Aalto University
School of Chemical Technology

Surface Plasmon Resonance (SPR)

CHEM-L2000

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Outline

(1) SPR – general issues

(2) Theory

- Existence of surface plasmons
- SPR phenomenon

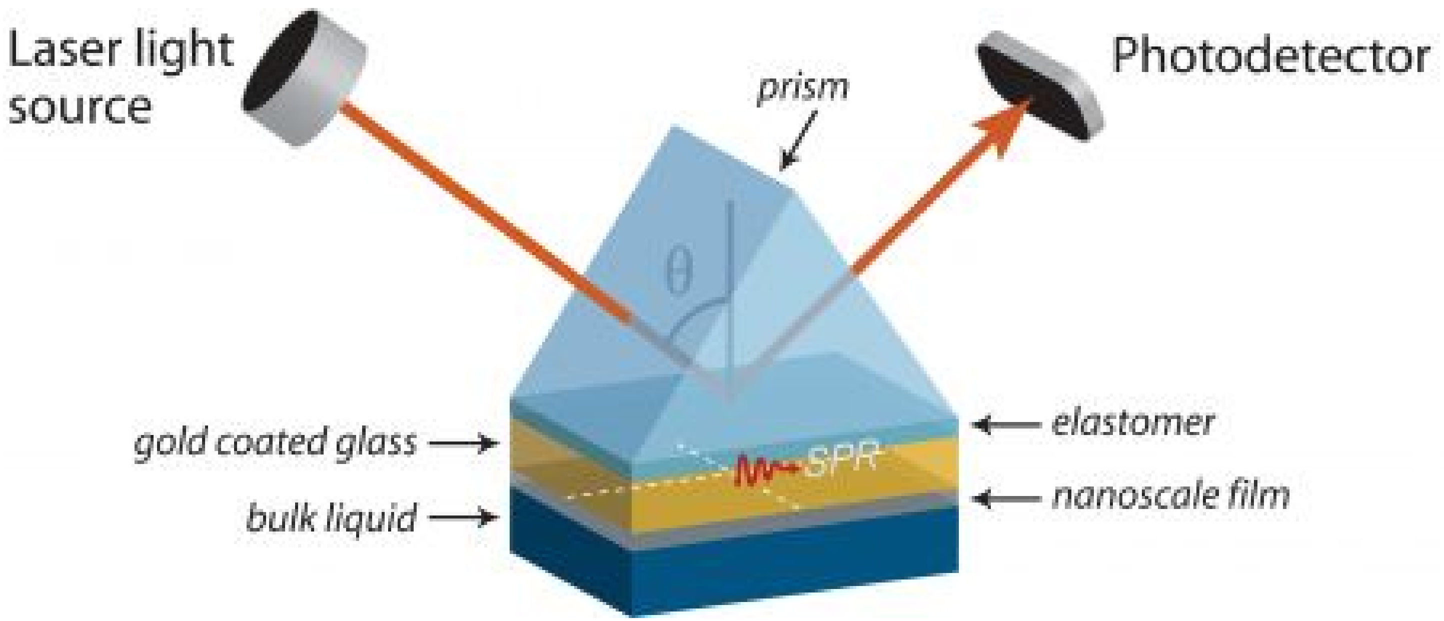
(3) Instrumentation, measuring and interpretation

(4) Applications of SPR

- Thickness and refractive index
- Following layer-by-layer deposition
- Following attachment of antibodies

SPR in general

- Light excitation causes the formation of plasmons on a metal surface
- The plasmons propagate on the metal-sample surface and are affected by the changes in the sample
- Enables *in situ* monitoring of, e.g., adsorption occurring on an ultrathin film



SPR in general

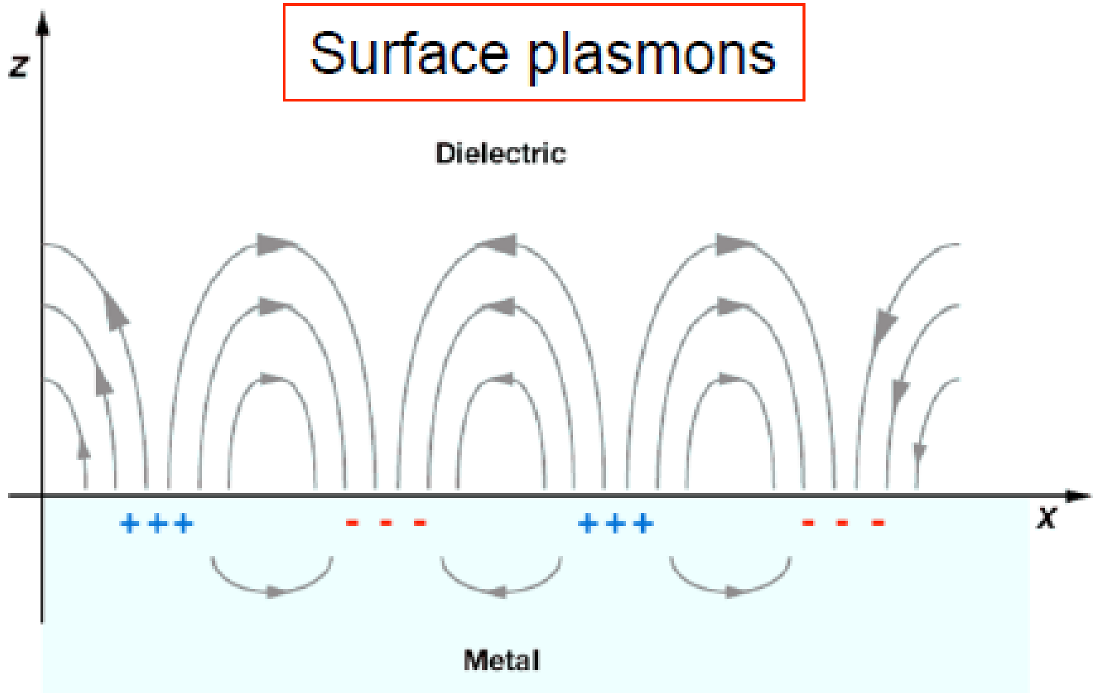
Main applications:

- Following protein immobilization
  - Binding of proteins (to antibodies)
  - Kinetics of binding
  - Analyzing mutant proteins
- Polymer adsorption studies
  - Adsorption kinetics
  - Following layer-by-layer deposition
  - Equilibrium measurements (affinity and enthalpy)
- Interfacial reactions

In essence, SPR is generally applied to follow binding of an object (macromolecule, nanoparticle, colloid) on a surface and the possible changes occurring in the bound layer.

Theory

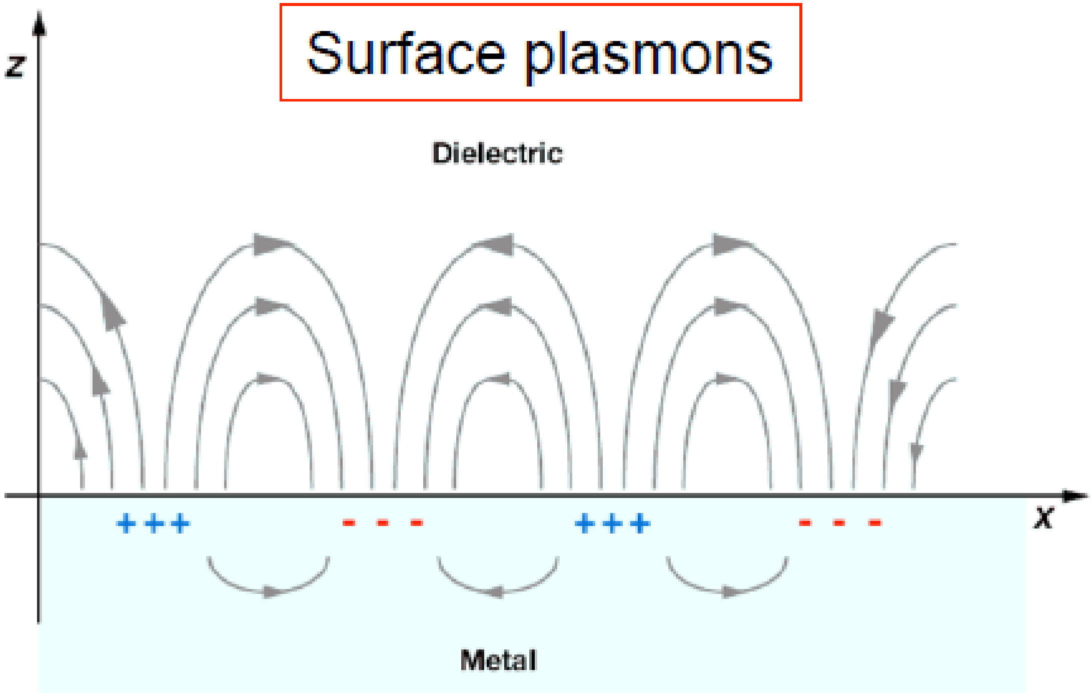
What are surface plasmons?



- **Plasmons** are charge density oscillations of the nearly free electron "liquid" or "gas" in metals
- **Surface plasmons** are electromagnetic waves that propagate along a metal/dielectric interface

Note: a dielectric is an electrical insulator that can be polarized by an electric field.

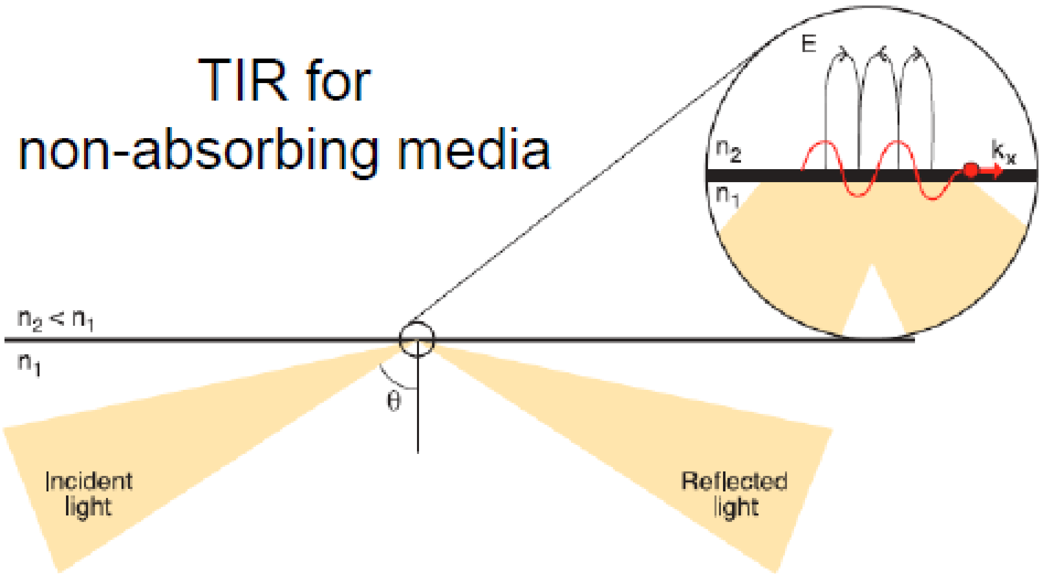
What are surface plasmons?

