Surface Sensitive Techniques

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Lecture: 3/7

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Outline

We will look mainly at the following techniques:

- Surface plasmon resonance (optical).
- Quartz crystal microbalance (mechanical).
- Impedance spectroscopy (electrical).

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Surface Sensitive Techniques

Detect <u>anything</u> that binds to a surface! Several instruments exist, most are based on optical measurements, some mechanical and also a few other.

The techniques will be used in the projects!

SPR Navi (Bionavis)
http://www.bionavis.com/

Qsense (Biolin Scientific)
http://www.qsense.com/

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What is a Plasmon?

Physical understanding: *Plasmons are collective oscillations in the free electrons of metals.*

Mathematical understanding: *Plasmons are solutions to Maxwell's equations for certain metal-dielectric geometries.*

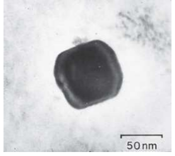
For a metal nanoparticle, the polarization enhanced at certain frequencies of light. For noble metals, this occurs in the visible region, which gives strong colors.

The *Lycurgus Cup* is the oldest example (year ~400) of this kind of staining.

We will dig a bit more into plasmons, but fear not, you do not have to learn the physics in detail!

ambient light light inside cup

British Museum
http://www.britishmuseum.org/



Freestone et al.
Gold Bulletin **2007**, 40 (4), 270-277.

Ag, Au, Cu (60%, 30%, 10%) in glass

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Absorption and Scattering

The polarizability (α) of the particle determines the *absorption* and *scattering* cross sections (σ). The *extinction* is the sum of absorption and scattering.

For gold nanoparticles (small and spherical):
Blue light is absorbed (true for all gold).
Green light is absorbed and scattered (by the plasmon).
Red light is transmitted (low extinction).

$$\sigma_{\text{ext}} = k\,\text{Im}(\alpha)$$

$$\sigma_{\text{sca}} = \frac{k^4}{6\pi}|\alpha|^2$$

$$\sigma_{\text{abs}} = \sigma_{\text{ext}} - \sigma_{\text{sca}}$$

Here k is the incident wavevector ($k = 2\pi/\lambda$). The polarizability is a volume and the cross sections are areas (shadows)!

British Biocell International
http://www.bbisolutions.com/

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Electrostatic Approximation

By considering the electric field of light as static, the polarizability can be calculated easily for a sphere:

$$\alpha_0 = 3V\frac{\varepsilon(\lambda) - \varepsilon_{\text{m}}}{\varepsilon(\lambda) + 2\varepsilon_{\text{m}}}$$

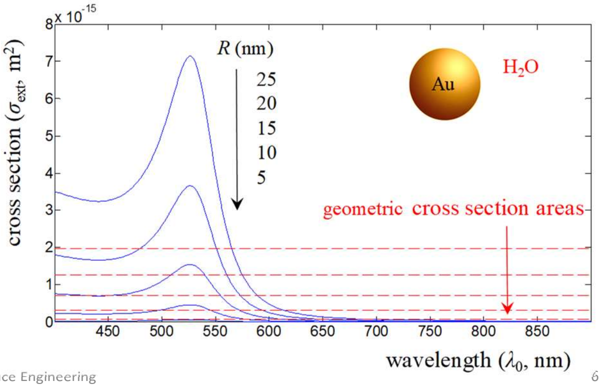
Jackson
Classical Electrodynamics **Wiley 1999**

This is valid for particles that are small compared to the wavelength of light (<50 nm).

Here ε is again (relative) permittivity, but now at optical frequencies.

For simple transparent materials, ε is roughly the square of the refractive index ($\varepsilon = n^2$).

For metals, the permittivity is complex (energy absorption) and *dispersive* (λ dependent).



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The Optical Near-Field

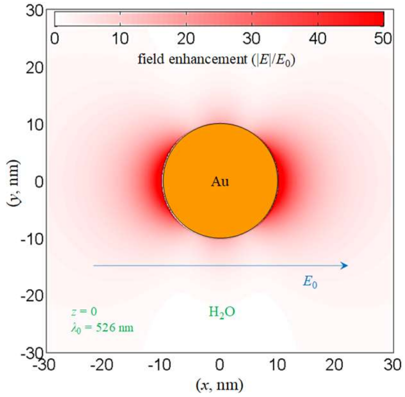
The absorption and scattering cross sections correspond to the “far field” properties of the nanoparticles, i.e. they describe what happens to a light wave that passed the particle.

One can also talk about the “near-field”, i.e. the local electromagnetic field distribution on the nanoscale.

The field is strongest at the metal and typically extends a distance approximately equal to the radius of the particle.

