Ellipsometry for probing sensor materials and sensing mechanisms

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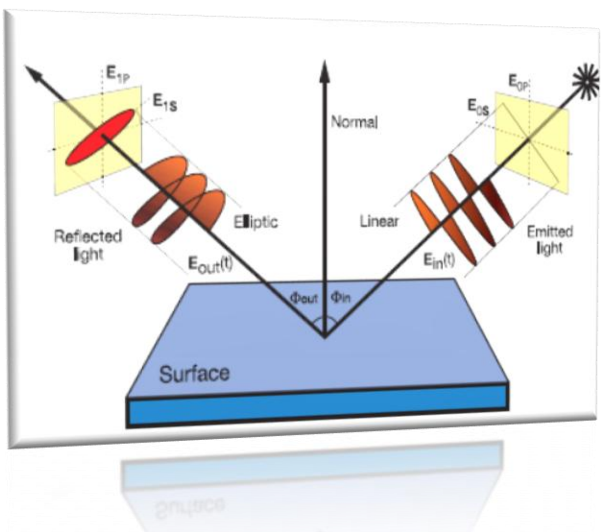
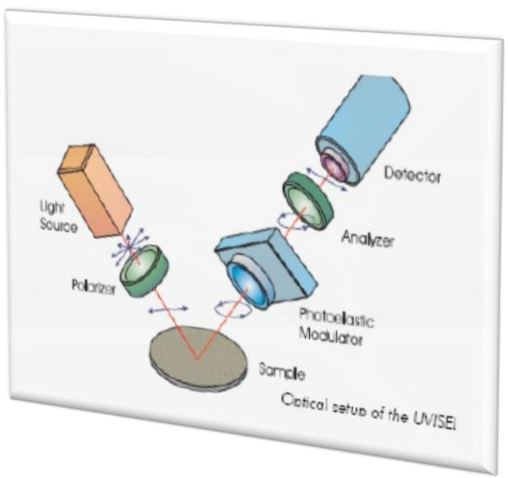


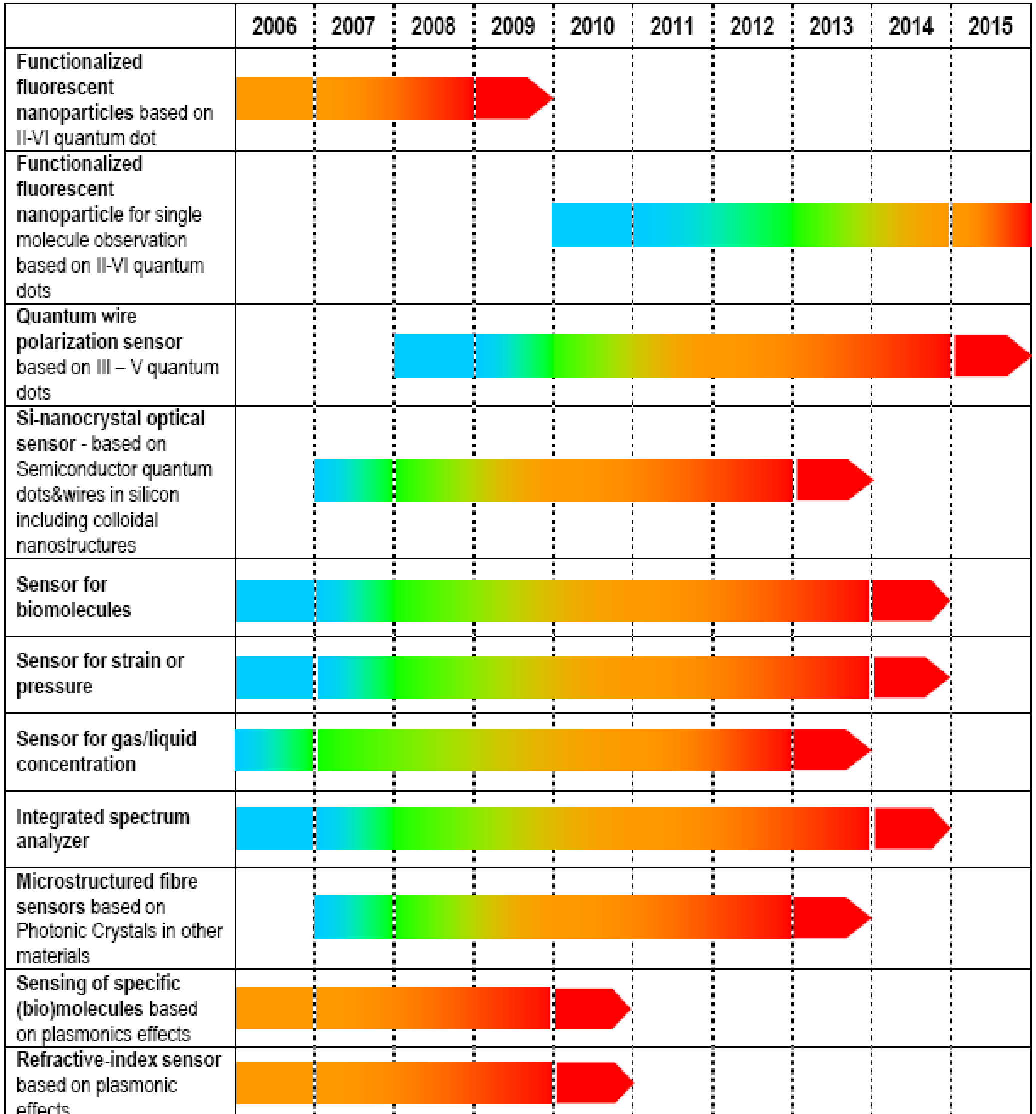


Outline

Overview of Ellipsometry potential and capabilities in materials characterization and technological applications

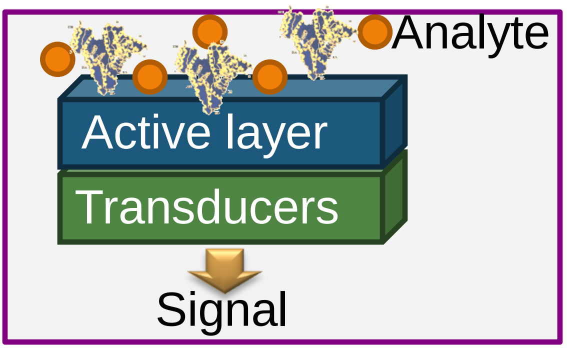
- optical sensors
  - surface plasmon resonance
  - single wavelength ellipsometry
  - spectroscopic ellipsometry
- what is ellipsometry and what it does?
- materials for sensors
- example of applications in biosensing

Sensors application time scale



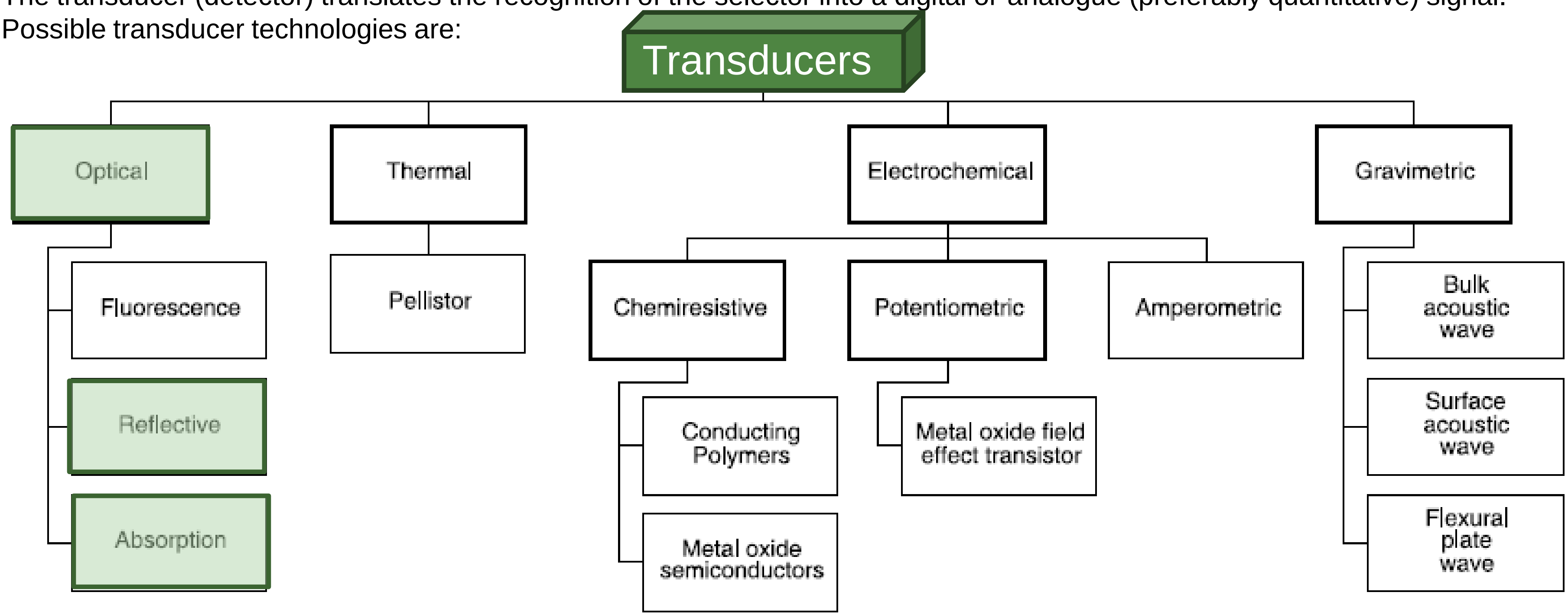
- Ellipsometry is today used for R&D phase
- And it is compatible for the future mass-production

Principle of Chemical Sensors



A chemical sensor is composed of an active layer and a transducer. The **active layer** represents the main part of the sensor. Many materials such as organic, inorganic or hybrid organic-inorganic polymers can be used as active layers, providing that pollutants can interact/diffuse into the matrix, thus modifying the physical or/and chemical properties of the material. The active layer can also be doped with specific probe-molecules able to react selectively with the targeted pollutants, thus providing the selectivity of the sensor. The **transducer** role is to convert the variation of a physical property (i.e. refraction index..), or a chemical interaction (i.e. H-bond formation, electrostatic interaction…) or a chemical reaction (covalent bond formation) into a measurable signal (optical, electrical, electrochemical, piezoelectrical, etc…) proportional to the analyte concentration

The transducer (detector) translates the recognition of the selector into a digital or analogue (preferably quantitative) signal. Possible transducer technologies are:



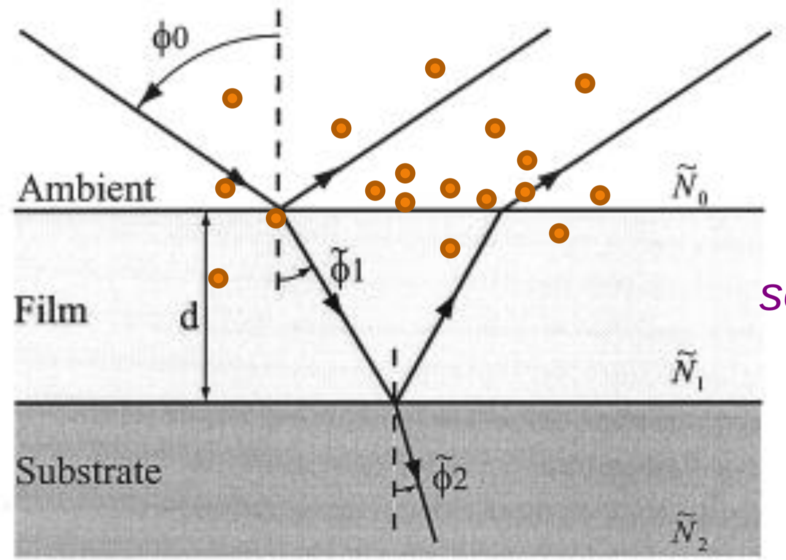
Bio and Gas Sensor Technologies

Type	Sensitive Material	Detection principle
Semiconducting metal oxide (MOS)	Doped semiconducting metal oxides (SnO_2, WO_3, MbO_3,..)	Resistance change
Field effect sensors, FET (also organic, OFET) (Transistors, capacitors, Schottky diodes)	Catalytic materials Organic polymers	Potential change at the gate
Conducting polymers	Modified conducting polymer	Resistance change
Optical sensors	Organic dyes, semiconductors oxides, metals	Changes in reflection, absorption, refractive index, layer thickness
Surface Plasmon Resonance (SPR) sensors	Thin films and metals	Changes in the angle of incidence of the light
Ion conducting metal oxides	Ionic conductors, ZrO_2, Ga_2O_3	Potential changes
Quartz crystal microbalance, QCM, Surface acustic wave, SAW	Organic or inorganic layers	Frequency change due to mass change
Thermistor/Pellistor/thermopile	Semiconductors/Catalytic materials/metals	Temperature change
Electrochemical cells	Solid or liquid electrolytes	Current or voltage change

Optical Sensors

Sensors based on optical methods have attracted considerable interest in recent decades. Different techniques utilize quantification of one or more of the fundamental characteristics of optical waves such as amplitude, frequency, phase and polarization by measuring e.g. **reflectance, transmittance, absorbance, fluorescence, ellipsometric response, surface plasmon resonance** effects etc.

e.g. ellipsometric response



sensing layer

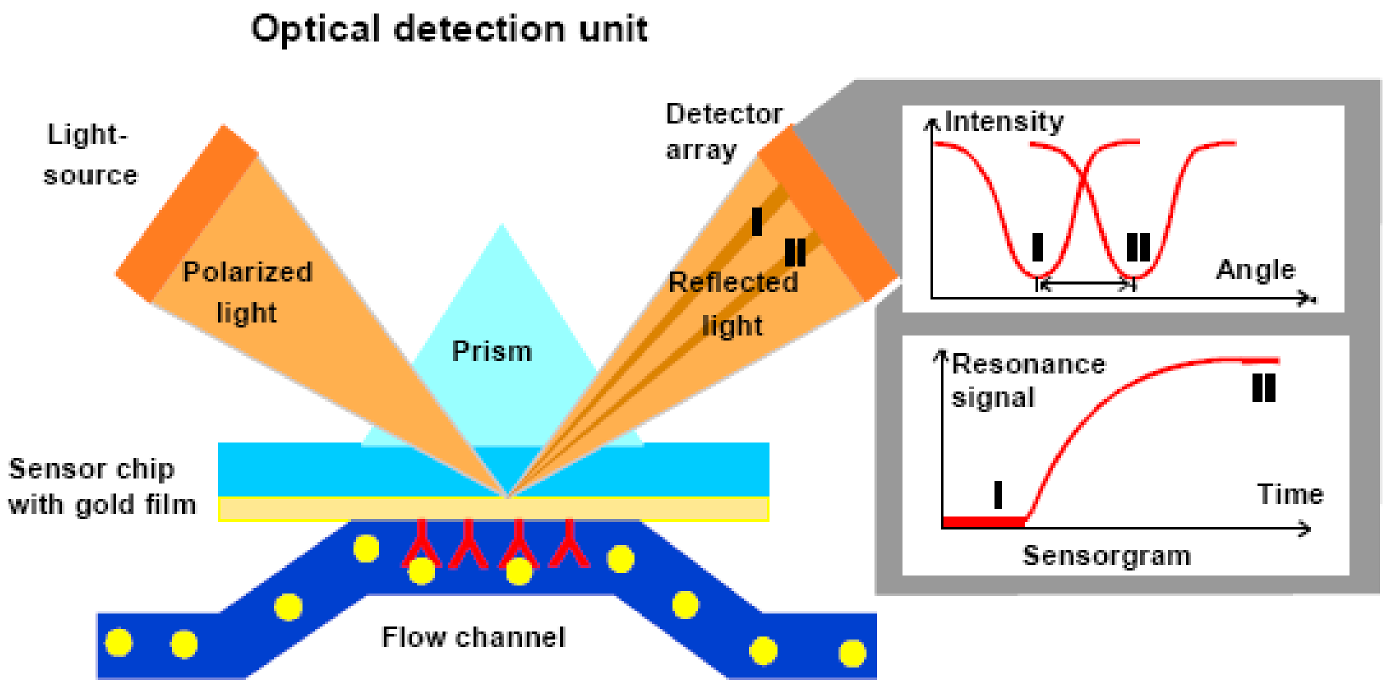
Three-phase model representing a substrate with a sensing layer

Since *ψ and Δ (the ellipsometric parameters)* are very sensitive to changes in the thickness and/or the refractive index of a thin layer, ellipsometry can be used in sensor applications if e.g. a substance in a liquid or a gas interacts with the layer.

Two basic transducer mechanisms are therefore that **the refractive index and/or the thickness of the sensing layer change** when exposed to the gas/substance under study.

Surface Plasmon Resonance (SPR) Optical Sensors – (mainly used)

The main advantage of optical sensors over electrochemical sensors is that they are resistant to electromagnetic interference, capable of performing remote sensing, and can provide multiplexed detection within a single device. In optical biosensing, generally, there are two broad detection protocols that can be implemented : labeled (e.g., fluorescence-based detection) and label-free detection. The most widely used label-free optical biosensor is the **surface plasmon resonance sensor (SPR)**

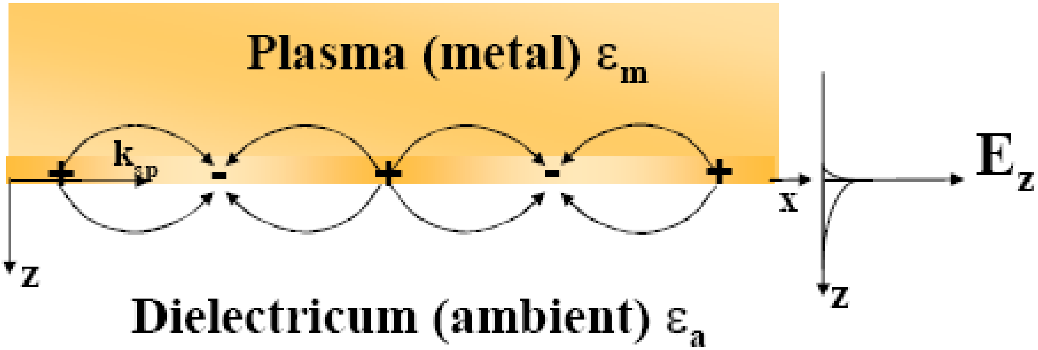


As biomolecular interaction events will lead to an increase in refractive index or thickness of the organic layer adsorbed by the gold sensor surface, which can in turn change the conditions for wave-vector matching, any biomolecular binding reaction taking place in the sensor surface will therefore result in a shift in the SPR dip both in the angular and spectral domains

Surface Plasmon Resonance (SPR) Sensor-Principle

Propagating plasmon

Surface plasmons are electromagnetic waves that propagate at the interface between a metal and a dielectric (such as air); they may be described as an oscillation in the electron density at the surface of the metal. Surface plasmons cannot be excited directly with light but may be excited by the evanescent field produced when light is reflected from the interface between two dielectric materials

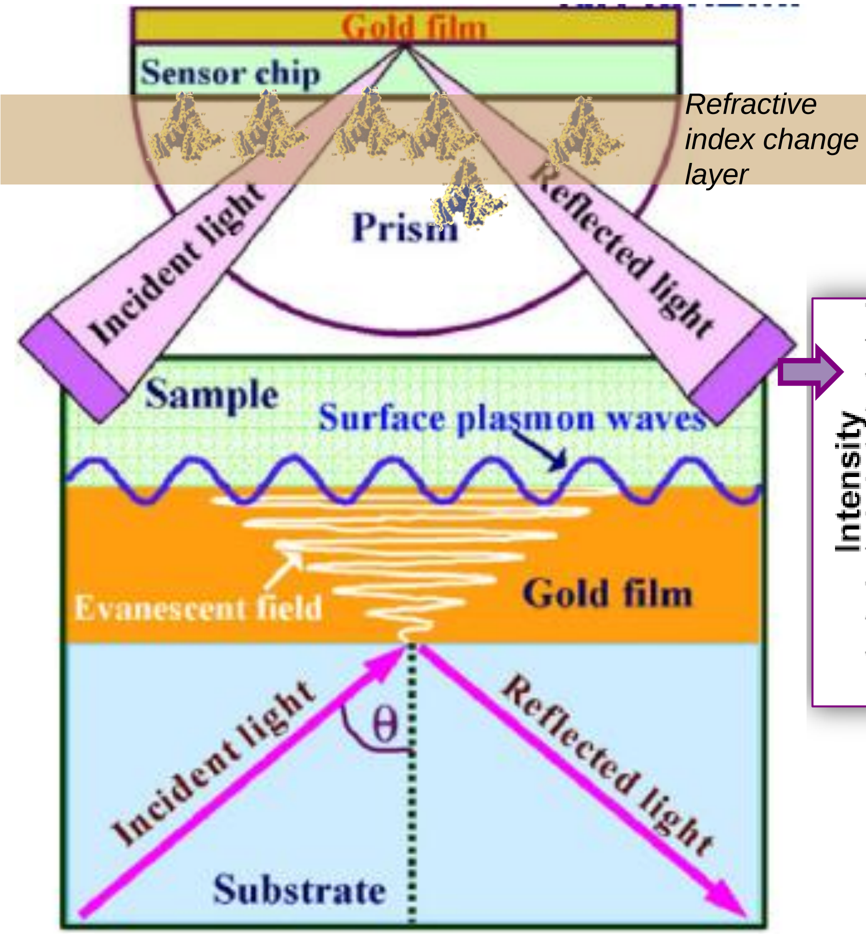


P-polarized surface bound electromagnetic wave.

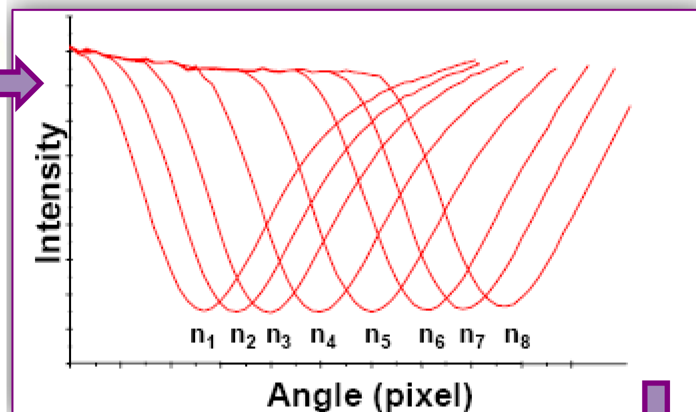
- Propagates in the interface between plasma and dielectricum.
- Coherent longitudinal charge fluctuations
- Evanescent field
- Surface sensitive
- Propagation length = f (λ,εm,na) ≈ 2 µm (gold, 633 nm).