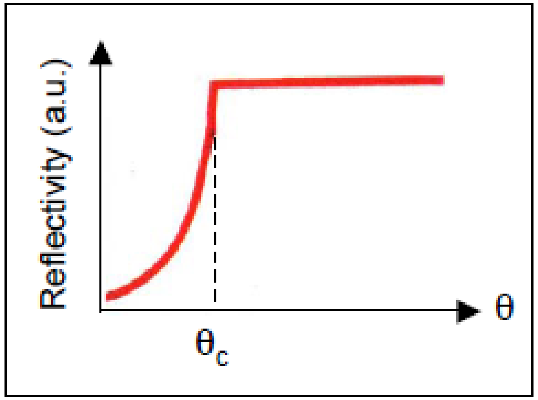


- **Plasmons** are charge density oscillations of the nearly free electron “liquid” or “gas” in metals
- **Surface plasmons** are electromagnetic waves that propagate along a metal/dielectric interface

Surface plasmons in SPR occur usually at air/metal or water/metal interface, excited by the electric field created by electromagnetic radiation from IR to visible light wavelengths.

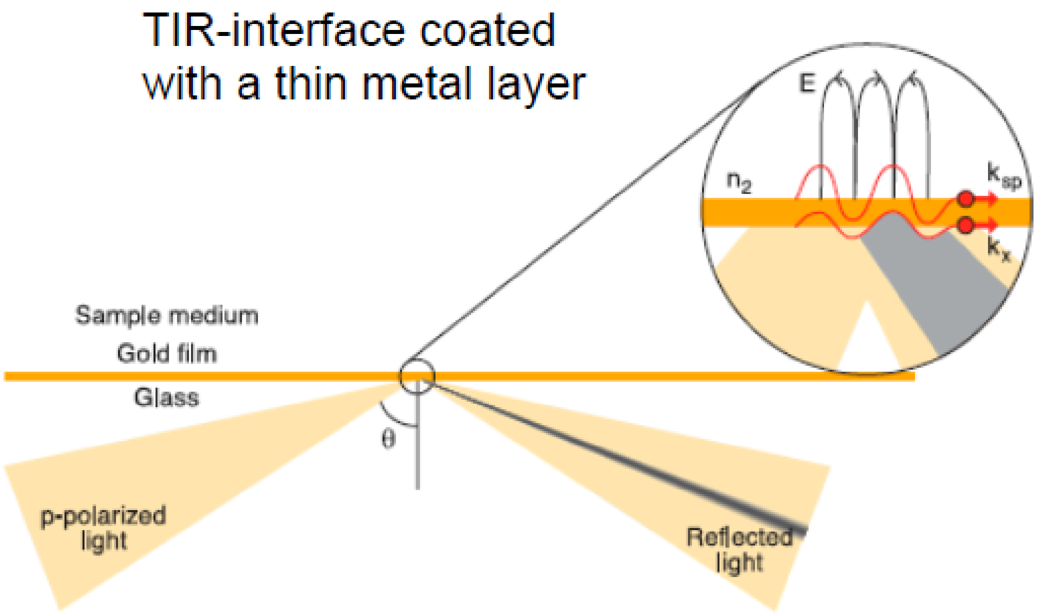
Total internal reflection (TIR) and surface plasmons

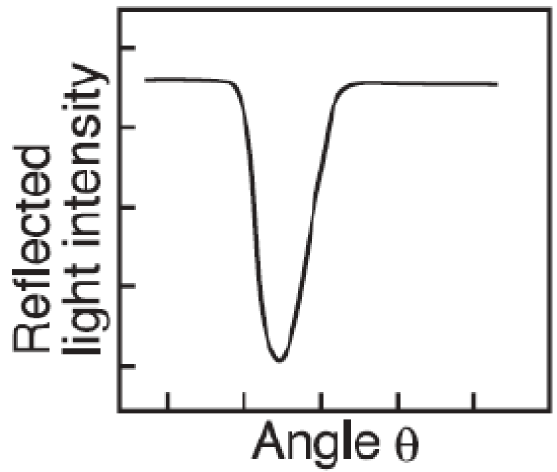




- A fully reflected beam causes an evanescent wave to penetrate into the low refractive index medium at the interface between two materials
- Evanescent wave is effectively an electric field that decays exponentially over a distance of ¼ wavelengths beyond the surface

Total internal reflection (TIR) and surface plasmons

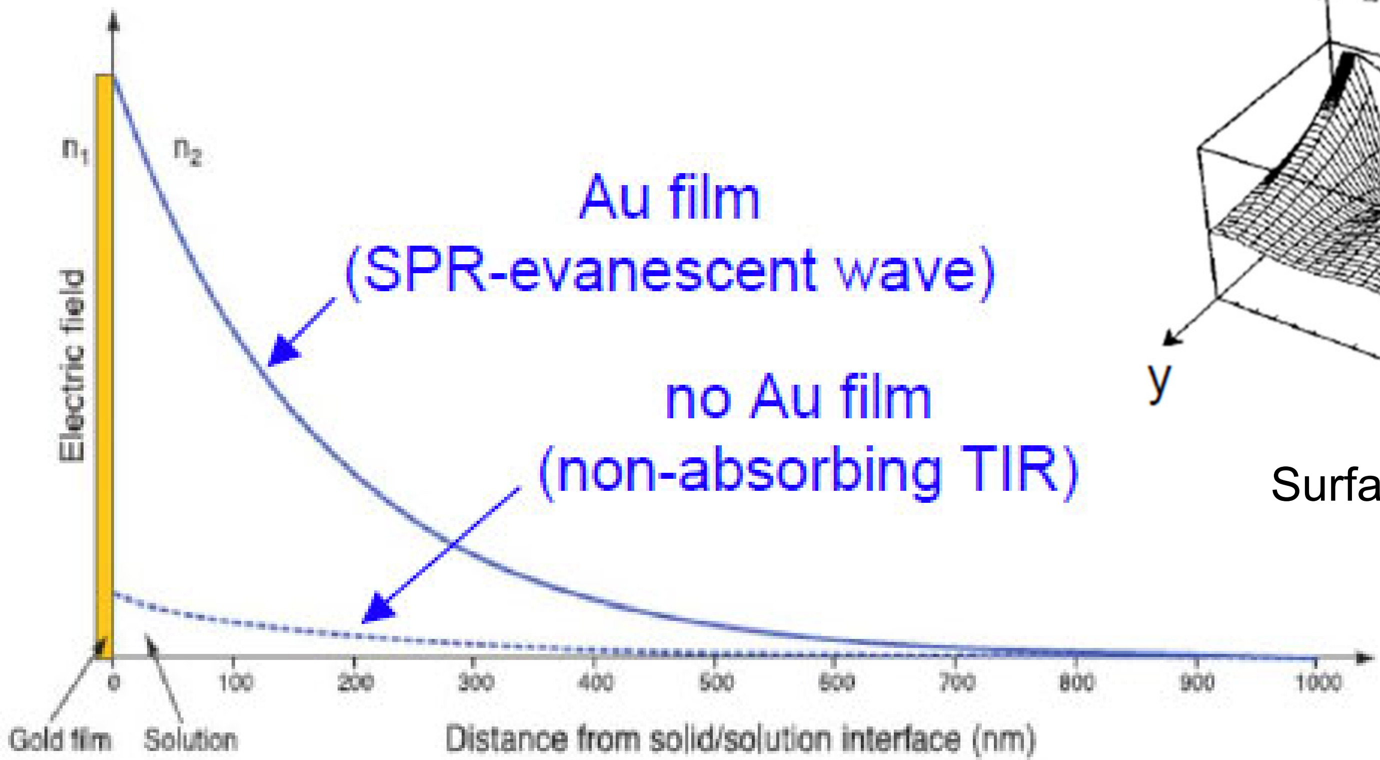


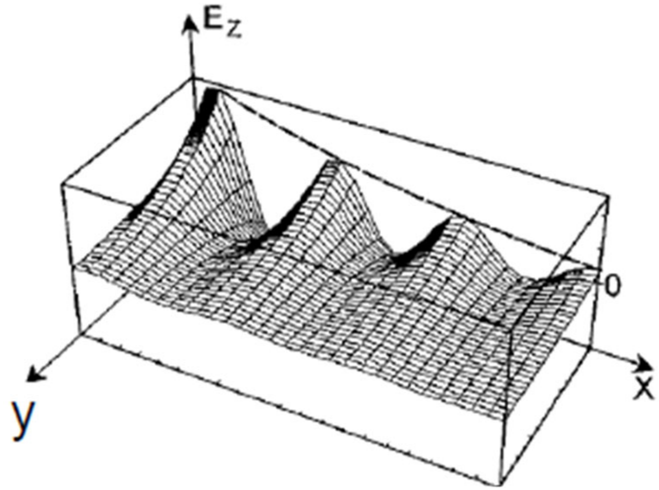


- At a certain incident angle or wavelength, the electromagnetic component of the p-polarized light penetrates the metal layer and energy is transferred to the metal's electrons → surface plasmons emerge

→ A decrease in reflected light intensity occurs

Surface plasmon resonance



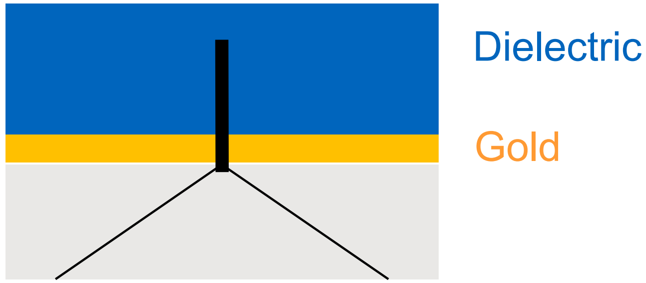


Surface plasmon wave

- The surface plasmon wave is electromagnetic and surface propagating

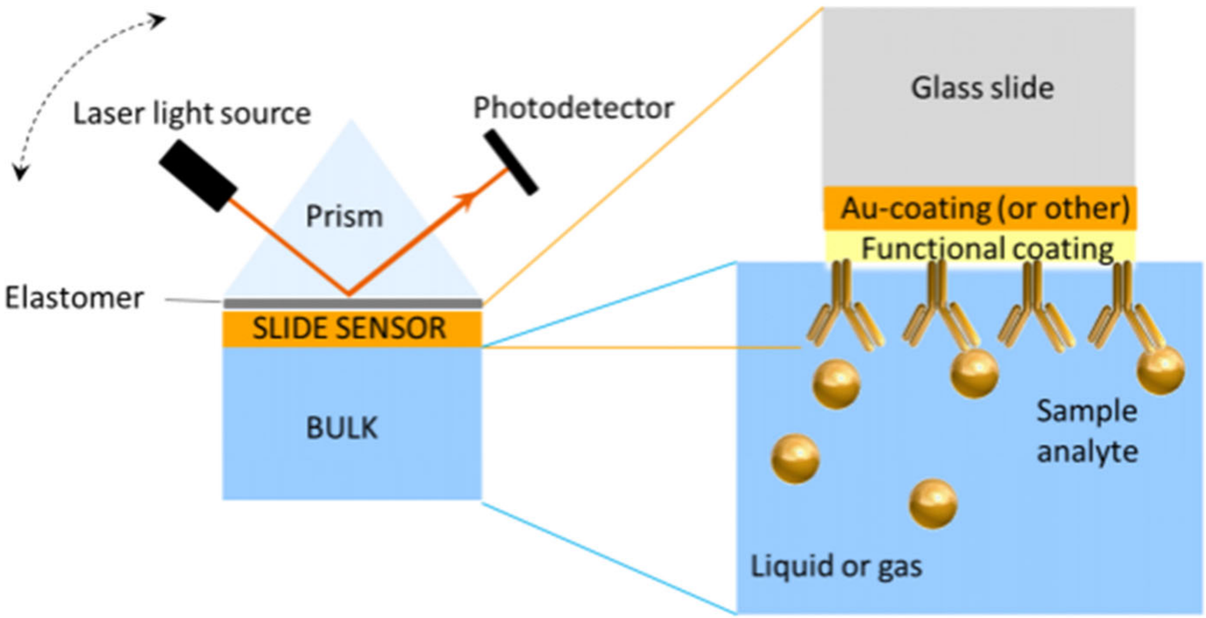
→ The surface plasmon wave significantly enhances the evanescent electric field amplitude