Electrostatic theory can be used to calculate the near field as well:

$$\vec{E}(x,y,z) = E_0\left[\vec{\mathrm{x}} - \mathrm{Re}(\alpha_0)\left[\frac{\vec{\mathrm{x}}}{r^3} - \frac{3x}{r^5}[x\vec{\mathrm{x}} + y\vec{\mathrm{y}} + z\vec{\mathrm{z}}]\right]\right]$$



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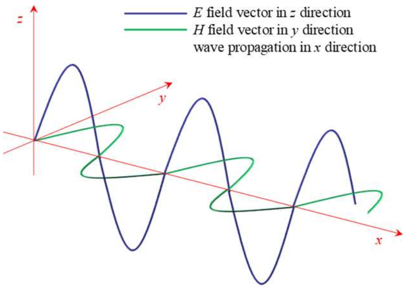
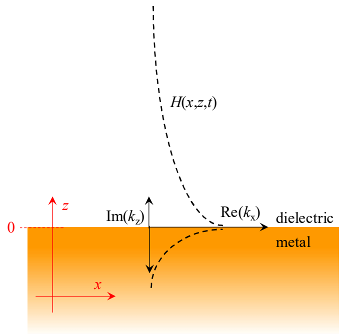
Surface Plasmons

Surface plasmons are similar to ordinary light (electromagnetic plane wave) but confined to the interface between a metal (conductor) and a dielectric (insulator).

The surface plasmon propagates along the interface (like a wave on a water surface). Eventually the wave energy has dissipated (normally by heating the metal).

The wave must have transverse magnetic polarization.





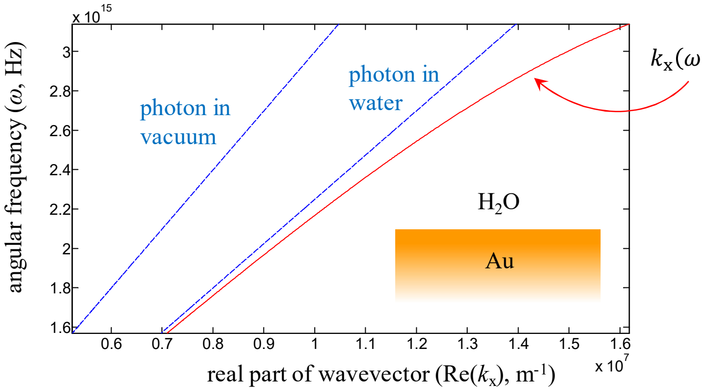
$$\vec{E}(x,t) = E_0\exp(-\mathrm{i}\omega t)\exp(-\mathrm{i}kx)\vec{\mathrm{x}}$$

$$\vec{H}(x,z,t) = H_0\exp(\mathrm{i}[k_x x \pm k_z z - \omega t])\vec{\mathrm{y}}$$

The Dispersion Relation

There exists a relation between the momentum and energy of a surface plasmon. In contrast to nanoparticles, surface plasmons exist in a *continuum* of frequencies!

Problem: Momentum is always lacking for the incident light! The dispersion relation does not cross that of photons in the dielectric medium!



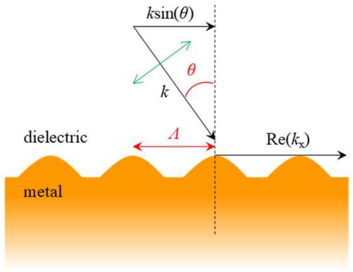
$$k_{\mathrm{x}}(\omega)=\frac{\omega}{c}\sqrt{\frac{\varepsilon(\omega)\varepsilon_{\mathrm{m}}}{\varepsilon(\omega)+\varepsilon_{\mathrm{m}}}}$$

Excitation of Surface Plasmons

The additional momentum needed can be added in several ways.

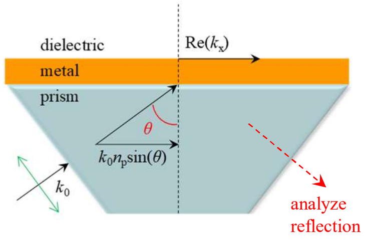
One is to introduce a periodic grating on the surface. The grating wavevector adds to that of the incident photons. The condition for excitation is:

$$\mathrm{Re}\left(\sqrt{\frac{\varepsilon(\lambda_0)\varepsilon_{\mathrm{m}}}{\varepsilon(\lambda_0)+\varepsilon_{\mathrm{m}}}}\right)=n_{\mathrm{m}}\sin(\theta)+\frac{j\lambda_0}{\Lambda}$$



One can also use a high refractive index material, a thin metal film and *total internal reflection* configuration. The prism adds to the photon momentum:

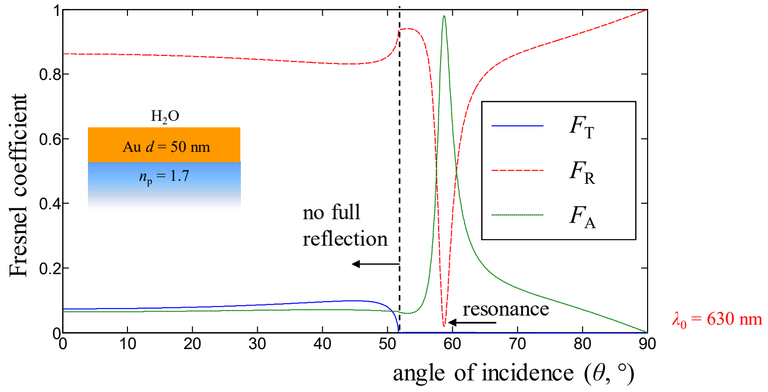
$$\mathrm{Re}\left(\sqrt{\frac{\varepsilon(\omega)\varepsilon_{\mathrm{m}}}{\varepsilon(\omega)+\varepsilon_{\mathrm{m}}}}\right)=n_{\mathrm{p}}\sin(\theta)$$



Total Internal Reflection

When measuring the reflected light one sees a minimum, representing coupling to surface plasmons!

One can vary either the angle or the wavelength of incident light.



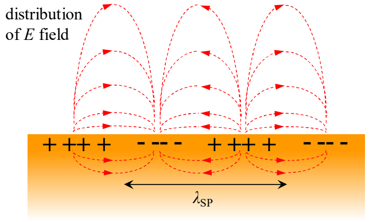
Surface Plasmon Near-Field

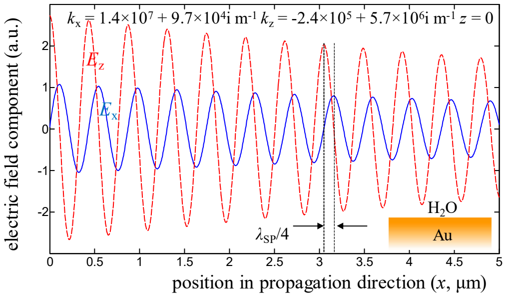
At any time, the surface plasmon has negative and positive poles on the metal surface.

The electric field has two components in the *xz* plane. Magnetic field only in *y*.

The time-averaged field strength only depends on the distance from the surface.

The field extends approximately half of the wavelength of light used to excite the surface plasmon.





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Detection with Plasmons

The most common plasmonic sensor principle is *refractometric detection*:

- When a molecule binds to the surface, the refractive index changes.

- The properties of the plasmon are changed because they depend on the refractive index close to the metal.

- By optical spectroscopy, changes in intensity of light can then be detected. The resonance will shift in the spectrum (angle or wavelength).

This holds both for surface plasmons and nanoparticle plasmons. The latter normally use wavelength shifts while SPR instruments measure angular shifts.

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Commercial SPR

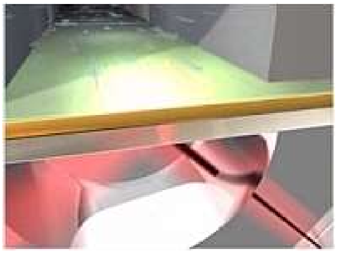
- First paper published in 1983.

- Pharmacia Biosensor started shortly after. Became Biacore later, then part of GE Healthcare, then eaten by Cytiva.

- SPR is now the most established technology for studying biomolecular interactions (later lecture).

- SPR has also become popular in material science.

- The instruments are expensive, but primarily because they contain efficient liquid handling and temperature stabilization etc. SPR can be cheap!



Label-free interaction analysis in real time

BIACORE cytiva

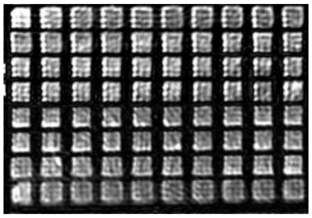
Liedberg et al.
Sensors and Actuators **1983**, 4 (2), 299-304.

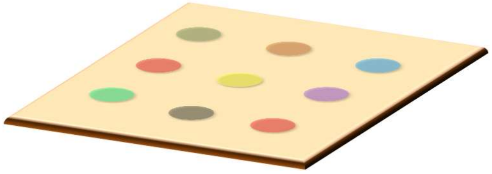
SPR Imaging

SPR can be operated in *imaging* mode which enables multiplexing: Several surface reactions can be probed simultaneously using the same sample solution.

Only intensity variations are measured (camera).

Ideal for use with patterned surfaces!