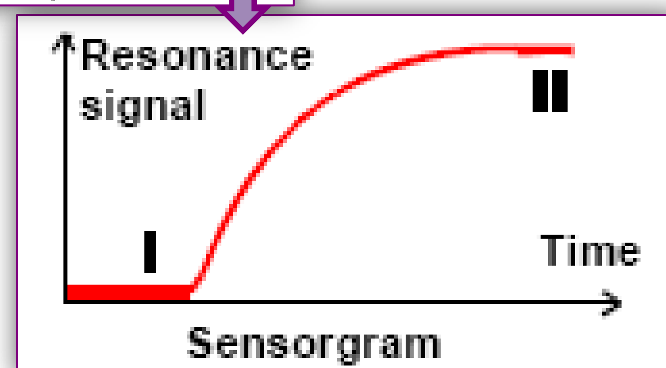
Surface Plasmon Resonance (SPR) Optical Sensors

SPR is a refractive index sensor



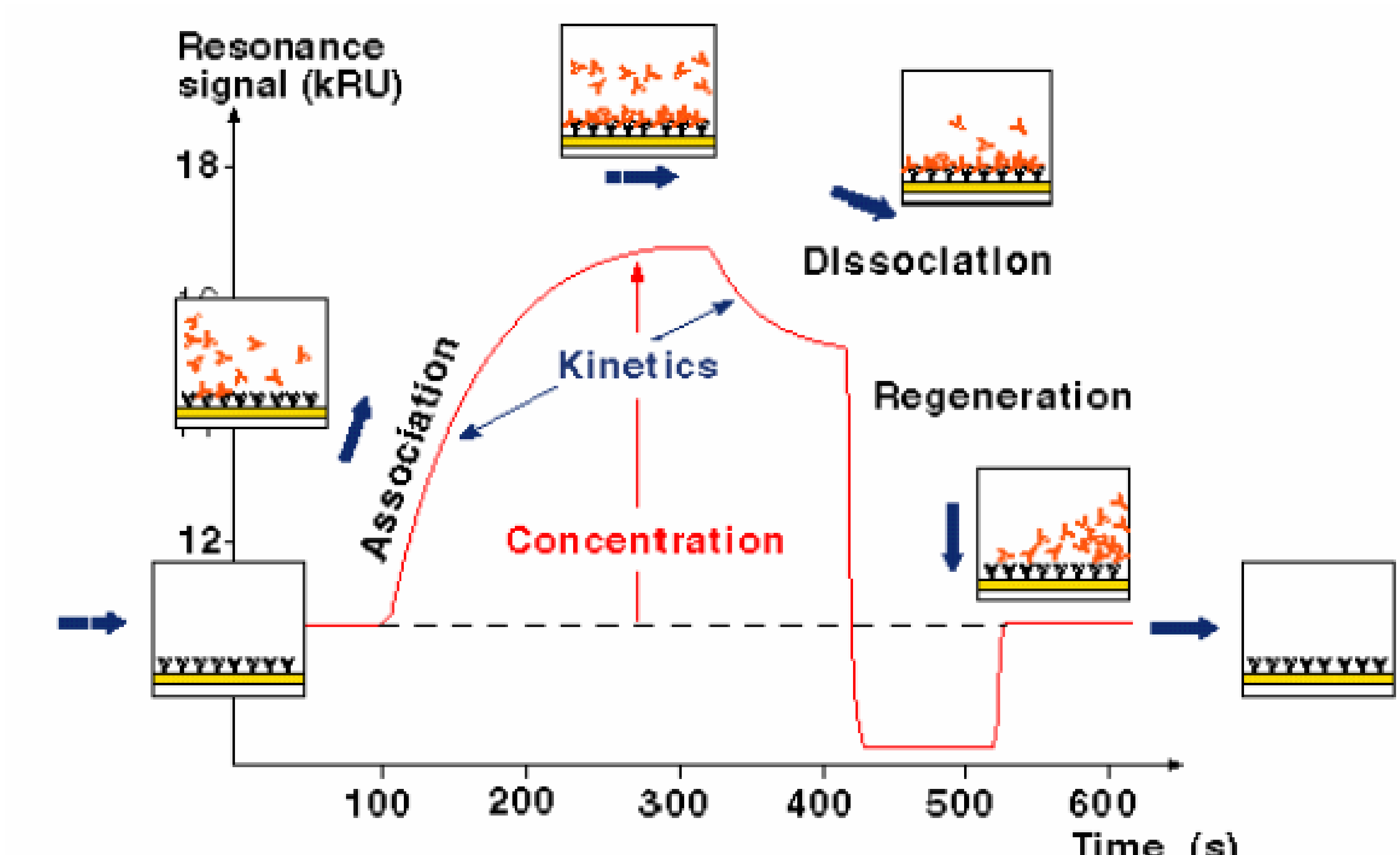
Adsorption of biomolecules (antigen or antibody) or gas on the metallic film as well as molecular interactions (antigen/antibody complex) will induce a change in the refractive index near the surface, thus giving rise to a shift of the resonance angle.





The SPR sensorgram

A plot of the SPR signal versus time

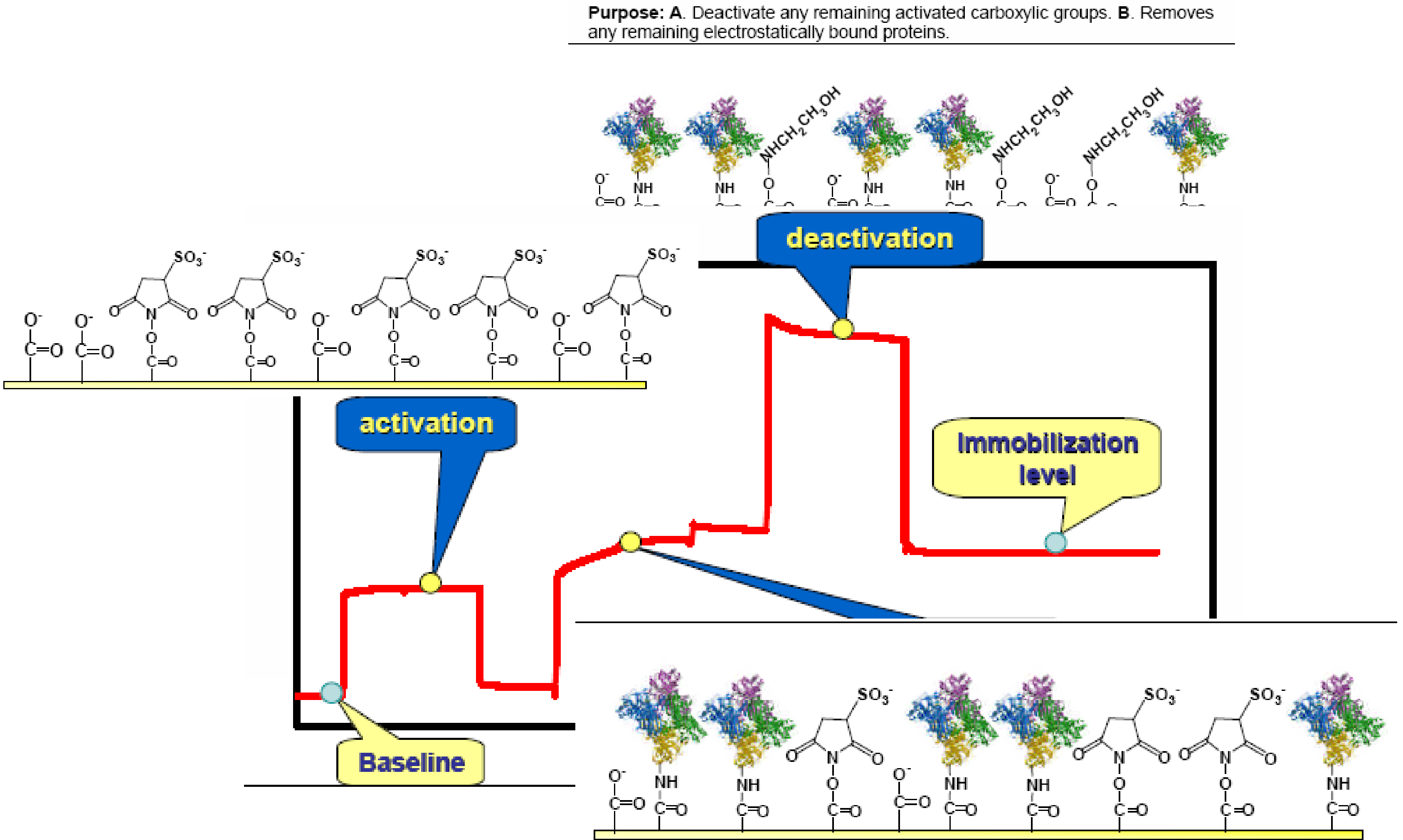


The progress of an interaction is monitored as a sensorgram. Analyte binds to the surface-attached ligand during sample injection, resulting in an increase in signal (injection start = 100 sec in diagram). At the end of the injection, the sample is replaced by a continuous flow of buffer and the decrease in signal now reflects dissociation of interactant from the surface-bound complex. A response of 1000 RU (Biacore specific terminology) corresponds to a change in surface concentration of 1 ng/mm^2.

[Source: Protein–Protein Interactions: A Molecular Cloning Manual, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 19, Figure 2]

SPR-Biosensing

Works were focusing mainly on antigen-antibody interactions, the streptatividin–biotin reaction. Current research includes the examination of protein–protein or protein–DNA interactions, even detecting conformational changes in an immobilized protein



SPR chemical gas sensing

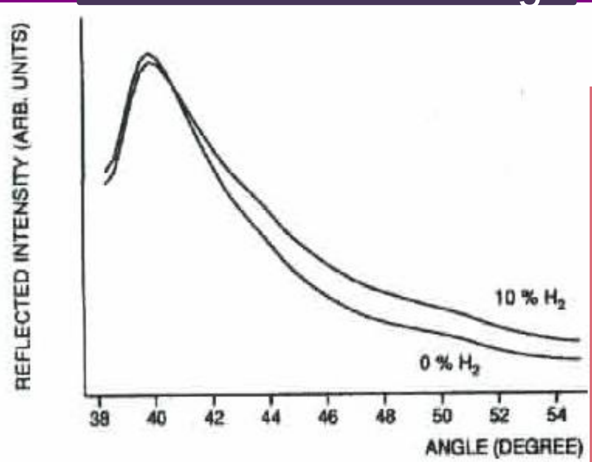
SPR-sensing devices using palladium as an SPR active metal can effectively detect H_2 because of intense adsorption of H_2 on palladium.

[B. Chadwick, M. Gal, A hydrogen sensor based on the optical generation of surface plasmons in a palladium alloy, Sensors Actuators B 17 (1994) 215]

A sensitive sensor for NO_2 detection utilizing chemisorption of NO_2 molecules in a gold SPR active layer has been reported.