Characteristics of the SPR evanescent wave



Metal layer supporting SPW	Gold	
Wavelength	$\lambda = 630$ nm	$\lambda = 850$ nm
Propagation length (μm)	3	24
Penetration depth into metal (nm)	29	25
Penetration depth into dielectric (nm)	162	400
Concentration of field in dielectric (%)	85	94

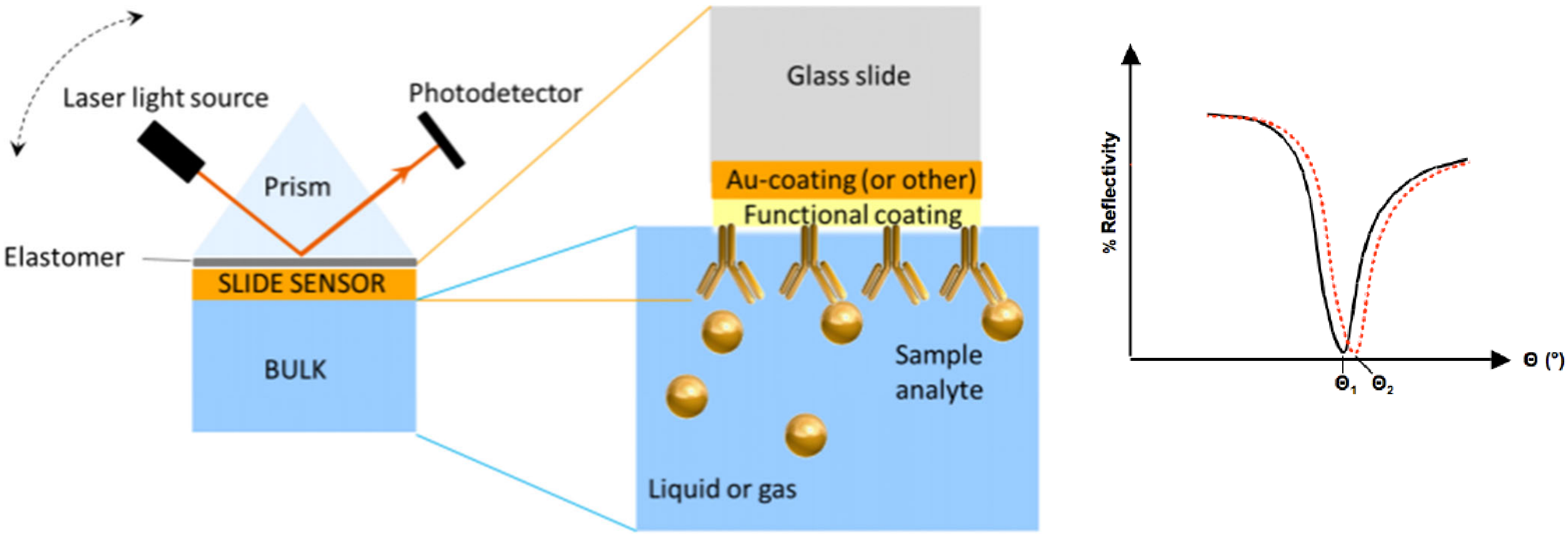
How SPR reacts on material changes on the surface



- The conditions of the SPR are sensitive to the refractive index at the metal-dielectric interface
- Added material / changes in the material alter the refractive index

→ Velocity of the surface plasmons is changed

How SPR reacts on material changes on the surface



- The change in plasmon velocity alters the incident light vector (e.g., angle) required for surface plasmon resonance and minimum reflection
- The exact position of minimum reflection (resonance) bears information on the interfacial mass coverage / thickness of the layer(s)

Instrumentation, measuring, and interpretation

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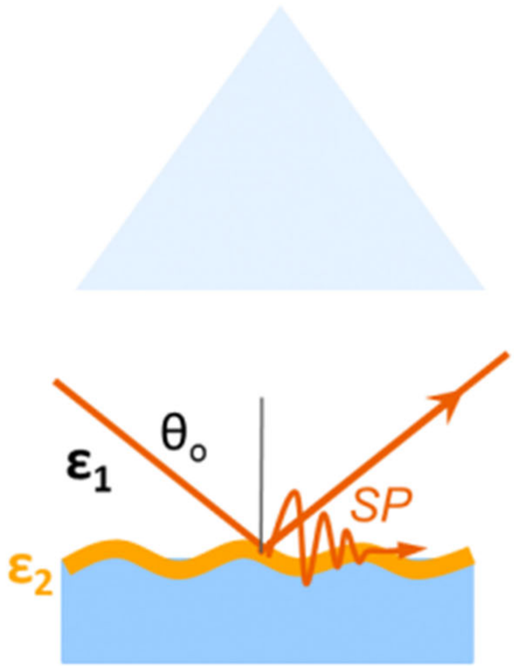
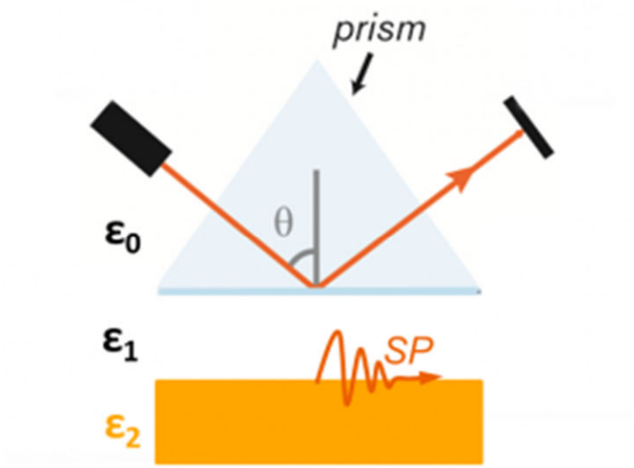
Basic instrumental configurations

Grating



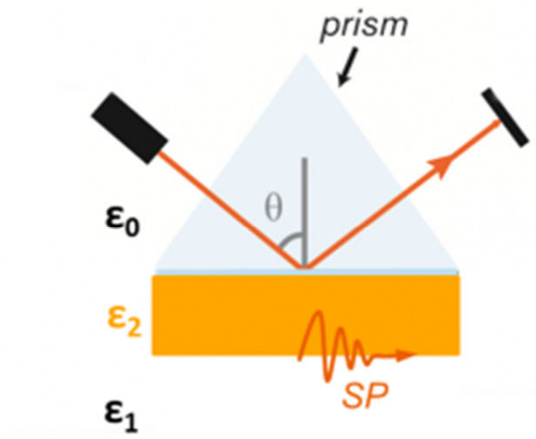
Incident light beam
goes through the sample

Otto



Gap between reflection
and the metal

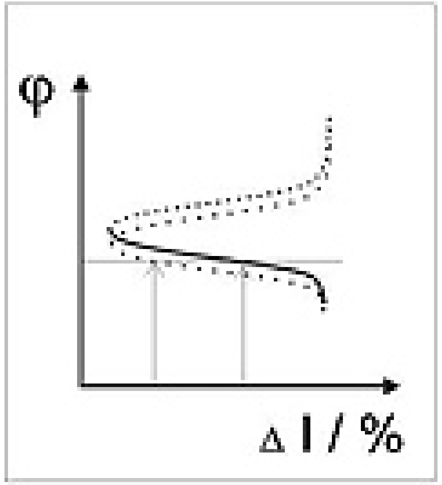
Kretschmann



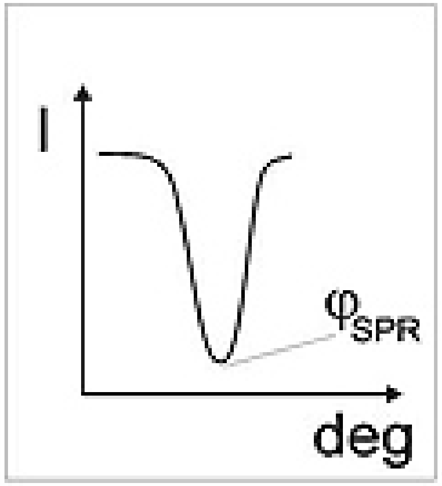
The most common
contemporary setup:
no gap and the beam does
not go through the sample

Basic detection modes

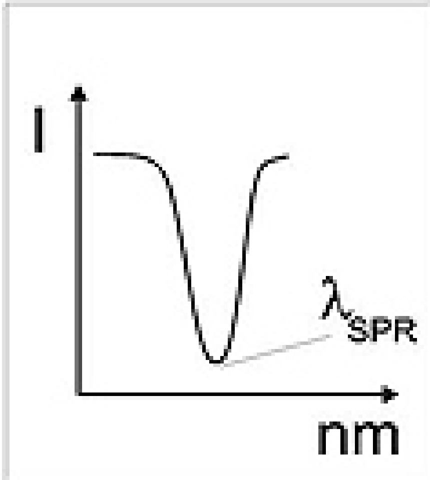
Intensity of the light is recorded at a fixed angle



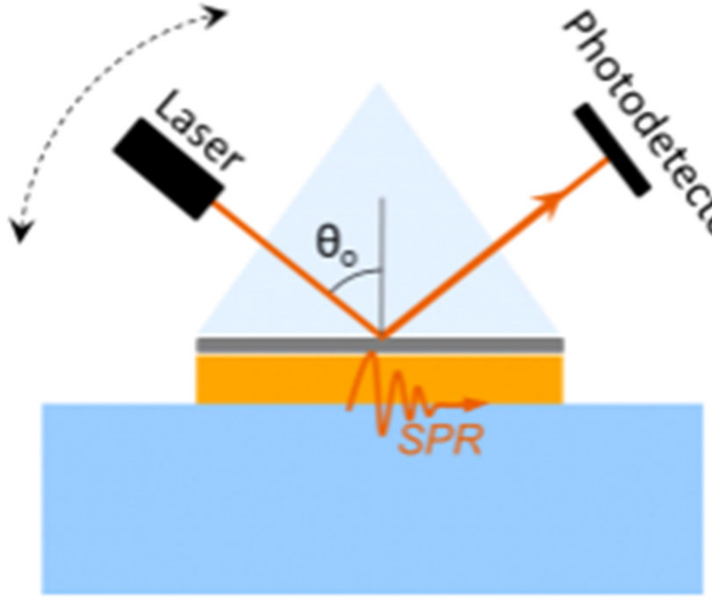
Monochromatic light source, angle varied



Fixed angle, the sample is irradiated with white light (whole spectrum) and the wavelength for resonance is detected