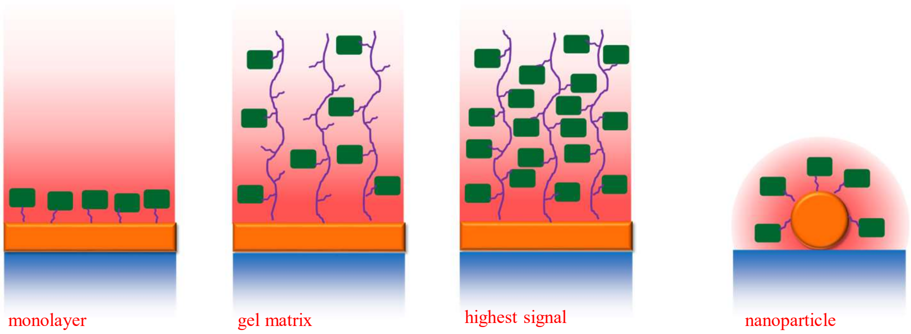
~100 µm spots, ~100 in total

Homola
Chemical Society Reviews **2008**, 108 (2), 462-493.

Importance of Field Extension

If the electromagnetic field extends very little, molecules far away will not be detected.

If the field extends far, only a part of the detection capability is utilized and the system may be more sensitive to “bulk effects” far away from the surface.



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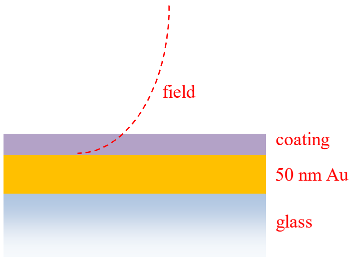
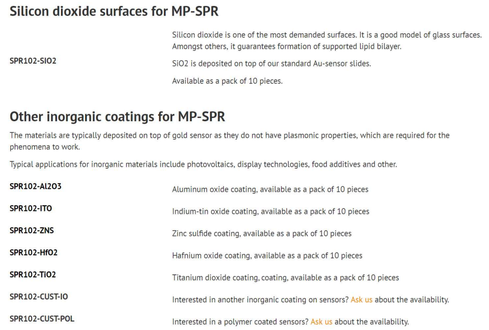
Additional Coatings

The metal does not have to be gold, but it is good due to chemical stability and low optical absorption.

Thin coatings on top are also possible if they are not too absorbing!





Silicon dioxide surfaces for MP-SPR

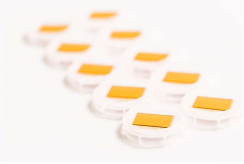
SPR102-SIO2	Silicon dioxide is one of the most demanded surfaces. It is a good model of glass surfaces. Amongst others, it guarantees formation of supported lipid bilayer. SiO2 is deposited on top of our standard Au-sensor slides. Available as a pack of 10 pieces.

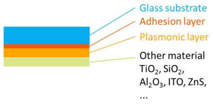
Other inorganic coatings for MP-SPR

The materials are typically deposited on top of gold sensor as they do not have plasmonic properties, which are required for the phenomena to work.

Typical applications for inorganic materials include photovoltaics, display technologies, food additives and other.

SPR102-Al2O3	Aluminum oxide coating, available as a pack of 10 pieces
SPR102-ITO	Indium-tin oxide coating, available as a pack of 10 pieces
SPR102-ZNS	Zinc sulfide coating, available as a pack of 10 pieces
SPR102-HfO2	Hafnium oxide coating, available as a pack of 10 pieces
SPR102-TiO2	Titanium dioxide coating, coating, available as a pack of 10 pieces
SPR102-CUST-IO	Interested in another inorganic coating on sensors? Ask us about the availability.
SPR102-CUST-POL	Interested in a polymer coated sensors? Ask us about the availability.





Bionavis
http://www.bionavis.com/

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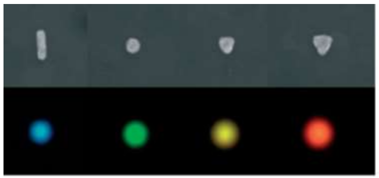
Miniaturized Nanoparticle Sensors

If SPR is so great, why bother with plasmonic biosensors based on nanoparticles?

One reason is that they are very small! In SPR, the sensing spot is at least microscale.



scattered light in dark field illumination

Murray, Barnes
Advanced Materials **2007**, 19 (22), 3771-3782.

Other Optical Techniques

Plasmons is definitely not the only way to go!

- Ellipsometry.
- Optical waveguide lightmode spectroscopy.
- Dual polarization interferometry.

Very similar since all are based on refractometric detection!

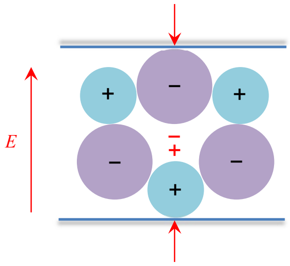
Microvacuum
http://www.owls-sensors.com/

Piezoelectricity

Some crystalline materials generate electric fields upon deformation because the atoms have different electronegativity.

Piezoelectricity is the connection between mechanical and electrical potential energy in a material.

Apply pressure to generate voltage or the other way around!