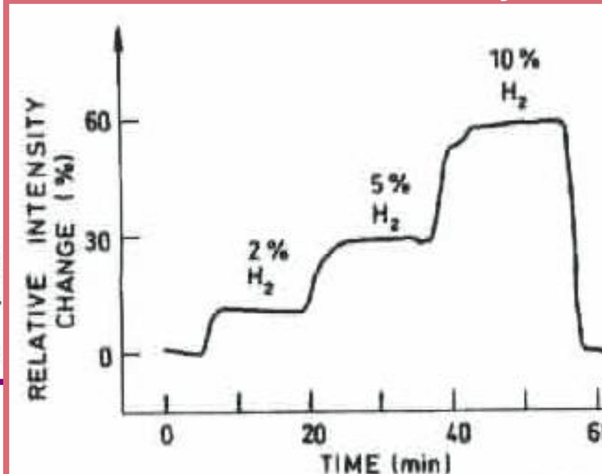
[G.J. Ashwell, M.P.S. Roberts, Highly selective surface plasmon resonance sensor for NO2, Electron. Lett. 32 (1996) 2089]

Reflectance-sensing



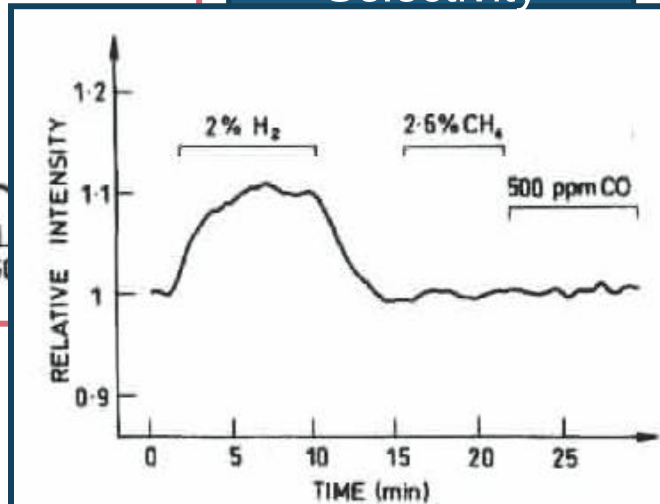
Reflected light intensity vs angle for a 200Å Pd film exposed to H_2

Sensitivity



Reflected light intensity change for the SPR sensor exposed to H_2

Selectivity



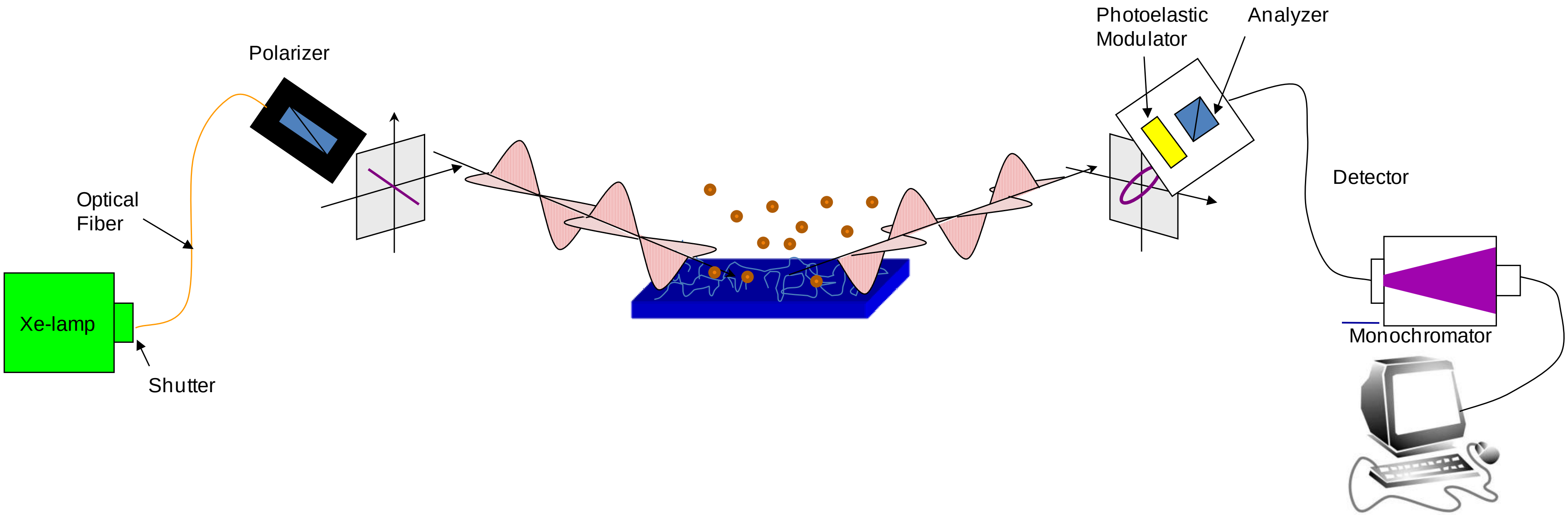
Limits of SPR sensors

- Until now, commercial SPR biosensor systems are predominantly based on the angular or intensity interrogation scheme, and their typical **limit of detection** is still **not comparable with** the level achievable by **fluorescence** tagging techniques, which can produce a signal associated with single molecular events.
- Despite of its less favorable sensitivity limit, SPR is still very attractive to the biomolecular research community because of **its label-free** and real-time quantification attributes.
- Current direct SPR biosensors are **limited to detection of about 1 pg/mm^2** surface coverage of biomaterial which is not sufficient for detecting low concentrations of low molecular weight analytes.
- Since commercial SPR instruments **limit direct detection** to molecules with molecular weights **above 10 kDa** or substances with high refractive index, some research is currently focusing on developing more sensitive SPR devices

For the **determination of small molecules**, the optical detection method based on total internal **reflection ellipsometry** (TIRE) **is more accurate and more sensitive**. TIRE was applied to detection of T-2, attaining LODs lower than those obtained by QCM or SPR [ref. below].

[A.V. Nabok, A. Tsargorodskaya, A. Holloway, N.F. Starodub, O. Gojster, Biosens. Bioelectron. 22 (2007) 885
A.V. Nabok, A. Tsargorodskaya, A.K. Hassan, N.F. Starodub, Appl. Surf. Sci. 246 (2005) 381]

Ellipsometry: a photon-in / photon-out non invasive Technique



Ellipsometry is focused on the reflection of a light beam from a reflective surface, where linearly-polarized light of a known orientation is reflected as elliptically-polarized light. From the changes in the ellipsometric angles (Ψ,Δ), the spectral dependence of optical properties (refractive index and dielectric function), thickness, morphology or roughness of layers or films on the surface can be calculated and used to determine *e.g. the amount* of adsorbed gas/protein on a surface

By analysing the change of a polarized light beam reflected at oblique incidence on a sensing layer during gas exposure, information about the concentration of the gas can be obtained

Why use ellipsometry?

- Ellipsometry is focused on the reflection of a light beam from a reflective surface, where linearly-polarized light of a known orientation is reflected as elliptically-polarized light. From the changes in the ellipsometric angles (Ψ,Δ), the spectral dependence of optical properties (refractive index and dielectric function), thickness, morphology or roughness of layers or films on the surface can be calculated and used to determine *e.g. the amount* of adsorbed gas/protein on a surface

- Ellipsometry is based on “photons-in” / “photons-out” and, therefore, does not require vacuum conditions and can be applied in gas and liquid ambients

- Ellipsometry has a thickness resolution of 0.01nm and therefore is suitable for studies of molecular layers on solid surfaces

- Ellipsometry is useful non destructive dynamics studies of gas adsorption on surfaces, gas sensing, molecules anchoring and film formation with a time resolution relevant for many biological processes

- Ellipsometry is also well adapted to sensor applications in explosive, corrosive and high temperature environments, as the probing light beam can access the sensing surface through windows

- Ellipsometry can be used for label-free biosensing or immunosensing

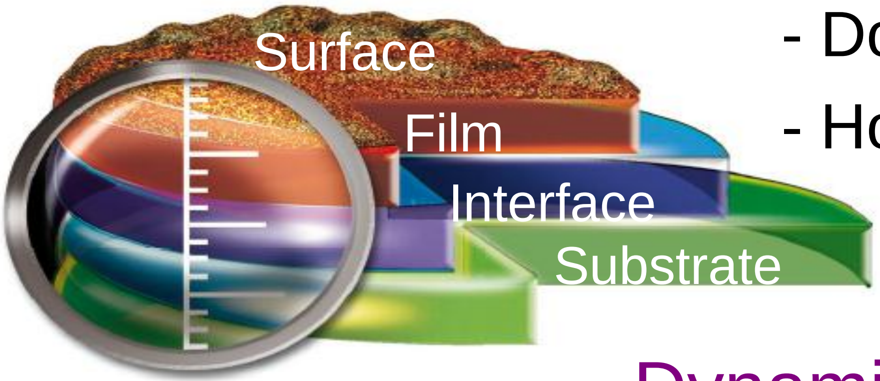
What Ellipsometry can do ?

Optical properties:

- Refractive index
- Absorption coefficients

Material properties:

- Composition
- Microstructure
- Doping level
- Homogeneity



Dimensional:

- Interface thickness
- Layer thickness
- Native thickness
- Roughness thickness

Dynamics & Kinetics:

- Sensing mechanism
- Optical read-out
- Kinetic constants

Selectivity

The optics in an **ellipsometric** gas and bio- sensor system **can only readout sensor responses** and **cannot change the selectivity** of the system, which is **totally determined by the sensing layer.**

If the sensing layer is a thin layer, its gas/biomolecule selectivity can be controlled by varying the fabrication parameters, and can also be changed by modification of the surface chemistry of the sensing layer

Ellipsometry an old diagnostic tool for today’s technologies

L. Tronstad, Trans Faraday Soc. 31, 1151 (1935)

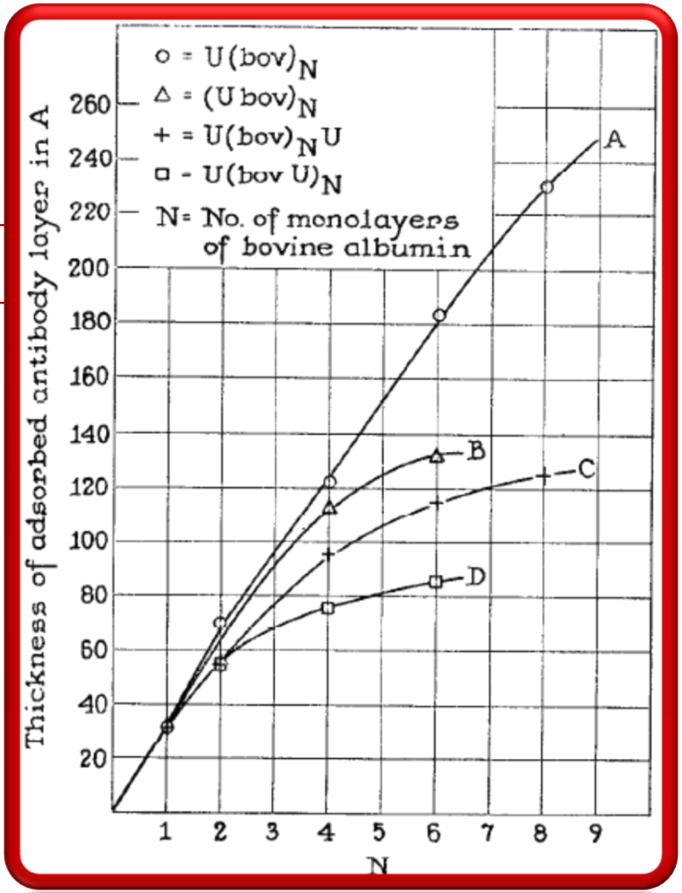
THE VALIDITY OF DRUDE’S OPTICAL METHOD OF INVESTIGATING TRANSPARENT FILMS ON METALS.