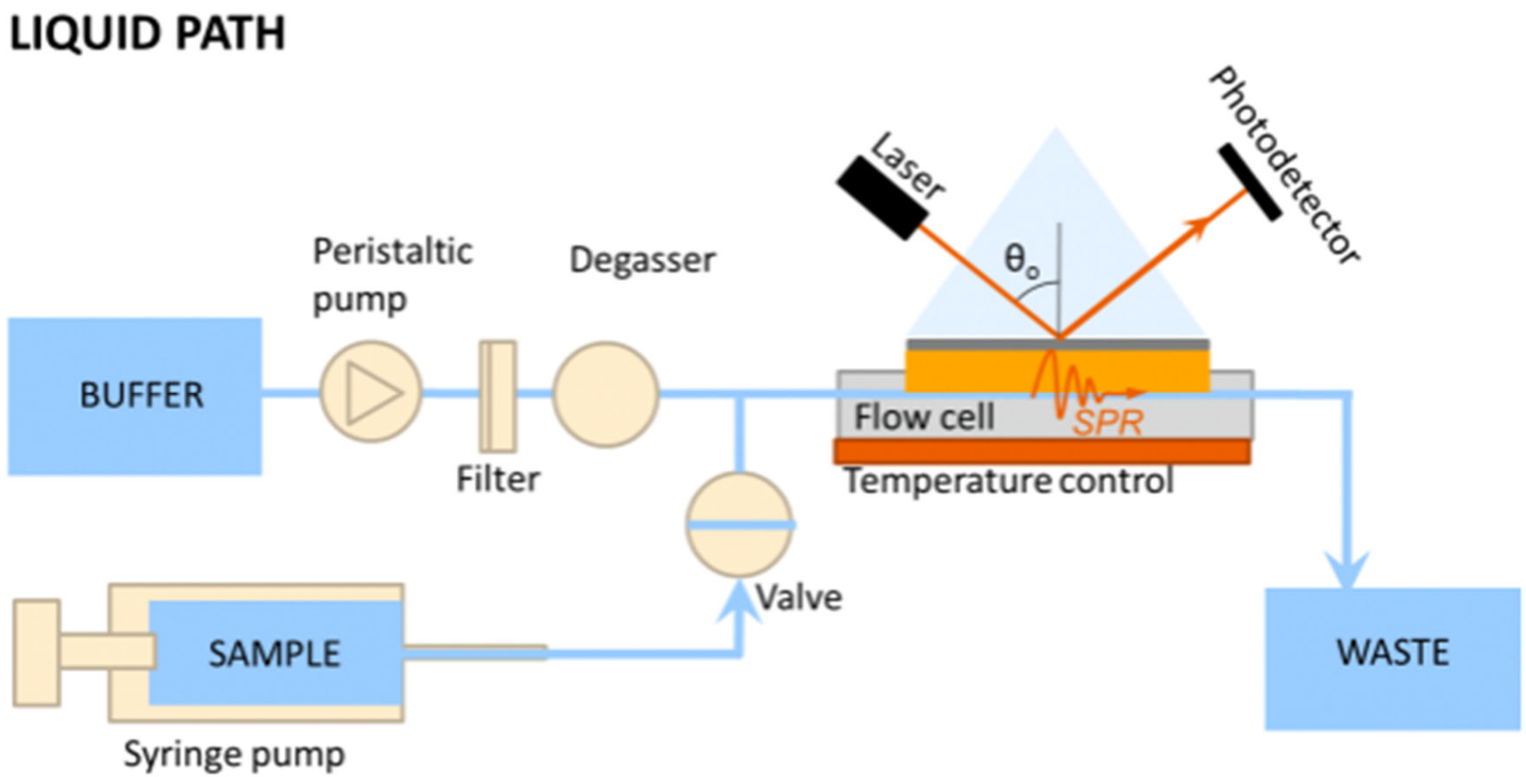
Basic detection modes



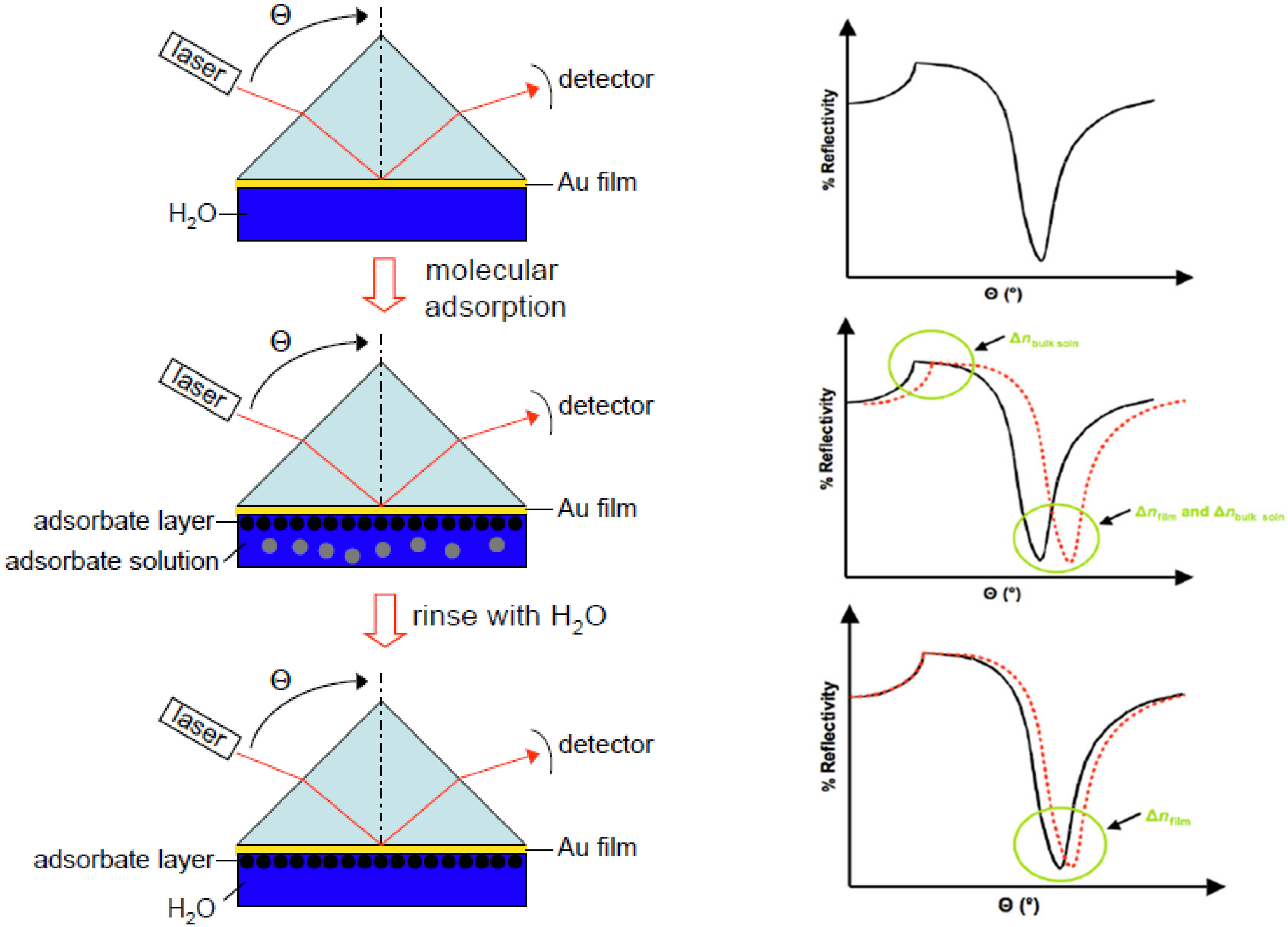
SPR Navi (commercial name)

- Merges fixed angle scan and angular scan
- Enables a wider range of angles *and* refractive indices