Wikipedia: *Quartz*

Quartz Crystal Microbalance

Gold plates on a quartz crystal deform it in shear mode (for the proper crystal angle cut).

With an AC voltage we can force the crystal into *oscillation*.

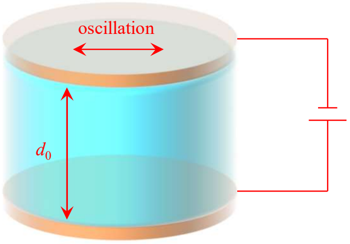
At the *acoustic* resonance frequencies *f* the amplitude is enhanced:

$$f = n\frac{v}{\lambda} = n\frac{v}{2d_0}$$

Here v is the speed of sound (in the material) and n is an integer.

In quartz $v = 3340$ m/s.

Crystal 334 µm thick gives $f = 5$ MHz for the fundamental resonance ($n = 1$).



QCM Sensors

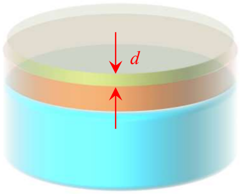
Sensing principle is that an adsorbed film becomes part of the oscillation. If a thin film of thickness d is bound to the resonator with initial thickness d_0 the frequency shift is:

$$\Delta f = \frac{nv}{2[d_0+d]} - \frac{nv}{2d_0} = \frac{nv}{2}\left[\frac{1}{d_0+d} - \frac{1}{d_0}\right] = \frac{nv}{2} \times \frac{d_0 - [d_0+d]}{d_0[d_0+d]}$$

$$= -\frac{nv}{2} \times \frac{d}{d_0[d_0+d]} = -\frac{nv}{2} \times \frac{1}{d_0\left[\underbrace{\frac{d_0}{d}}_{\gg 1} + 1\right]} \approx -\frac{nv}{2d_0} \times \frac{d}{d_0} = -\frac{f_0}{d_0} \times d$$

The QCM sensor is thus essentially a thickness meter!

Higher Δf for higher f_0, but also more noise (thinner crystals).



Dissipation Monitoring

If we excite the oscillation with an AC voltage close to *f* and then disconnect, the crystal will continue to oscillate freely at its resonance frequency for a short time.

The amplitude *A* will decay exponentially with a certain *dissipation D*:

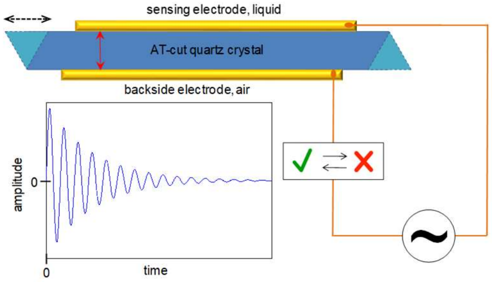
$$A(t) = A_0 \sin(2\pi f t)\exp(-Dft)$$

Both *f* and *D* are obtained. The definition of *D* is energy loss per cycle, so it is dimensionless.

In liquids *D* becomes high and:

$$\Delta f = -{f_0}^{3/2}\left[\frac{\rho_\mathrm{l}\eta_\mathrm{l}}{D\rho_\mathrm{q}G_\mathrm{q}}\right]^{1/2}$$

G_q is the elastic storage modulus of quartz, which is 2.95×10^{10} Pa.



Quantification of Frequency Shift

The frequency shift can be rewritten in terms of mass coverage and film density:

$$\Delta f = -\frac{f_0}{d_0}\times d = -\frac{f_0}{d_0}\times\frac{\Gamma M}{\rho}$$

In 1952 a German guy called Sauerbrey said that the adsorbed film can be assumed to have the density of quartz as long as it is rigid and much thinner than the acoustic wavelength. (It kind of makes sense.) This means that:

$$\Delta f = -\frac{f_0}{d_0}\times d = -\frac{f_0}{d_0\rho_\mathrm{q}}\times \Gamma M$$

This gives a linear sensitivity and a *Sauerbrey constant*:

$$S = \frac{d_0\rho_\mathrm{q}}{f_0}$$

For a typical 5 MHz quartz crystal ($\rho_\mathrm{q} = 2.65$ g/cm^3) becomes 17.7 ng/cm^2 per Hz.

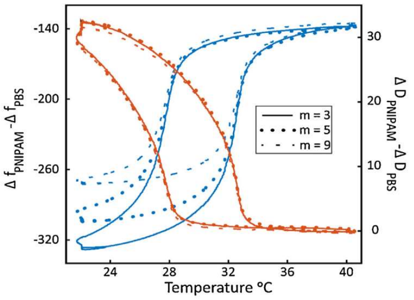
If $\Delta D/\Delta f > 4\times10^{-7}$ Hz^{-1} Sauerbrey does not work and the film must be treated as *viscoelastic* (more advanced).

