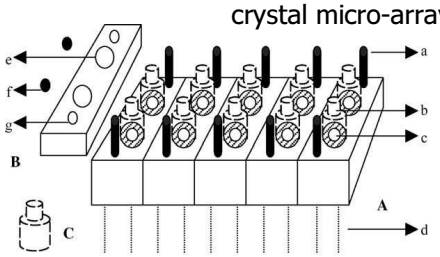
- Direct measurements at air/water interfaces
- Continuous flow measurements
- Crystals easily mounted in measuring chamber or crystal holder
- Multi-frequency and temperature controlled measurements
- Real time measurements (seconds)
- Easy to use Windows based software and Straightforward operation
- Mass sensitivity (5 MHz crystal)
  - ~ 1 ng/cm^2 in air/gas
  - ~ 5 ng/cm^2 in liquid
- Active sensor area ~ 20 mm2

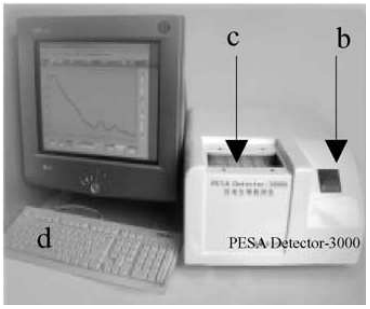
- Frequency range 3-50 MHz
- Max film thickness ~ 5 mm



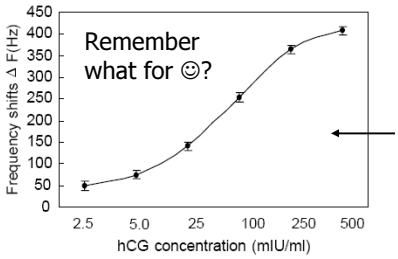
Can be multi-channel too

Schematic diagram of 2 × 5 quartz crystal micro-array





a) Detector, (b) thermal controller, (c) 2 × 5 crystal micro-array, (d) PC



Standard curve for determination of human chorionic gonadotropin with hCG micro-array immunosensor

Biosensors and Bioelectronics 19 (2004) 711–720

QCM in a nutshell

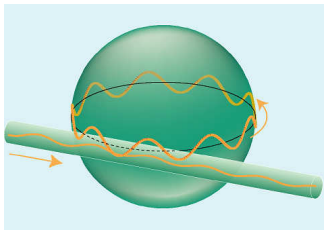
- The QCM sensor is a thin quartz crystal disk sandwiched between two gold electrodes
- The disk is made to oscillate in an oscillating filed and it is possible to both electrically excite the quartz into oscillation and measure its resonance properties
- The resonant frequency, which is proportional to mass, can be measured with great precision
- As a result the binding event at the surface can be easily detected and monitored in real time

VERY SIMPLE ☺

How do they compare?

Sensor	LoD (pg/mm^2)	Sensor area, mm^2	Min mass detected (pg)
SPR	~10	~1	~10
QCM	~0.5	~100	~50

And there are other too, including the remarkable WGM*



*whispering-gallery mode biosensor – **pioneered at Poly**

It's about mln times more sensitive than SPR (!!!)

Have fun and see you next week