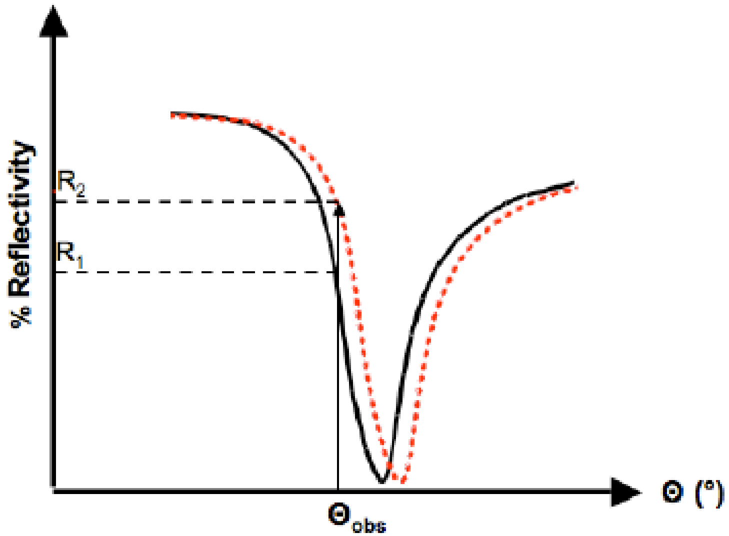
Basic instrumentation

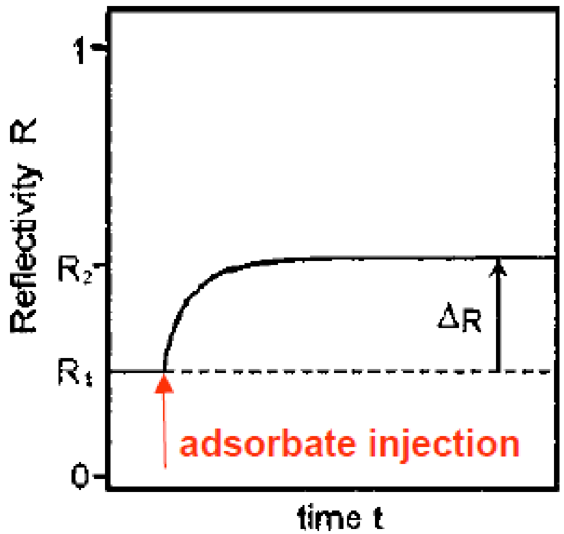


Data interpretation during adsorption



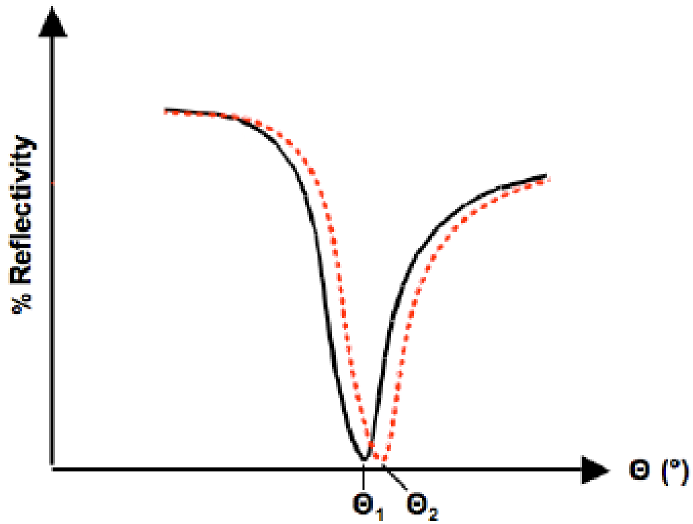
Data interpretation during adsorption

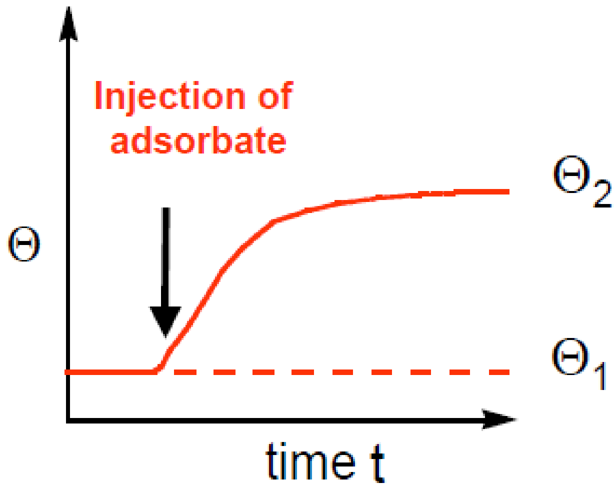




Time-dependent curve may be constructed from either reflectivity differences

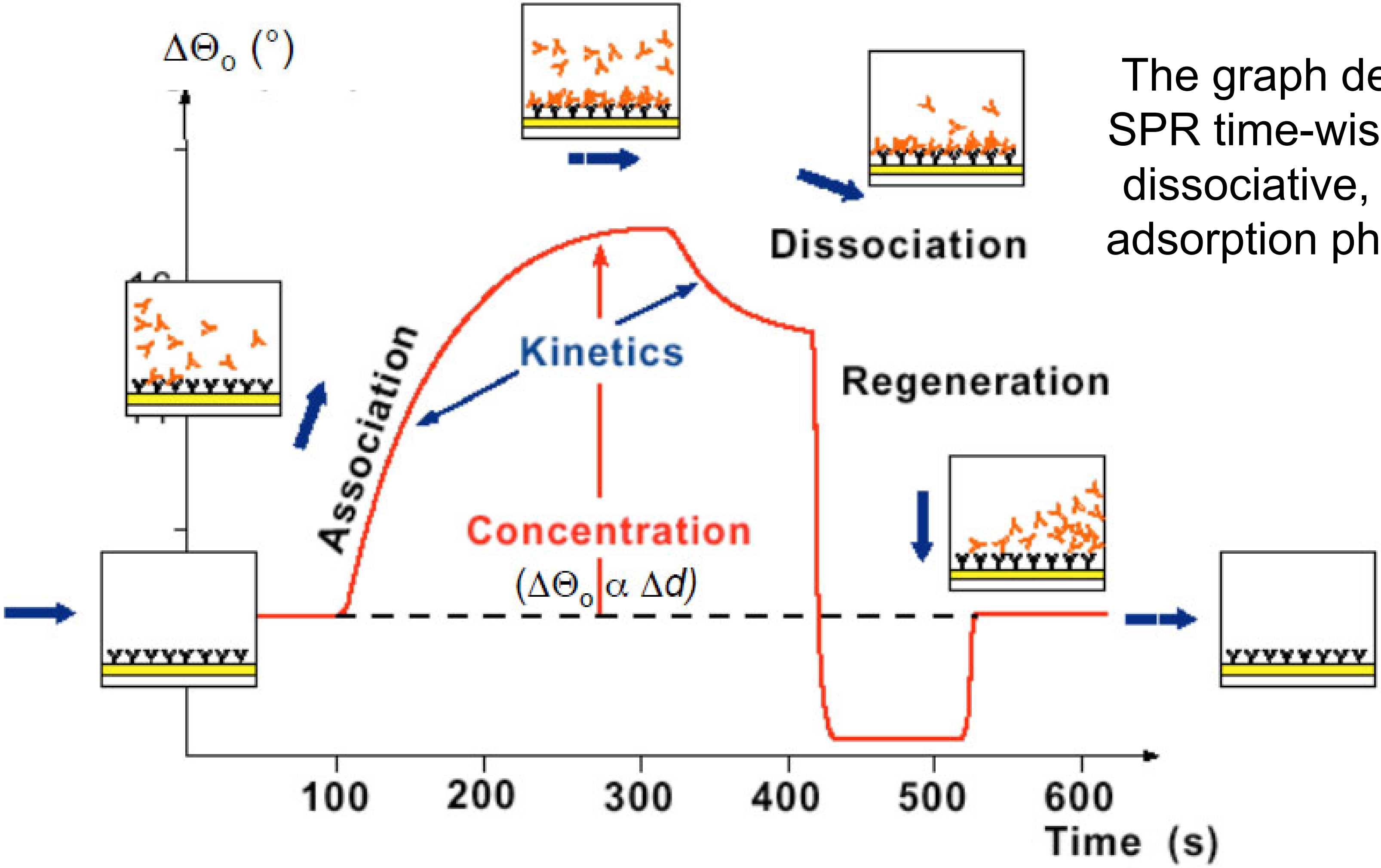
or





angular differences (at reflection minimum)

Data interpretation: sensorgram



The graph depicts how SPR time-wise follows a dissociative, reversible adsorption phenomenon

Data interpretation: sensorgram

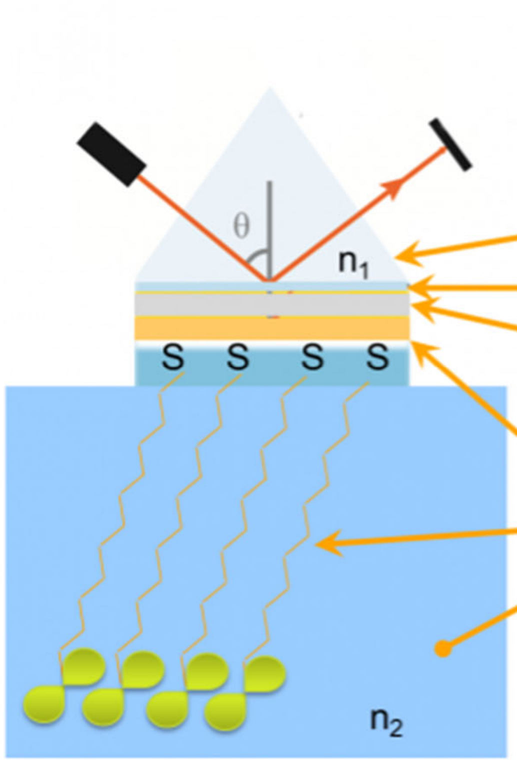
- Following of adsorption (or binding) is the most common use for SPR
- Nowadays, the time-dependent sensorgram is used very often in the adsorption measurements
- When using sensorgrams, the analyst does usually not even see the raw data (reflection curves)
- Often, the SPR software converts the sensorgram directly into mass or thickness data if the user inputs the refractive indices of the elements there beforehand

Modelling of SPR

- SPR curves can be modelled using the Fresnel equations (see previous lectures) for a multilayered structure
- Accurate, more reliable data on the properties of the adsorbed layer can be gained this way

$$\Delta\Theta_o = c_1\Delta n + c_2\Delta d$$

n – refractive index
d – thickness of adsorbed layer



Layer	Thickness [nm]	Real n	Imag. k
Prism	0	1.514	0
Elastomer	100 000	1.514	0
Glass slide	550 000	1.514	0
Cr	1.97	2.9512	2.9584
Au	49.88	0.1995	3.6525
(Thiol)	1.9	1.442	0
Air or Water	0	1.000273 1.33166	0

$n_2 < n_1$

Applications

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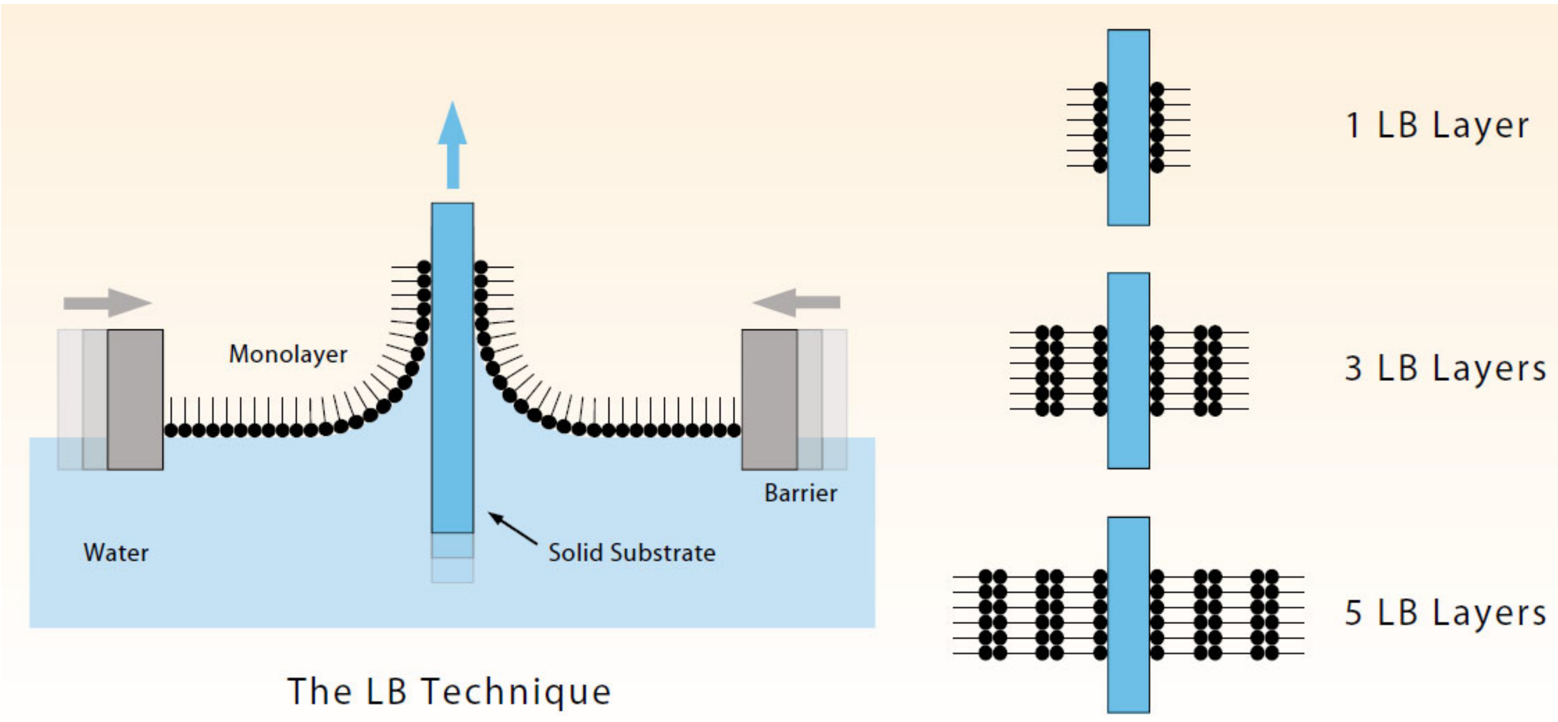
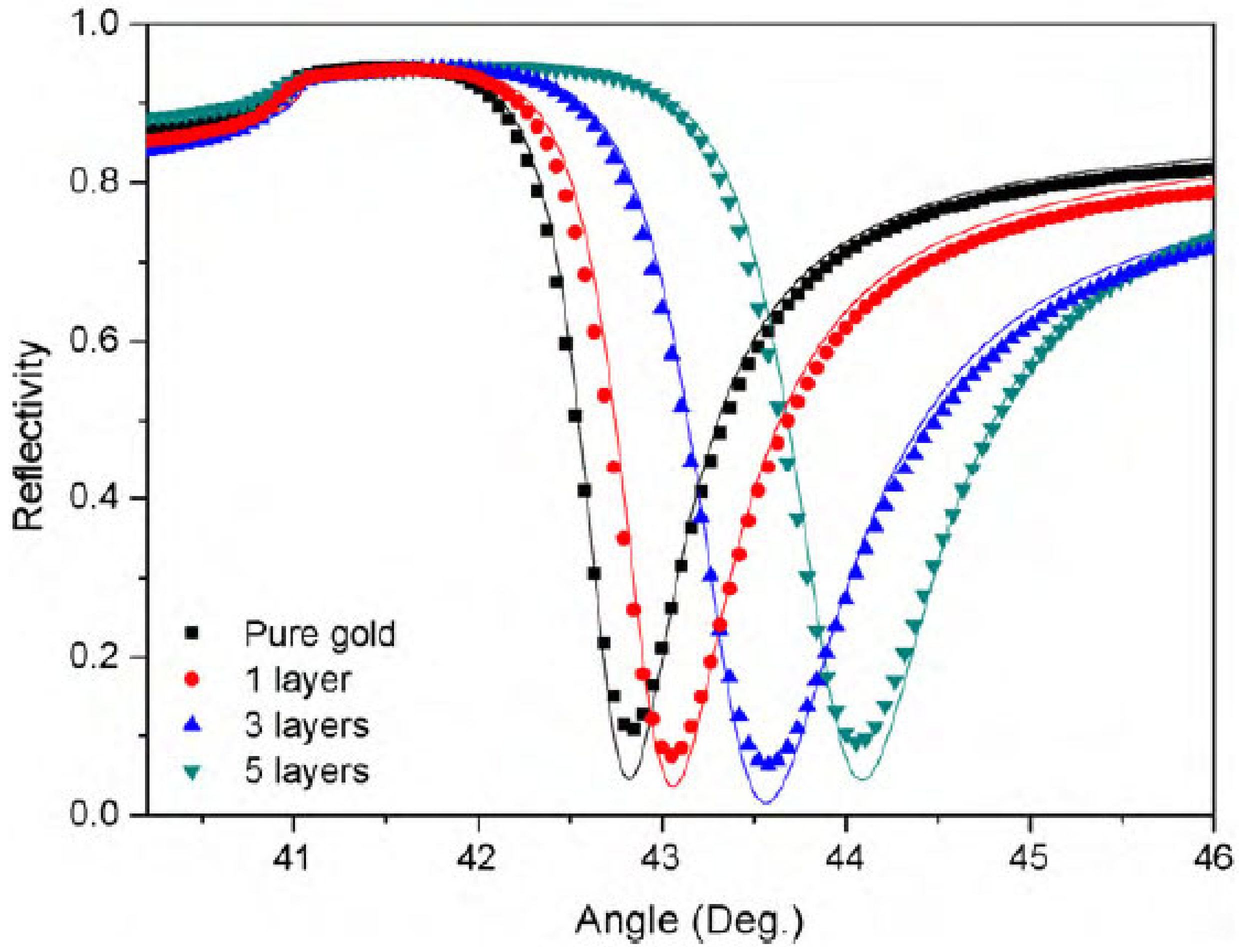
Thickness of Langmuir-Blodgett films