Early biosensor study

A. Rothen, Rev. Sci. Instrum. 16, 26 (1945)

The Ellipsometer, an Apparatus to Measure Thicknesses of Thin Surface Films

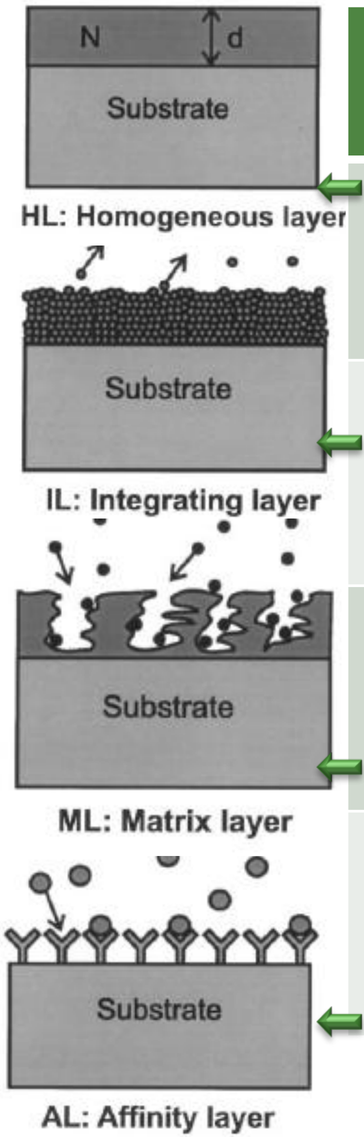
IMMUNOLOGICAL REACTIONS BETWEEN FILMS OF ANTIGEN AND ANTIBODY MOLECULES



L. Vroman, A. Lukosevicius, Nature 204, 701 (1964)

Ellipsometer Recordings of Changes in Optical Thickness of Adsorbed Films associated with Surface Activation of Blood Clotting

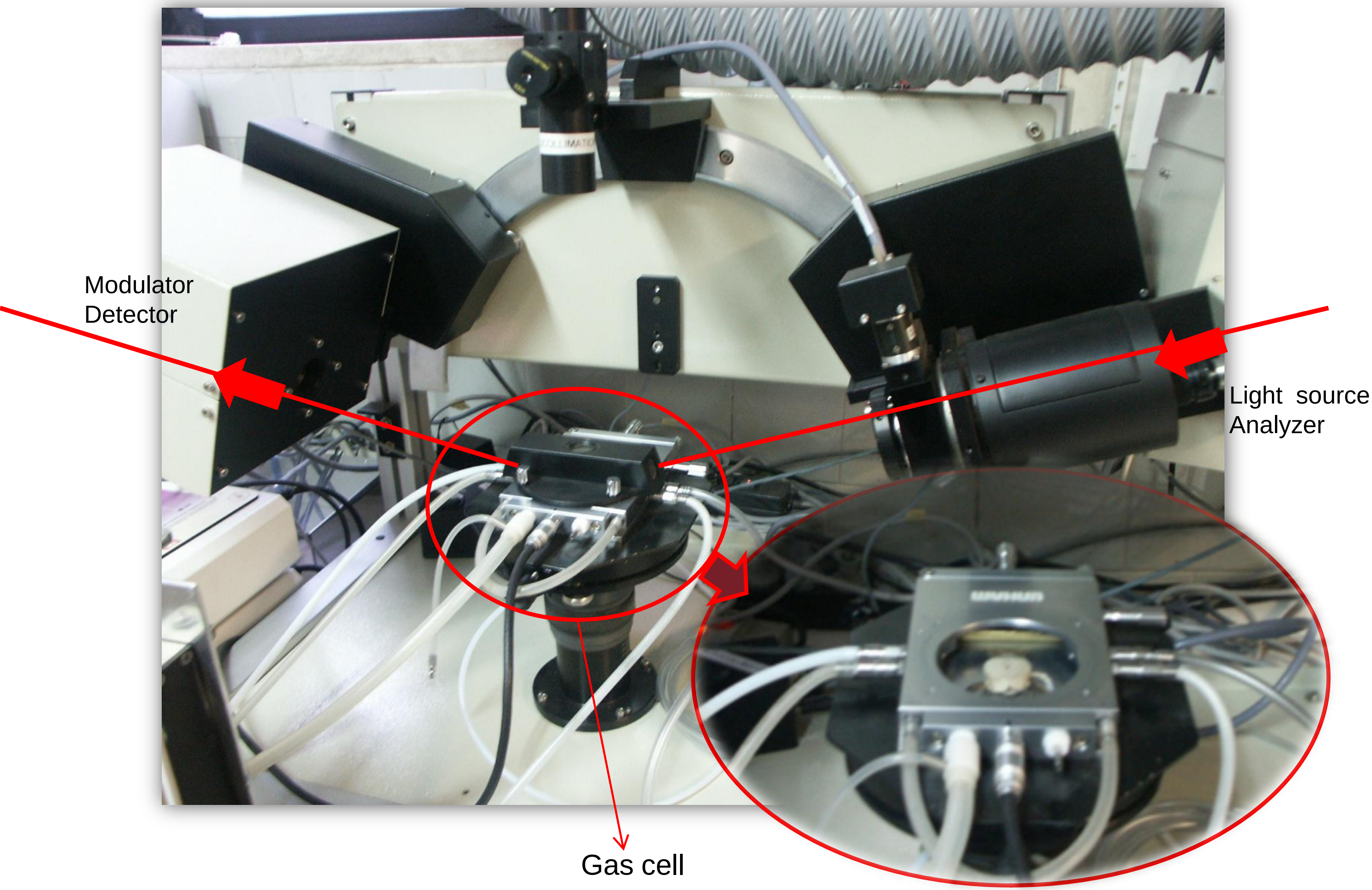
Sensing Layers by Ellipsometry



Sensor Type	Mechanism	Physical/ chemical effect	Layer parameter	Application
Homogeneous layer	Adsorption Electronic effect Thermal Solvation	Chemical bond Energy level shifts Thermal expansion Chemical ineraction	Thickness/index	Gas sensors NOx sensors Temperature sensor
Integrating layer	Desorption	Dissolution Enzymatic cleavage	Thickness	Corrosion
Matrix Layer	Pore filling	Capillary condensation Internal adsorption Biointeraction Size selectivity	Refractive index	Immunosensors Gas sensors
Affinity Layer	Adsorption Chemical bonding	biointeraction	Thickness Refractive index	Immunosensors DNA-hybridization

H. Arwin / Sensors and Actuators A 92 (2001) 43–51

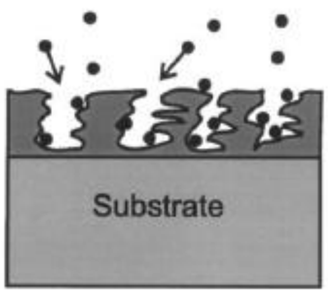
Gas Sensing by Ellipsometry



Gas Sensing by Single Wavelength Ellipsometry

The sensing layers used in ellipsometric gas sensing systems are usually thin layers with typical thickness in the range 0*.01–10 μm on silicon or metal substrates.* The basic requirements on a sensing layer are that its refractive index and/or thickness are changed by the gas under study and that this effect is reversible.

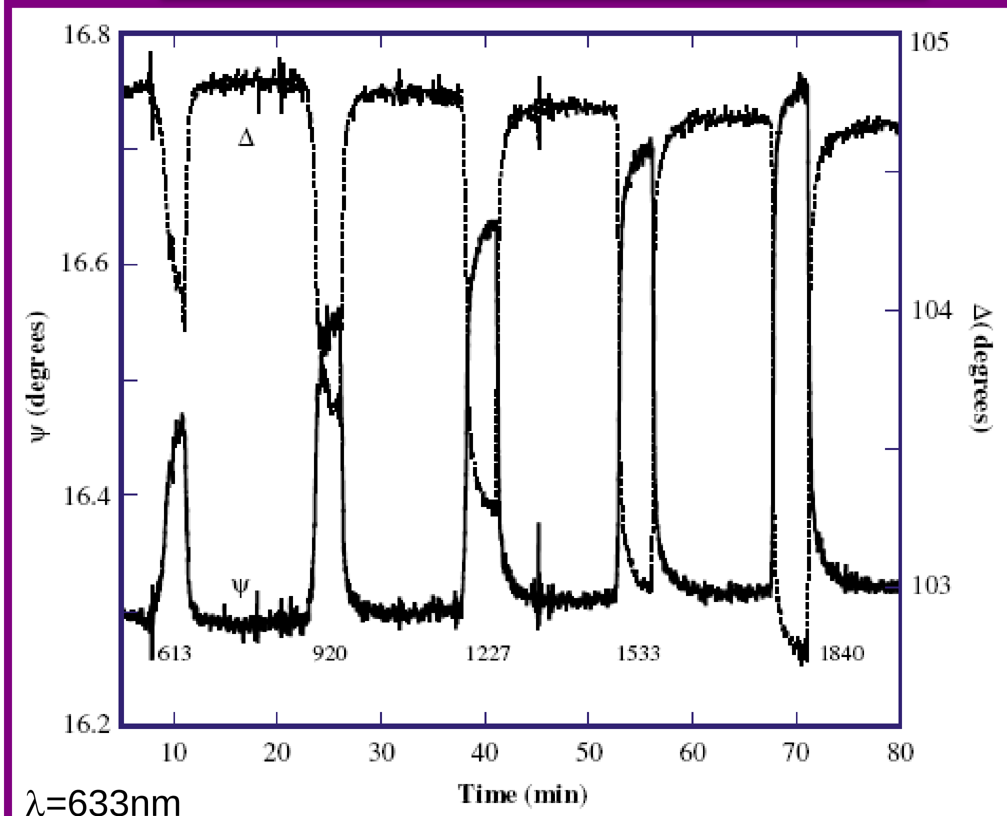
The gas sensing mechanism of porous silicon used in this work involves changes in its effective refractive index due to pore filling by gas molecules (gas adsorption or capillary condensation).



ML: Matrix layer

ψ and Δ of a porous silicon sensing layer during the exposure of ethanol vapours of different concentrations as indicated
at each pulse in parts per million

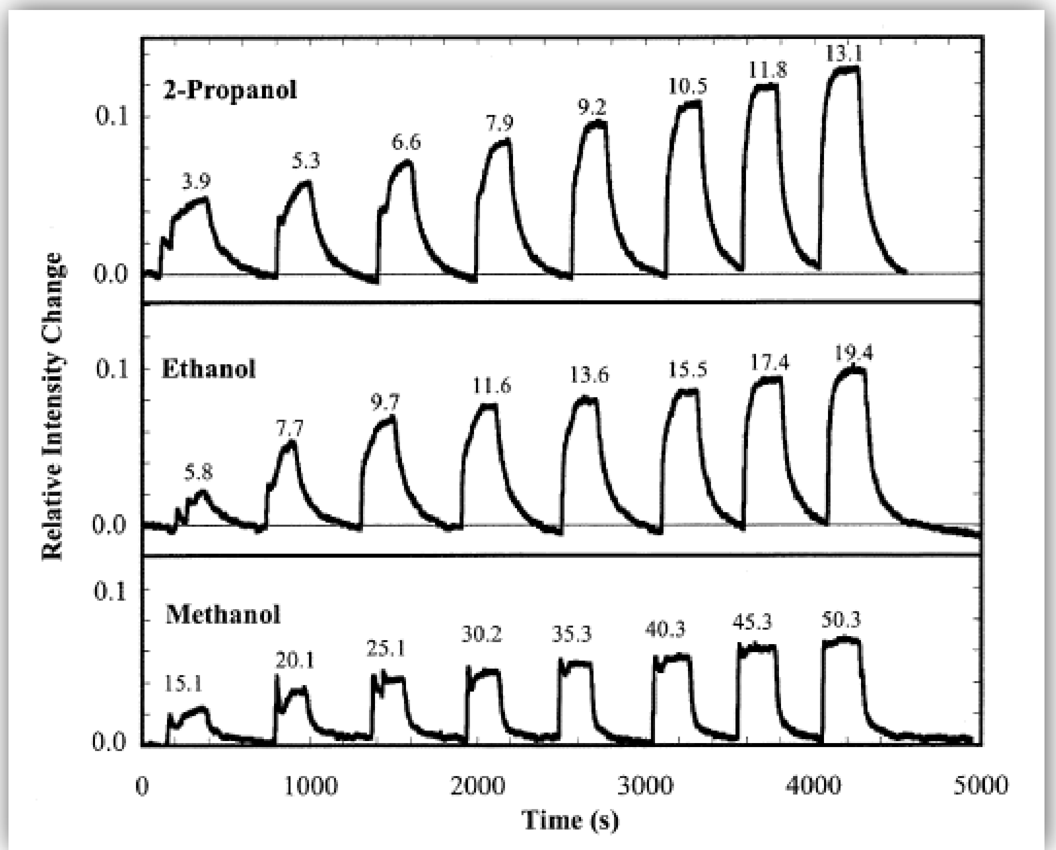
It can be seen that *ψ* increases whereas Δ *decreases during the exposure of ethanol* vapours in the concentration range used