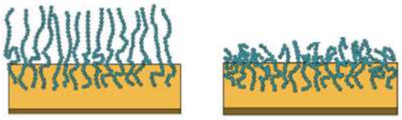
“Dry” and “Wet” Mass

Especially for highly hydrated ”floppy” molecules on the surface a lot of water will be coupled to the oscillation and thus also detected as mass.

The mass determined by an optical sensor and the QCM differs!

Example: Change in frequency and dissipation when a polymer becomes dehydrated.





the “dry” Γ is constant

Emilsson et al.
Applied Surface Science **2017**, 396, 384-392.

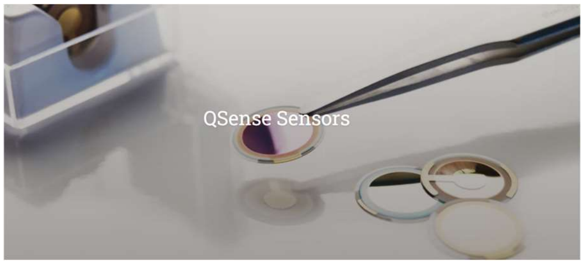
Video: QCM-D

Generic tool for studies in liquids.

Surface Material Variation

Just like for SPR, additional coatings can be used on the sensor crystals. It does not have to be gold!

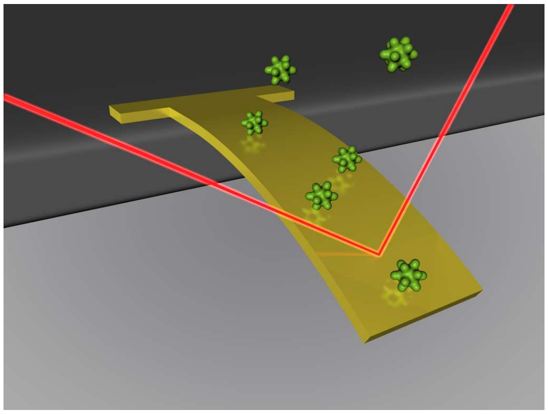
Even more flexibility: the films can be thicker than in SPR and it is not a problem if they are optically absorbing (which kills plasmons). Carbon is one example!



https://www.biolinscientific.com/qsense/sensors

Other Mechanical Sensors

Microcantilevers can oscillate or bend due to surface binding.



Electrochemical Impedance Spectroscopy

Electrochemistry can be used to characterize the surface of electrodes and to detect molecular binding.

The method that gives the most information is *electrochemical impedance spectroscopy* (EIS).

We apply an AC voltage with angular frequency $\omega = 2\pi f$:

$$U(t) = U_0 \sin(\omega t)$$

The amplitude is kept low (~10 mV) so that the system responds linearly. The current is then also sinusoidal with a certain amplitude and a *phase shift* φ:

$$I(t) = I_0 \sin(\omega t - \phi)$$

The impedance Z is a more general term than electrical resistance. It is complex and frequency dependent. In principle it is still defined as voltage divided by current:

$$Z = \frac{U_0 \sin(\omega t)}{I_0 \sin(\omega t - \phi)}$$

Complex Denotation

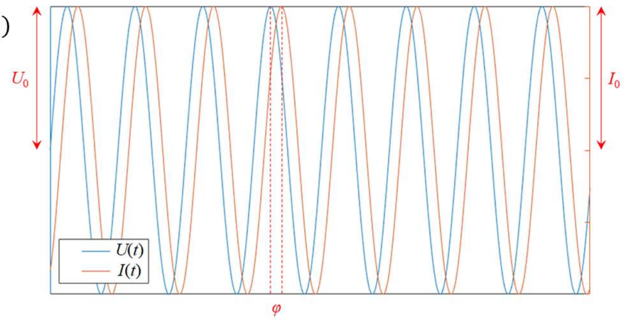
Everything becomes easier if we use Euler’s formula:

$$U = U_0 \exp(\mathrm{i}\omega t)$$

$$I = I_0 \exp(\mathrm{i}[\omega t - \phi])$$

The impedance is then represented by a *magnitude* Z_0 (of the complex number) and a *phase* φ:

$$Z = \frac{U}{I} = \frac{U_0 \exp(\mathrm{i}\omega t)}{I_0 \exp(\mathrm{i}[\omega t - \phi])} = Z_0 \exp(\mathrm{i}\phi)$$



Circuit Elements

An ideal resistor has no capacitive effects and Z is real with no dependence on ω:

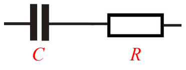
$$Z = \frac{U_0}{I_0} = Z_0 = R$$

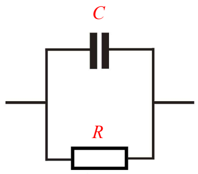
An ideal capacitor has zero steady state current and Z is purely imaginary:

$$Z = Z_0 \exp\left(\mathrm{i}\frac{\pi}{2}\right) = -\frac{\mathrm{i}}{\omega C}$$