- Langmuir-Blodgett (LB) films of different thicknesses are prepared on a gold substrate (SPR sensor)
- The exact thickness values are gained from SPR measurements after the film preparation (*ex situ*)



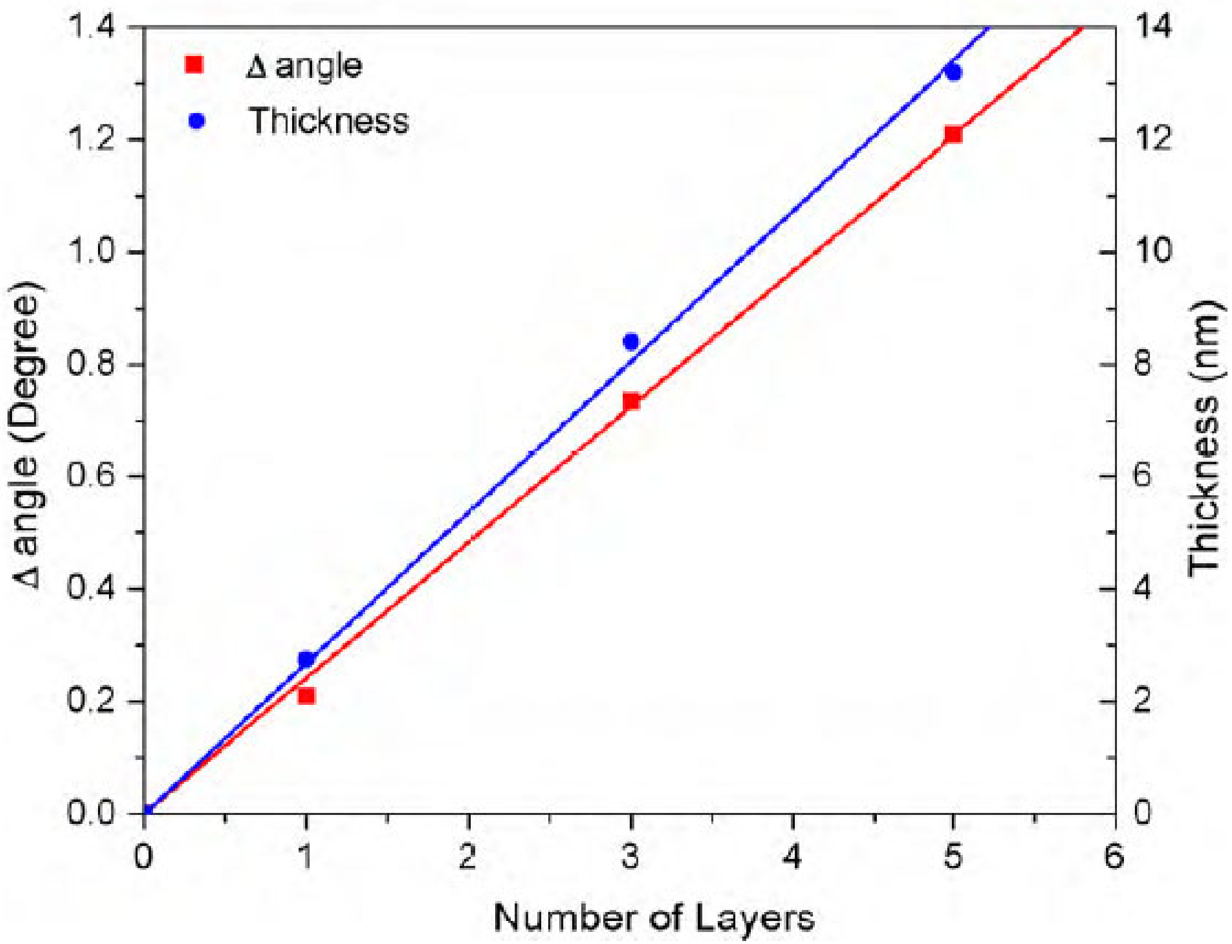
Thickness of Langmuir-Blodgett films



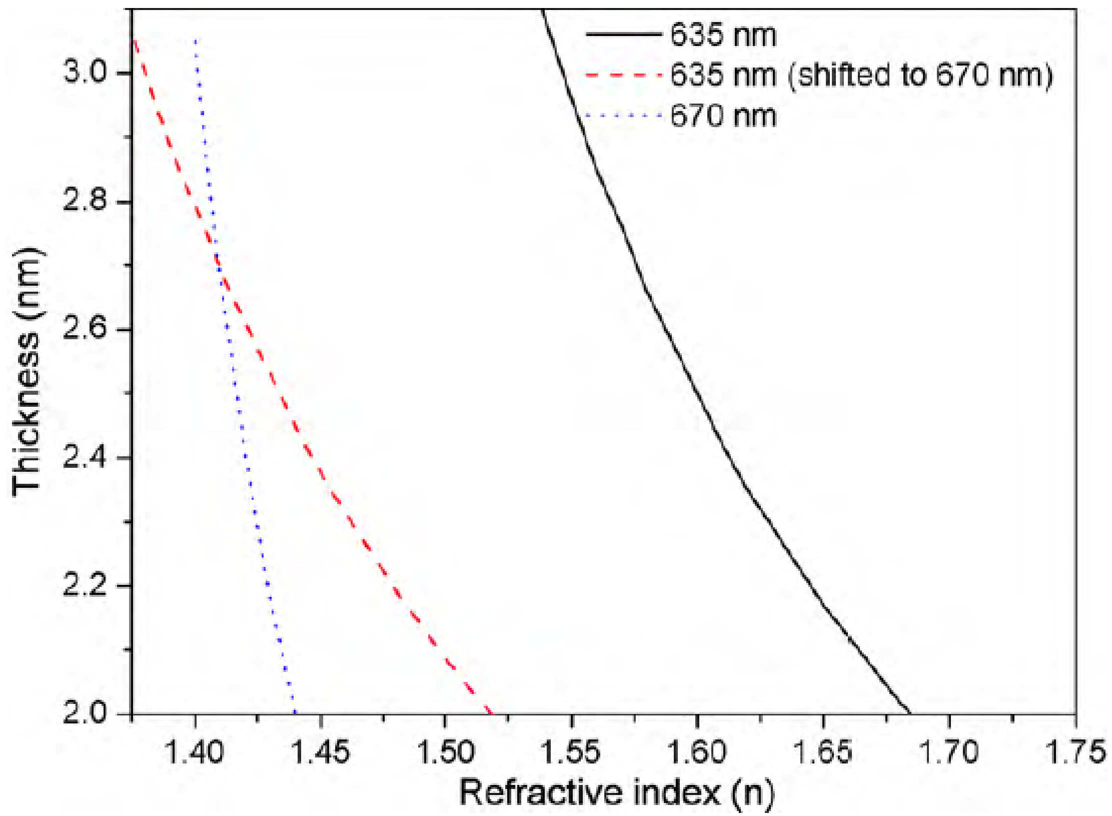
- Layers of stearic acid are deposited on gold
- Data is fitted to find out the thickness in high accuracy

Thickness of Langmuir-Blodgett films

- Angle change in SPR indicates the thickness (deduced from modelling)