[G. Wang et al.Meas. Sci. Technol. 15 (2004) 216–220]

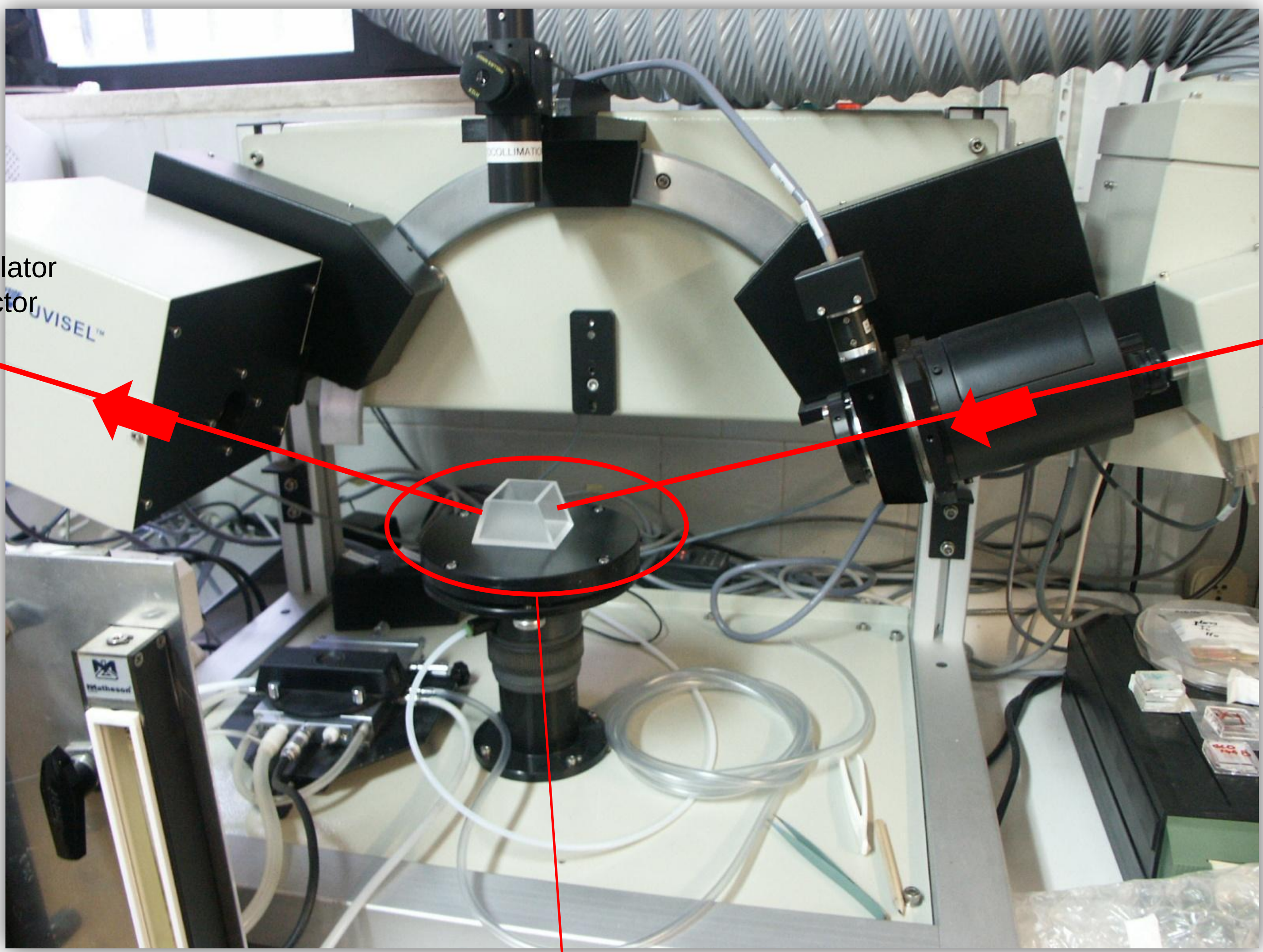
Optical Gas Sensor Based on Single Wavelength Ellipsometry

Gas-induced changes are in most cases due to general physical effects, such as adsorption, layer swelling, pore filling, etc., and therefore the selectivity is in general relatively poor

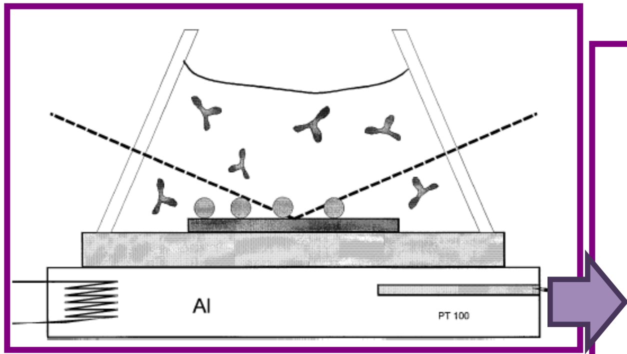


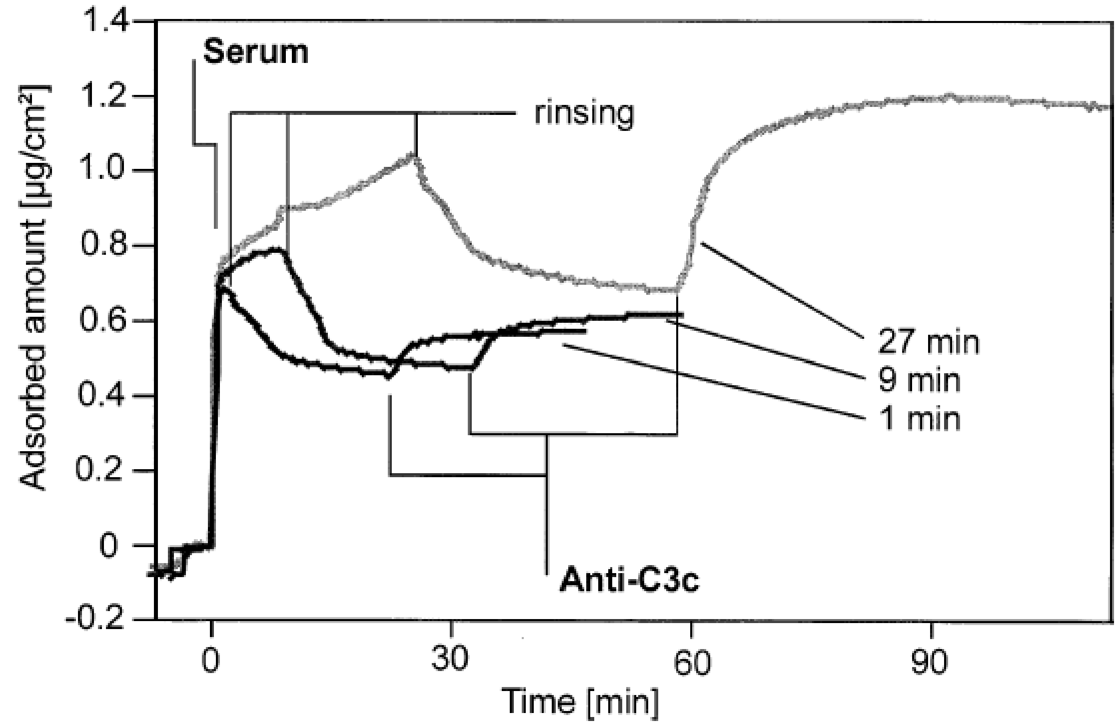
a thermally oxidized porous silicon layer exposed to ppm concentration vapors of 2-propanol, ethanol and methanol

Ellipsometry for monitoring of processes in a liquid cell



Plasma protein adsorption on TiO_2 – Ellipsometry monitoring





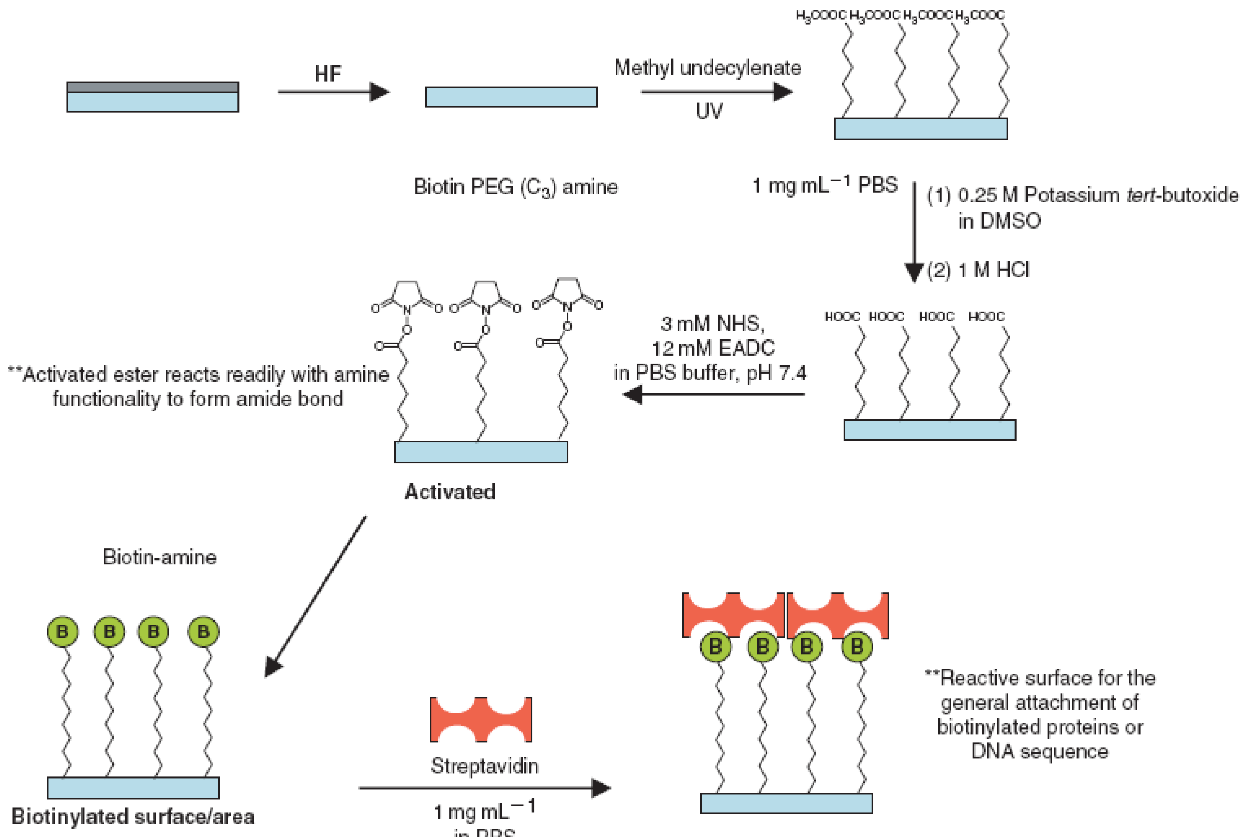
Serum adsorption versus time measured on TiO_2 followed by rinsing in buffer and subsequent binding of anti-C3c. The three adsorption curves correspond to different serum incubation times: (a) 1 min (b) 9 min and (c) 27 min.