We can combine impedances (just like resistors). Resistor and capacitor in series:

$$Z = Z_1 + Z_2 = R - \frac{\mathrm{i}}{\omega C}$$



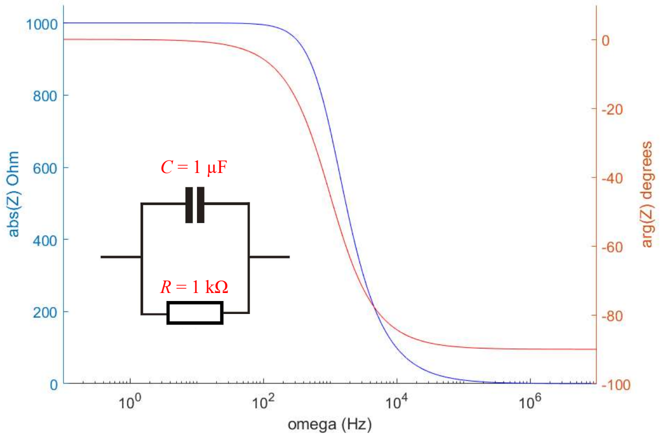


More important, in parallel:

$$Z = \frac{Z_1 Z_2}{Z_1 + Z_2} = \frac{\frac{\mathrm{i}R}{\omega C}}{R - \frac{\mathrm{i}}{\omega C}} = \frac{\frac{\mathrm{i}R}{\omega C}\left[R + \frac{\mathrm{i}}{\omega C}\right]}{R^2 + \frac{1}{\omega^2 C^2}} = \frac{R}{1 + R^2\omega^2 C^2} - \frac{R^2 \omega C}{1 + R^2\omega^2 C^2} \times \mathrm{i}$$

Impedance Spectra

EIS data can be visualized in the complex plane (Nyquist plot), but to see the frequency dependence more clearly a *Bode plot* of $Z_0(\omega)$ and $\varphi(\omega)$ (double y-axis) is usually better.

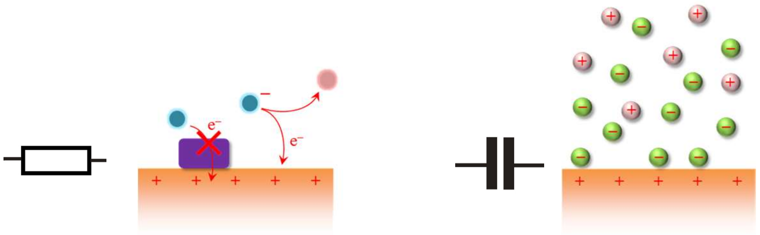


Equivalent Circuits

So what is the relation to surface analysis? EIS data can be modelled by *equivalent circuits*, i.e. elements like resistors and capacitors (even though the real system is not like that at all).

We can distinguish from *charge transfer* (Faradaic reactions) and *capacitive currents* (typically the double layer). We will always have both at the surface!

Especially with a redox active molecule in solution, EIS will detect if something binds to the surface. Changes in double layer capacitance can also be detected.

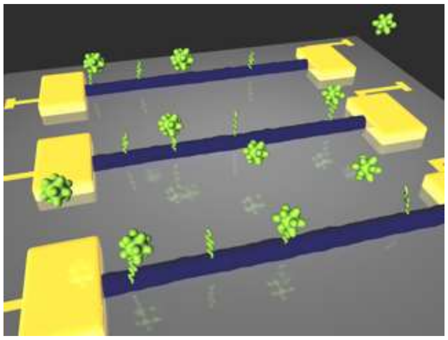


Other Electrical Techniques

Field effect sensors monitor changes in *conductivity*.

Many versions exist, such as *nanowires*. They essentially respond to <u>charge</u> of analytes.

Do not work well at physiological ionic strength because of *screening*.



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Conclusions

Surface sensitive techniques are generic tools that detects binding of <u>all</u> molecules to surfaces <u>without</u> any chemical identification.

They normally provide data in real-time so you can get information of rate constants.

Signal transduction is normally based on something of the following:

- Refractive index (optical sensors).

- Mass (mechanical sensors).

- Charge (electrical sensors).

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Implications from Binding Kinetics

Remember the models for binding kinetics to surfaces!

If the surface is a sensor that gives a measurable response <u>proportional</u> to Γ (e.g. SPR and QCM)

- Real-time operation gives k_{on} and k_{off} and confirms equilibrium. Measuring the equilibrium coverage gives only K_{D} from C_0 or vice versa.