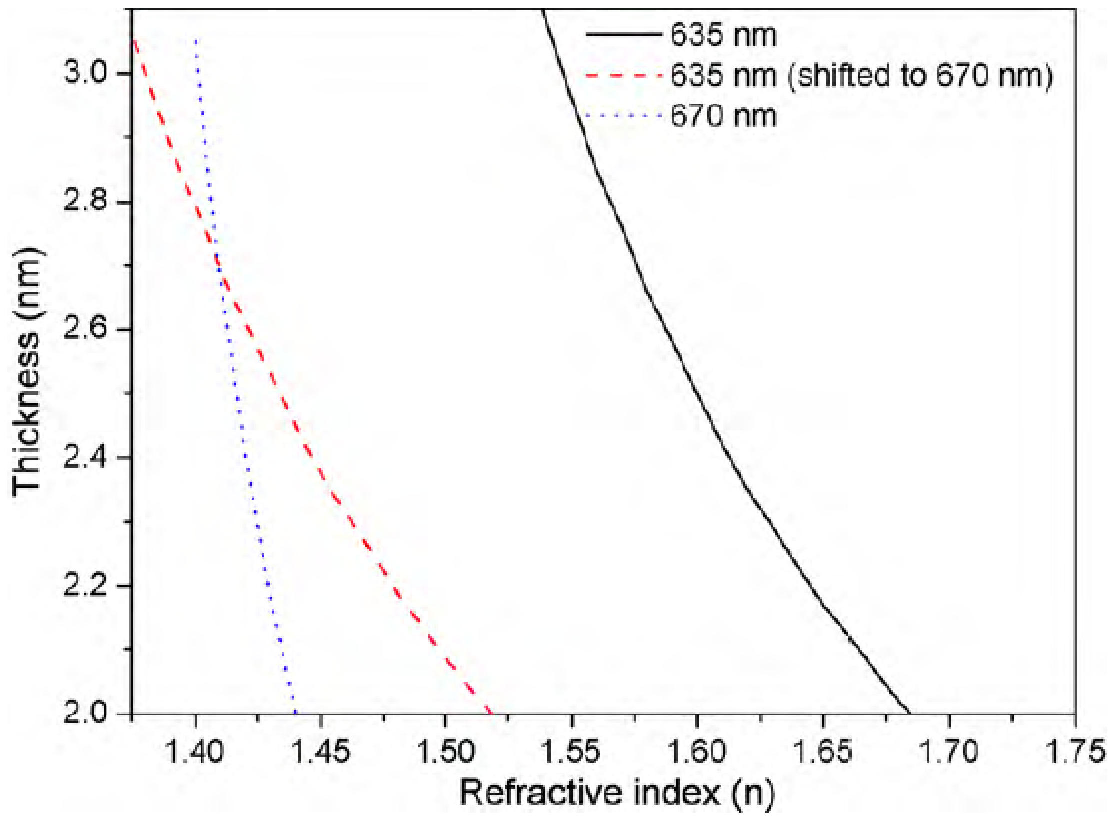


Refractive index of Langmuir-Blodgett films



- The LB film is monitored by SPR with two distinct wavelengths (635 and 670 nm)
- Refractive index depends slightly on the wavelength
- Film thickness is plotted as a function of refractive index (gained from modelling)

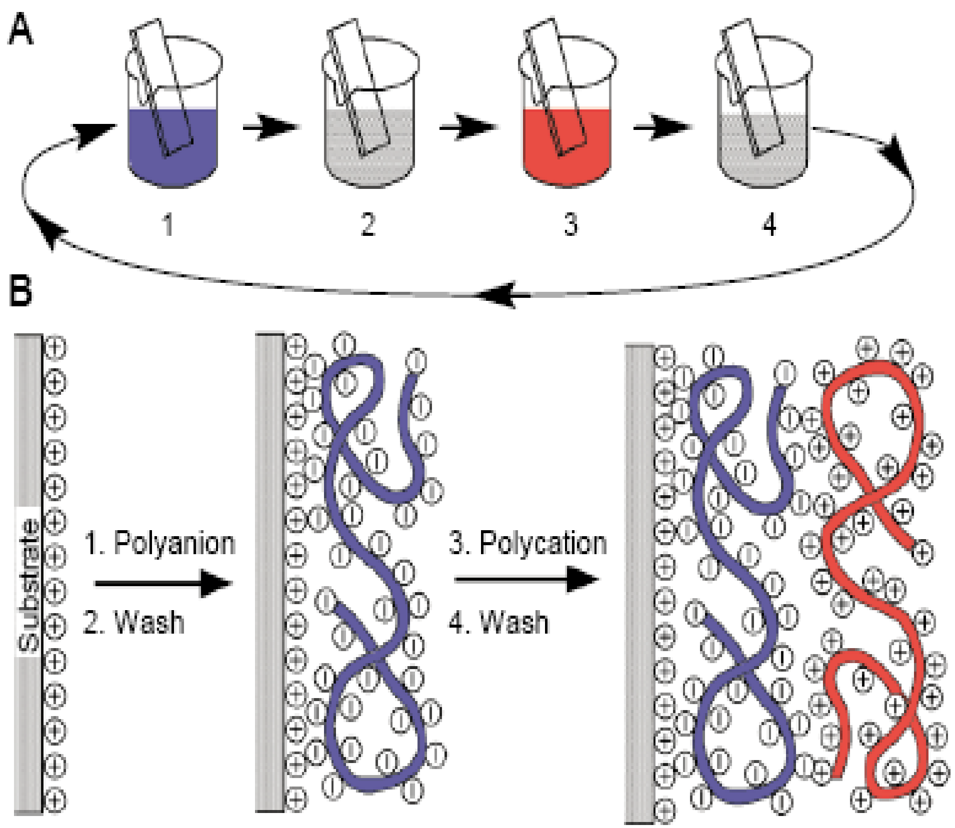
Refractive index of Langmuir-Blodgett films



- The other refractive index is mathematically shifted to match the other one
- The intersection point yields the definitive refractive index of the material

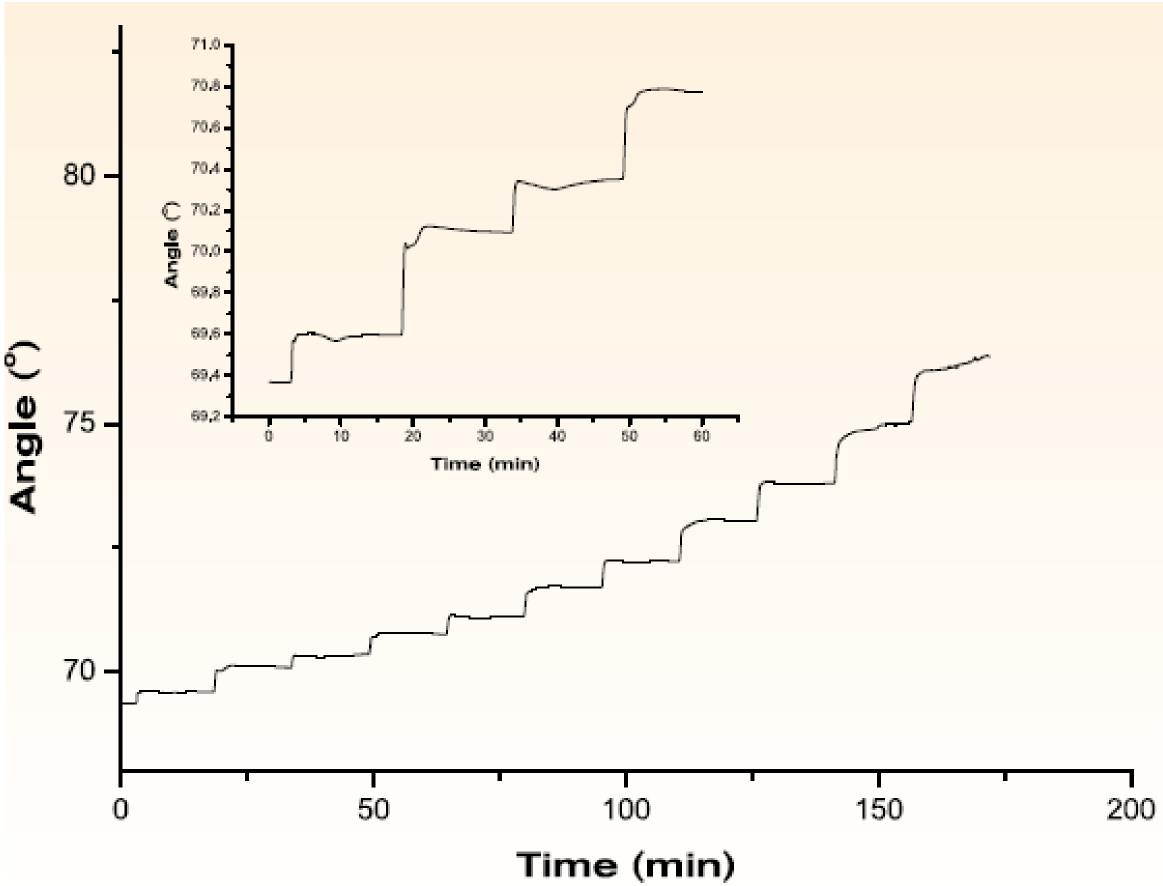
Investigating layer-by-layer deposition

- polyelectrolytes of opposite charges can be deposited one after another
- experimentally the easiest technique: requires only polyelectrolyte solutions and washing in between



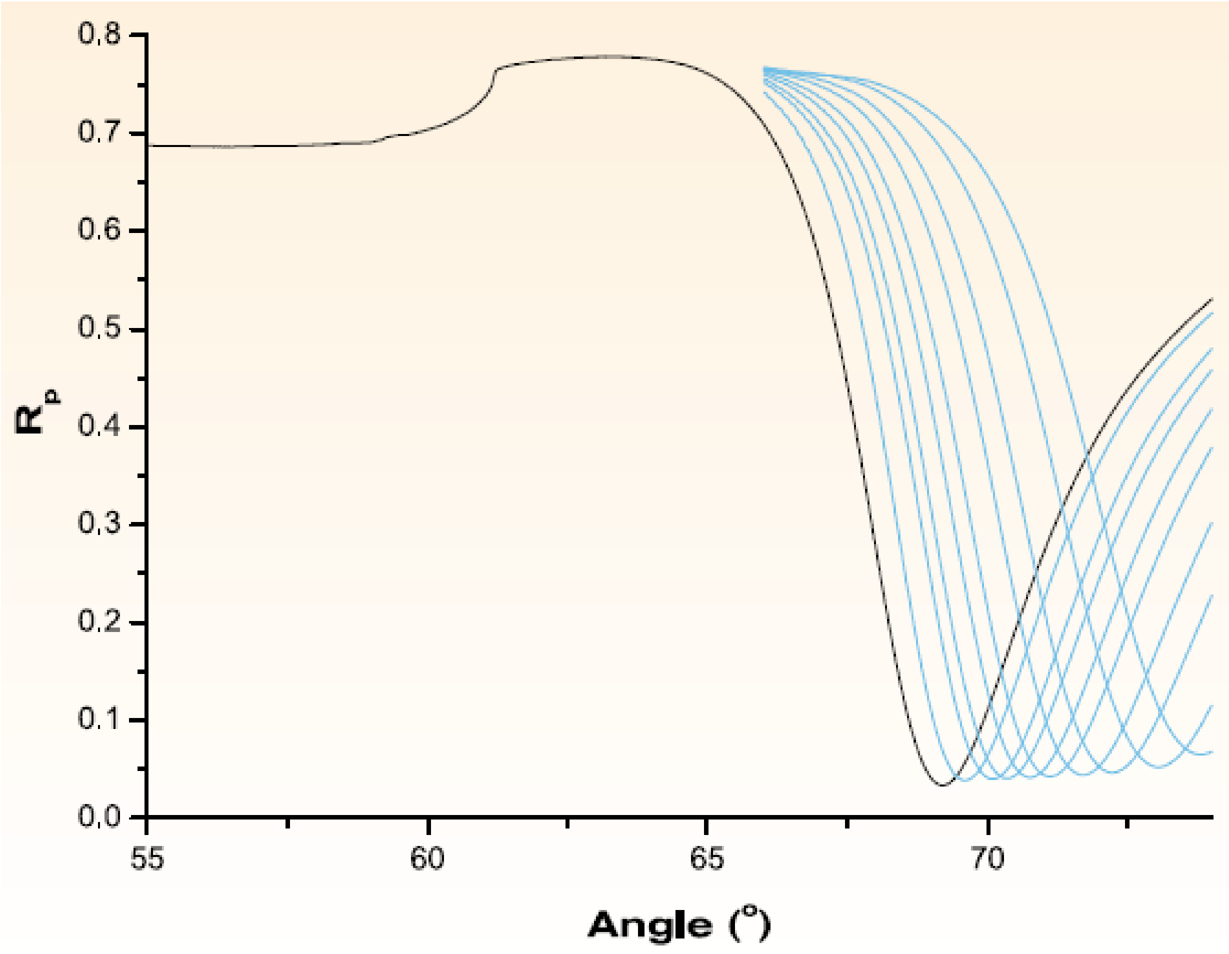
Science **1997**, *227*, 1232.