All operations were performed with a serum flow rate of 1.5 ml s−1 (corresponding to an approximate shear rate of 4.5 s−1). The flow rate during antibody exposure was 0.5 ml s−1.

[R. Kurrat et al. Colloids and Surfaces B: Biointerfaces 11 (1998) 187–201]

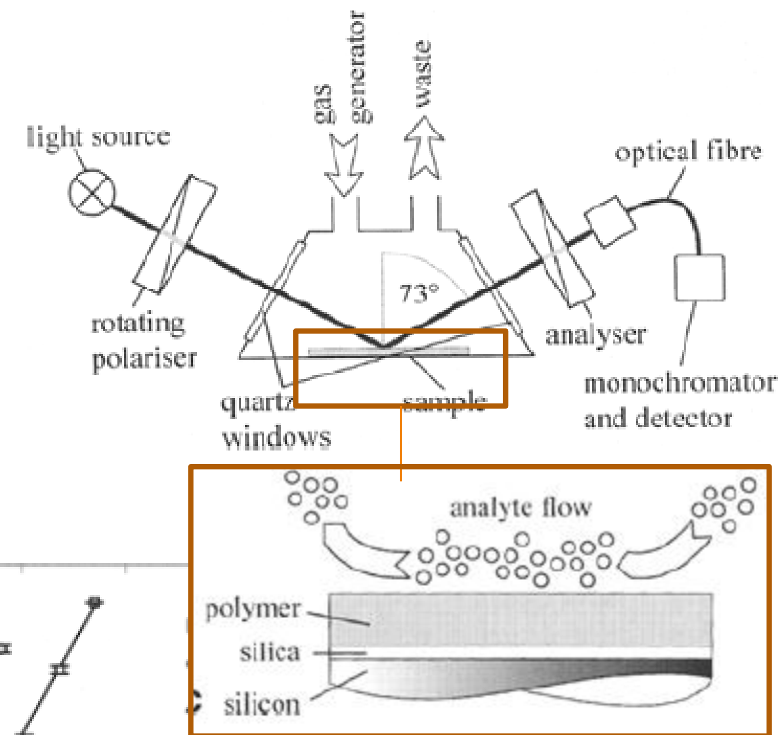
Detection of Antigen–Antibody Reactions by Ellipsometry

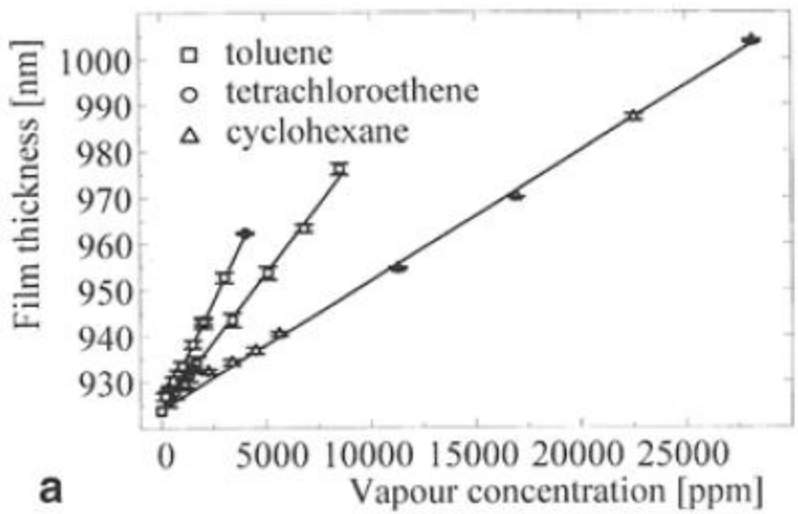
The substrate for **imaging ellipsometry** is **not limited to a gold** surface, unlike surface plasmon resonance, which makes it **possible to orient** our **research towards** crystalline **silicon substrates**.
The silicon surfaces are highly favourable because of their purity and well-defined structure, and the functionalization of silicon for immobilization of biomolecules has been studied extensively.

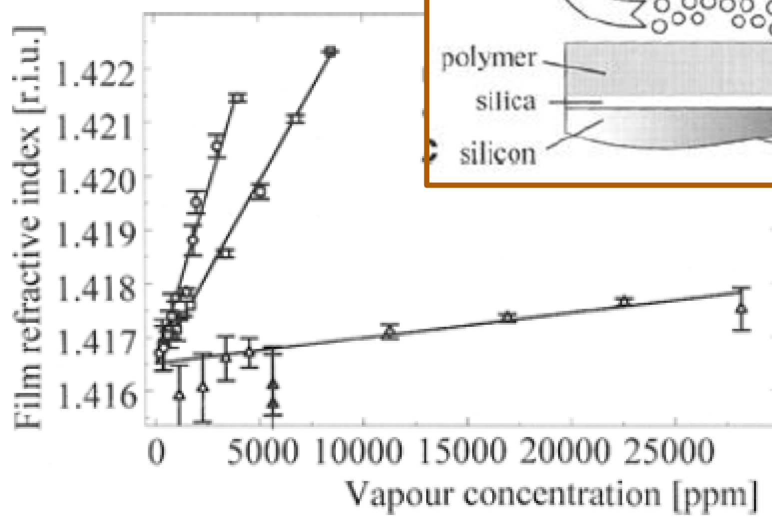


Conductive polymers for chemical sensing of volatile organic compounds

The interaction of polymer films with solvent vapours is considerably fast with typical coefficients of diffusion within the range of 10 -12 m^2/s to 10-11 mz/s at
room temperature and fully reversible within a few seconds.
The permeation of analyte molecules into the polymer
film leads to an increase of film thickness and a
change of refractive index.
The resulting refractive index of the polymer/analyte mixture phase can be higher or lower than the initial refractive index, depending on the refractive index of the analyte.





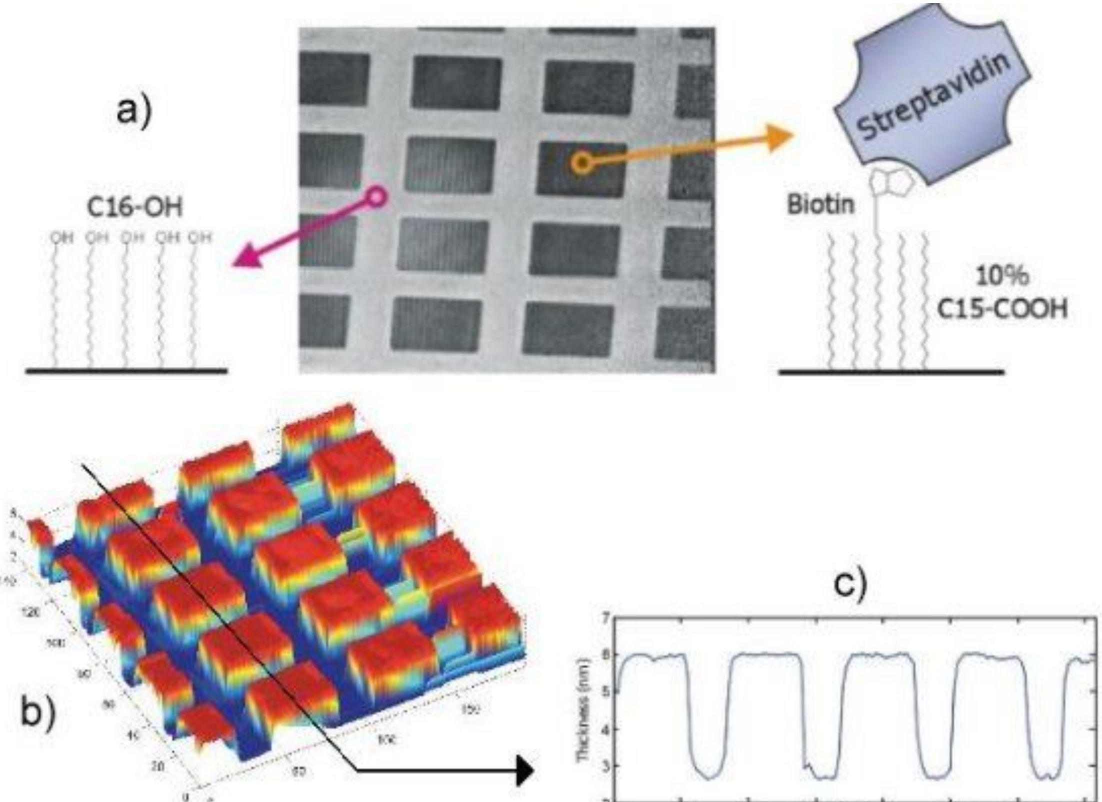


[K. Spaeth et al. Fresenius J Anal Chem 357 (1997) 292-296]

Imaging ellipsometry: applications to Biotechnology

Imaging elliposmetry is a **label-free** technique sensitive to the formation of a monolayer of biomolecules, and in-vitro interactions between proteins can be imaged.

protein array chip

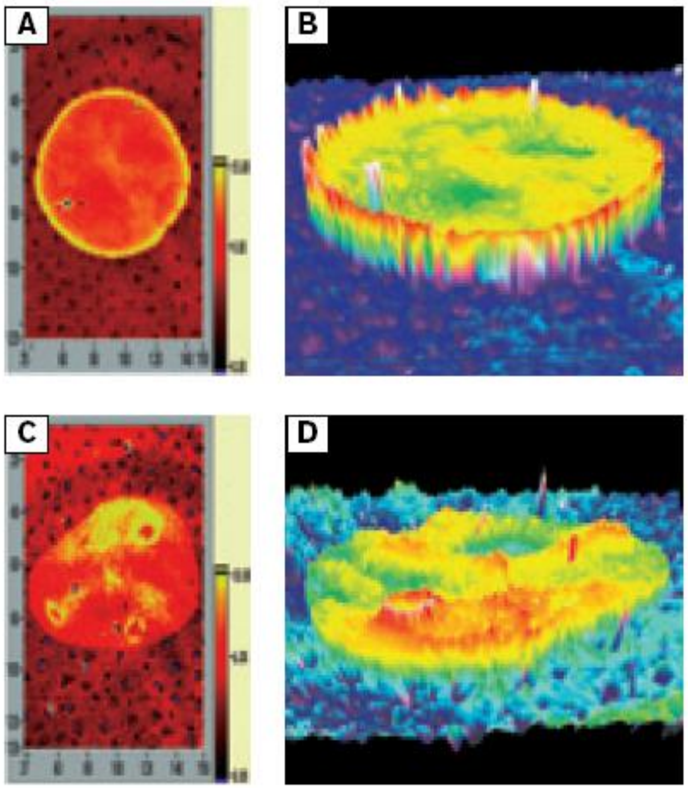


a)A gold surface was patterned with two different alkanethiols, biotin was coupled to carboxyle groups in the squares and streptavidin was washed over the surface. The image is taken with imaging ellipsometry and a clear contrast can be seen. **b)** The data in the image is transformed into a thickness map of the surface. **c)** A line profile taken from the thickness-map; the squares are approximately 3 nm higher than the frames.