- Diffusion models can estimate mass coverage limits: A molecule can diffuse to the sensor surface without binding to it, but not bind to the surface without first diffusing to it.

- Flow is often needed to prevent diffusion effects and reach Langmuir behavior. The depletion zone needs to be eliminated!

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Checklist 3

- Plasmons in nanoparticles: absorption and scattering
- Electrostatic approximation as well as near-field vs far-field
- Plasmons on surfaces, dispersion relations and excitation mechanisms
- Piezoelectricity
- Quartz crystal microbalance principle of detection
- The dissipation parameter
- The “wet” mass (vs dry mass)
- Impedance spectrum measurements
- Capacitive and resistive contributions in electrochemistry

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Exercise 3.1

A SPR sensor monitors the resonance wavelength λ_{res}, which changes with the refractive index of the environment (n) according to $\lambda_{\mathrm{res}} = S_0 n$, where $S_0 = 10^4$ nm (per refractive index unit). The refractive index change from proteins in water is 0.185 mL/g (unit is inverse concentration). A protein binds to the surface and forms a 10 nm thick film. What is Γ (here in mass per area) if the experiment shows $\Delta\lambda_{\mathrm{res}} = 5$ nm? Assume that the sensitivity distribution follows a one dimensional exponential decay with $\delta = 100$ nm:

$$\Delta\lambda_{\mathrm{res}} = \frac{S_0}{\delta}\int_0^{\infty} \Delta n(z) \exp\left(-\frac{z}{\delta}\right) \mathrm{d}z$$

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Exercise 3.1

We need to calculate the refractive index change in the thin film based on the SPR response. The idea is that Δn is constant in the film and zero otherwise:

$$\Delta\lambda_{\text{res}} = \frac{\Delta n S_0}{\delta}\int_0^{10} \exp\left(-\frac{z}{\delta}\right) \mathrm{d}z = \frac{\Delta n \times 10^4}{\delta}\left\{-\delta \times \exp\left(-\frac{z}{100}\right)\right\}_0^{10} =$$

$$\Delta n \times 10^4 \left[1 - \exp\left(-\frac{10}{100}\right)\right]$$

This gives $\Delta n = 0.0053$ inside the 10 nm film. The concentration of proteins is then $0.0053/0.185 = 0.0284$ g/cm^3. To get the surface coverage we multiply with the thickness (10 nm):

$$\Gamma = 0.0284 \frac{\text{g}}{\text{cm}^3} \times 10 \times 10^{-7}\text{cm} = 2.84 \ldots \times 10^{-8} \frac{\text{g}}{\text{cm}^2} = 28 \frac{\text{ng}}{\text{cm}^2}$$

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Exercise 3.2

A QCM sensor detects colloidal particles adsorbing to the sensor surface. The noise level in the measurement is 1 Hz. The colloids are spherical polystyrene particles 100 nm in diameter ($\rho = 1.04$ g/cm^3). What is the lowest number of particles on 1 µm^2 that the system can detect? You can use the Sauerbrey relation, but comment on the main error!

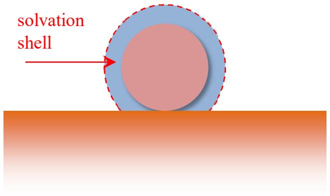
Exercise 3.2

We translate the noise level into a surface coverage by Sauerbrey:

$$\Gamma M = \Delta f \times S = 17.7\ \mathrm{ng/cm^2} = 1.77 \times 10^{-16}\mathrm{g/\mu m^2}$$

How many particles correspond to this mass? Each particle has a weight of $4\pi R^3/3 \times 1.04\ \mathrm{g/cm^3} = 5.4 \times 10^{-16}$ g, so it is enough with 0.32 particles per $\mu\mathrm{m}^2$.

The particles are solid so probably ΔD is low, but each particle still gives a higher signal than that corresponding to its mass because of coupled solvent!



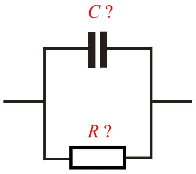
Exercise 3.3

EIS is useful but it is complicated to interpret and analyze results. At an electrode interface, it is easy to see that both capacitive and Faradaic processes occur by just applying a DC voltage U_0. For a simple capacitor and resistor in parallel, the current is then expected to change as:

$$I(t) = I_0 \exp(-kt) + I_{\mathrm{ss}}$$

The voltage is applied at $t = 0$.

Draw the $I(t)$ function and explain how to obtain R and C from such data!



Exercise 3.3

After sufficient time, the capacitor is fully charged and the current goes only through the resistor. This current corresponds to I_{ss}, so R = U_0/I_{ss}.

The capacitance can be obtained by integrating the function $I(t)$ in time, but you must subtract the contribution from the resistor (I_{ss}). This gives the total charge Q stored in the capacitor. The capacitance is then $C = Q/U_0$.