Investigating layer-by-layer deposition



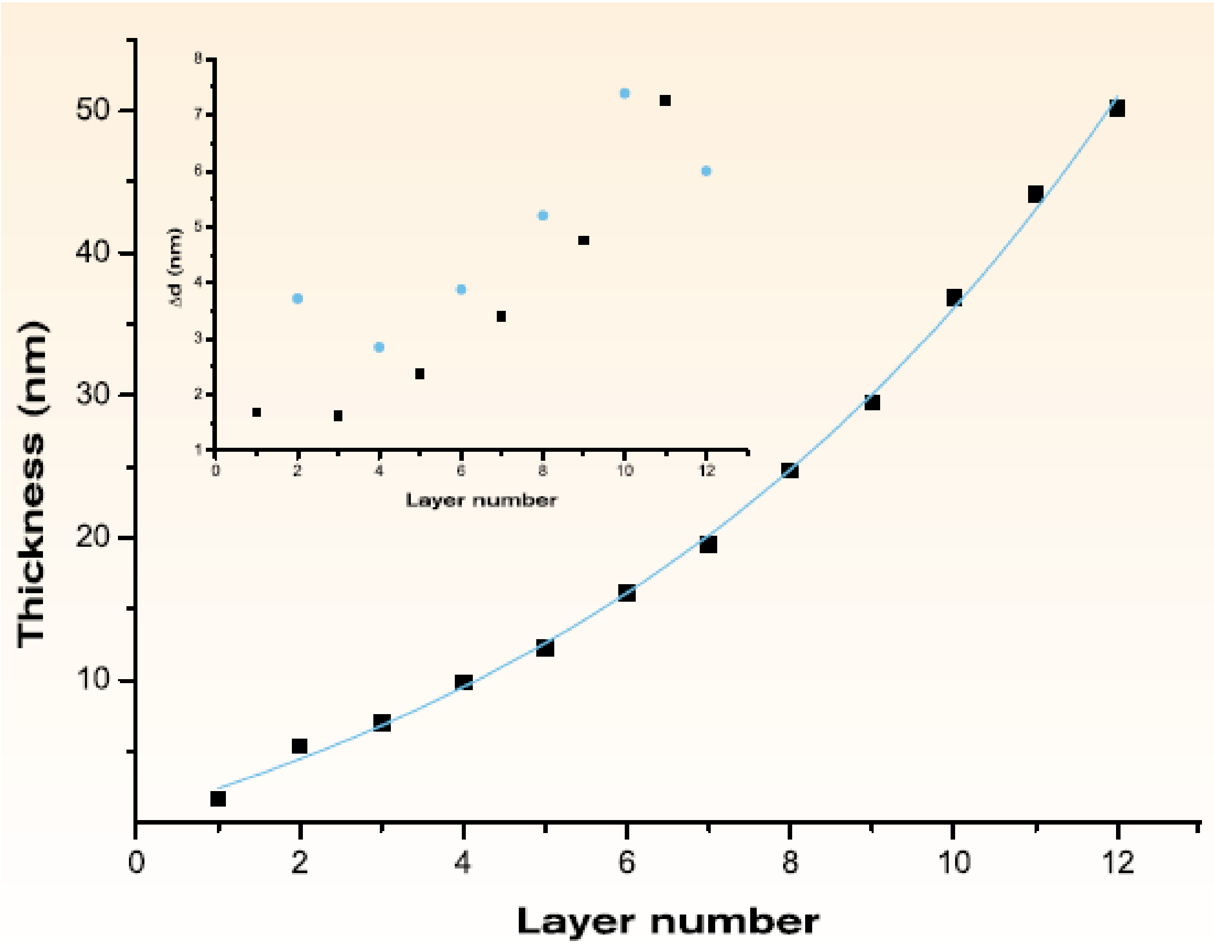
- Sensorgram can be used to follow the in situ build-up of multilayer
- It appears that in the case of these particular polyelectrolytes, the adsorption is very fast and rinsing brings about little desorption

Investigating layer-by-layer deposition



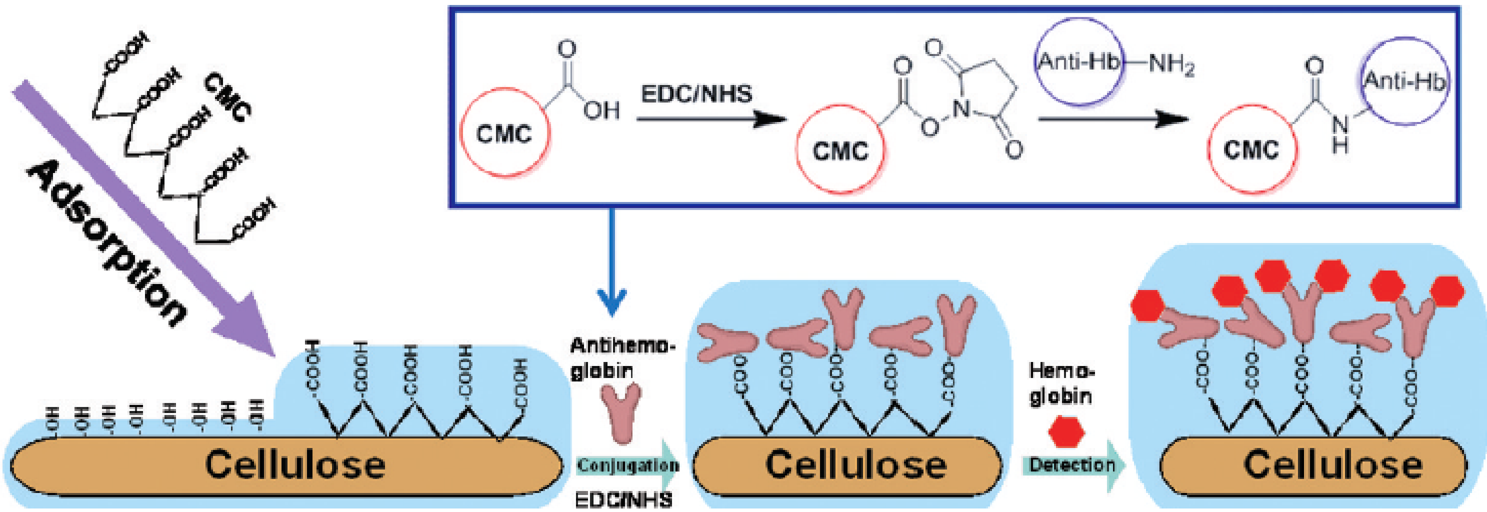
- Modelling of the raw data leads to accurate determination of the thickness

Investigating layer-by-layer deposition



Result:
In situ investigation of layer-by-layer deposition

Antibody conjugation on cellulose

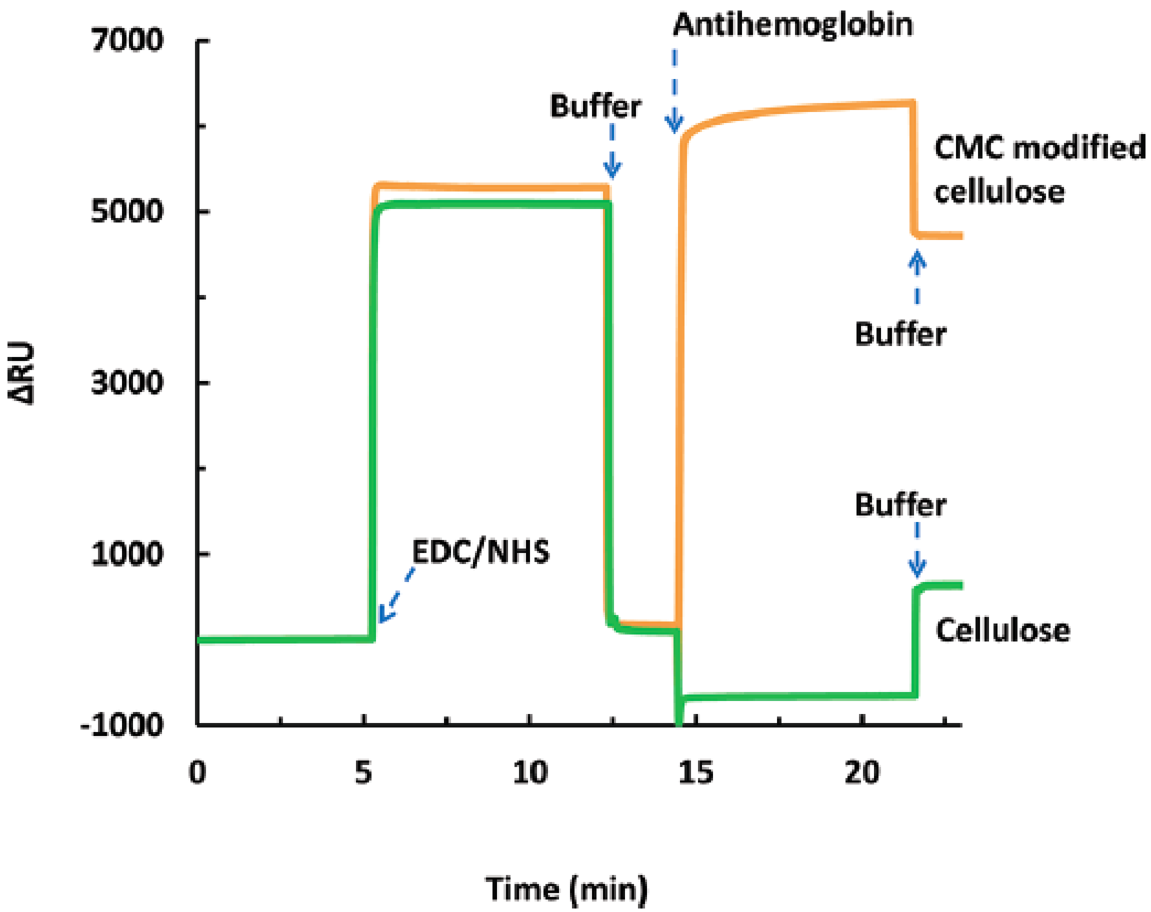


- Carboxymethyl cellulose (CMC) is adsorbed on cellulose surface
- Carboxylic groups are modified with so-called EDC/NHS mechanism
- An antibody (antihemoglobin, Anti-Hb) is attached on the surface
- Because hemoglobin binds selectively onto its antibody, the system can be used as a sensor for hemoglobin

Biomacromolecules **2012**, *13*, 1051.

Antibody conjugation on cellulose

EDC/NHS + antibody attachment followed in an SPR sensorgram



- Initial attachment of CMC by adsorption is absolutely necessary for the antibody binding to occur on cellulose

Biomacromolecules **2012**, *13*, 1051.

Summary

Summary

- SPR is based on the sensitivity of surface plasmons on a metal/dielectric surface: small changes in the refractive index of the dielectric (sample) affect the plasmon speed significantly

→ Small mass changes on the surface can be monitored

→ SPR can be used to monitor, e.g., adsorption (mass change on surface)

- Sensorgrams yield in situ information on, e.g., adsorption or interfacial reactions

- Modelling can be used to extract the exact thickness and refractive index of a film / adsorbed layer