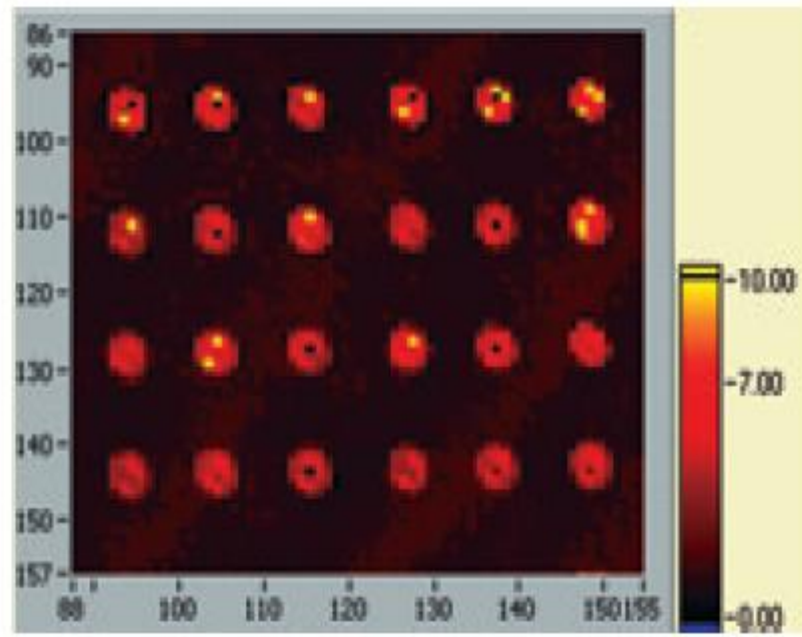
[A. Eing, M. Vaupel, Imaging ellipsometry in biotechnology, July 2002, www.nanofilm.de]

Imaging ellipsometry: applications to Biotechnology

Imaging ellipsometry can simultaneously give thickness profiles and maps of inner layers of a multi-layer stack, maps of material properties like the composition, the refractive index and extinction coefficient, can measure the thickness of multiple spots simultaneously and to monitor hundreds of reaction channels in parallel. This makes the instrument perfectly suited for high throughput screening.



The diameter, homogeneity, and shape of 2 DNA spots on glass displayed as ellipsometric Thickness-map [z in nm and x/y in pixel] (A) and the corresponding 3D-profile



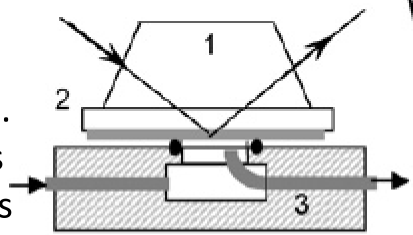
Thickness-map [z in nm and x/y in pixel] of protein array on a gold surface

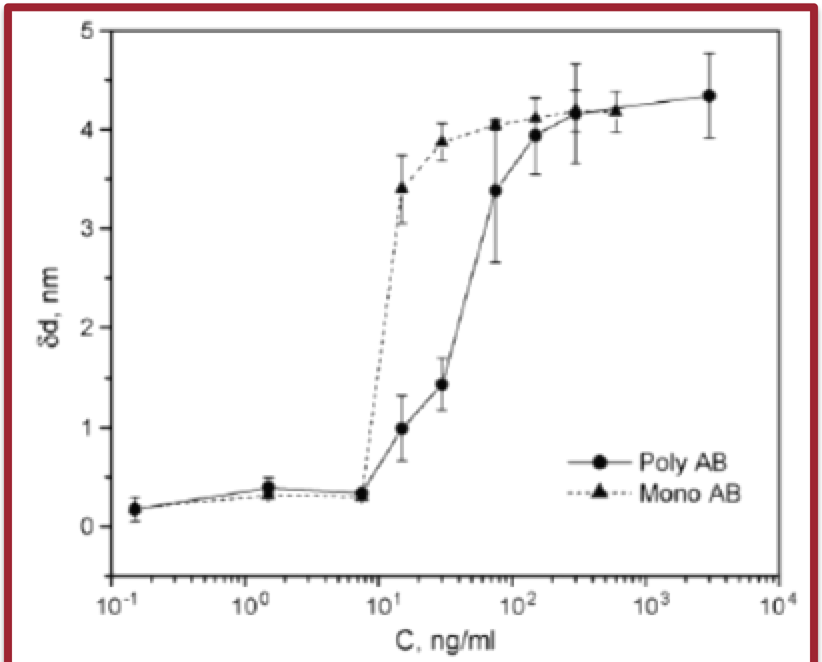
Because the thickness is related to the amount of immobilized material this method can be used to evaluate the amount of immobilized material on the surface

[A. Eing, M. Vaupel, Imaging ellipsometry in biotechnology, July 2002, www.nanofilm.de]

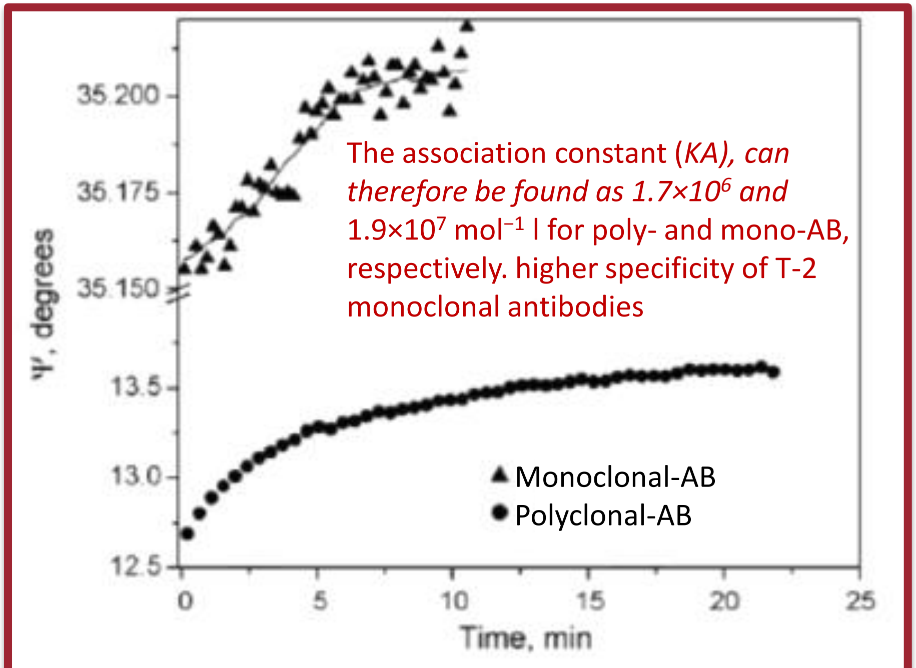
Registration of T-2 mycotoxyn with ellipsometry

Target: development of the new sensitive technique capable of registration of T-2 mycotoxins, as well as other low molecular weight toxins, in lowconcentrations in ppb range. Such great interest in the registration of T-2 mycotoxin, a byproduct of fungal metabolism, is caused by its extremely high toxicity, relative simplicity of synthesis and thus potential use as a bio-terrorism agent





Changes in the adsorption layer thickness caused by T-2 mycotoxin adsorption *vs* T-2 concentration in solution

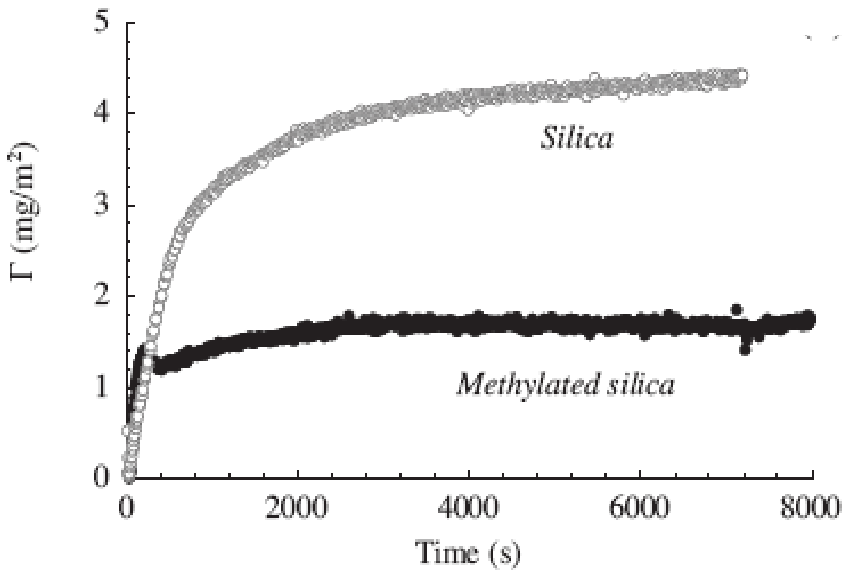


Kinetics during binding T-2 mycotoxin to mono-AB and poly-AB

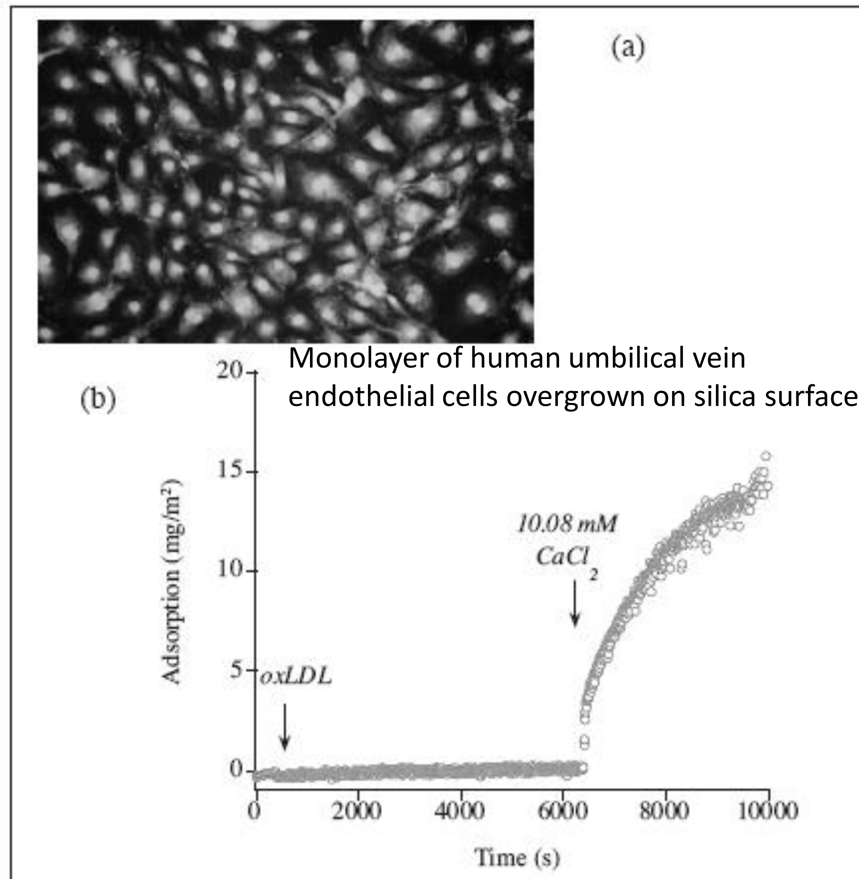
A.V. Nabok et al. Biosensors and Bioelectronics 22 (2007) 885

Ellipsometry studies of lipoprotein adsorption in atherosclerosis and Alzheimer’s

The deposition of lipoproteins at a range of model surfaces was investigated by in situ **ellipsometry** in the context of lipoprotein plaque formation in atherosclerosis.



the use of ellipsometry for monitoring lipoprotein deposition at endothelial cell substrates at a microscopic level seems feasible, although unphysiologically high Ca2+-concentrations are used to accelerate the interfacial deposition process.



Monolayer of human umbilical vein endothelial cells overgrown on silica surface

[M. Malmsten et al. J. Drug. Del. Sci. Tech. 17 (2007) 245]

Further reading

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