BIOSENSING SURFACES - DEVELOPMENT IN SWEDEN

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It was demonstrated more than ten years ago that so-called surface plasmon resonance (SPR) could be used for biosensing purposes [1, 2]. Since then, there has been an industrial development in Sweden by Pharmacia Biosensor of an instrumentation for real time biospecific interaction analysis without the use of labelled molecules [3]. Such an instrumentation, called BIAcore, was launched on the market in 1990. In this instrumentation optics, surface physics and chemistry, and biochemistry are combined with microfluidics and electronics [3, 4]. The biospecific interaction takes place in an interaction matrix consisting of a hydrogel on a gold surface, where changes in the optical properties occurring upon biomolecular interaction in the matrix are measured with SPR. Since the method does not need specially labelled molecules (e.g. fluorescente or radioactive) it can be used for direct real time analysis of biomolecular interactions and is therefore shown a large interest at present. Competitors to Pharmacia Biosensor also utilizing optical effects at surfaces have recently occurred on the market. (Fisons: "IAsys" and ASI: "BIOS-1").

The development of BIAcore contains several interesting examples related to material science and engineering. In surface plasmon resonance charge density waves in a thin metal film are excited by light at a given angle of incidence as illustrated in Fig. 1. The reflected light disappears at this angle. The thickness of the metal should be a fraction of the wavelength of the light. The dielectric function of the metal should have a negative real part at the chosen wavelength for SPR to occur. Thin layers (ca 50 nm) of gold or silver on glass are possible candidates in the visible region. The metal films can be made e.g. by (electrongun or thermal) evaporation and sputtering. Research is related e.g. to the choice of glass, the properties of the metal-glass interface, the influence of the microstructure of the metal on the SPR and to the use of multiple metal or metal-dielectric layers to optimize the dependence of the SPR on the optical properties of the ambient of the metal surface.

An "interaction matrix" on the metal (gold) surface makes use of the evanescent electric field outside the metal surface in an efficient way for biosensing purposes. In BIAcore a dextran layer is attached to the sensing surface via a so-called self assembled monolayer. This monolayer is created by alkane-thiols which form strong bonds between gold and the sulphur atoms. The stability of

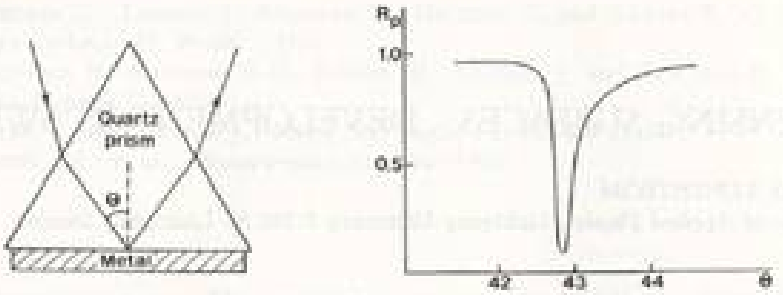

Fig. 1 - Schematic illustration of surface plasmon resonance. Light falling through glass towards (air) buffer under total reflection conditions hits a thin metal film; At a given angle of incidence to so-called surface plasmon resonance angle, Θ_{sp}, the photon energy is transferred to the electrons in the metal surface. The reflected light disappears; An evanescent electric field exists outside the metal which interacts with the ambient. The decay length of this field is approximately (0.2 – 0.3) λ where λ is the wavelength of the light.

the monolayer formed is further increased by the interaction between the hydrocarbon chains. With thiol chemistry the gold surface can thus be provided with coupling groups for an efficient binding of dextran molecules to the gold surface [5]. It is here important to obtain an extended, swollen, hydrogel that each dextran molecule is anchored to the surface only at a few points. Thiol modified gold surfaces are today used in many studies of the influence of surface functionality on chemical and biological surface phenomena. It is interesting to note that Pharmacia Biosensors' use of the thiol-gold chemistry is one of the first commercial applications of this possibility.

Another detail of the developed instrumentation, related to material science and technology, is the microfluidic system used to deliver the sample (analyte) to the sensing surface. It is made in silicon rubber using modern micromachining methods. The microfluidic cartridge contains sample and buffer loops and forms a thin measurement cell together with the sensing chip. The cell formed consists of four parallel measurement channels, each channel 50 μm high and 0.5 mm wide, with a total volume of 50 nl. The liquid flow is controlled by pneumatically driven valves also made in silicone rubber. In BIAcore the liquid handling is fully automatized and computer controlled. A simpler version, called BIAlite, has recently been introduced. BIAlite has the same microfluidic system as BIAcore but sample and buffers are manually injected into the instrument. An efficient optical system without moving parts using a convergent light beam and a photodiode array determines the location of the surface plasmon resonance angle. The light source used is a light emitting diode ($\lambda = 760$ nm).

It is outside the scope of this communication to describe in detail the applications for real time biospecific interaction analysis. It should be pointed out,

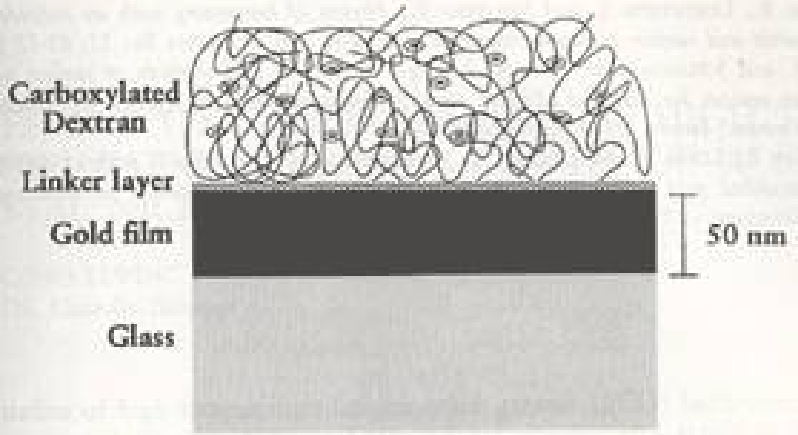

Fig. 2 - Schematic illustration of the sensing chip developed by Pharmacia Biosensor. The linker layer consists of alkane thiols (see the text). The carboxy methylated dextran layer is about 100 nm thick in swollen form and corresponds to a mass of 1 ng/mm². It contains 97-98% water. The carboxyl-groups are used to couple biomolecules (e.g. antibodies) to the sensing matrix using well developed coupling chemistries. Interaction between biomolecules in the coupling matrix (e.g. antigen-antibody) changes the optical properties of the sensing layer and hence the SPR-resonance angle. Changes in organic mass corresponding to about 10 pg/mm² can be resolved with the developed instrumentation.

however, that it can be used not only for biosensing purposes, i.e. to determine concentrations of molecules (antigens, antibodies ...) but also for more complicated studies regarding the interaction between biomolecules, like

— determination of kinetic- and binding constants
— elucidation of relative binding patterns (cooperativity, epitope mapping) etc. [3,4].

Biospecific interaction analysis without the use of labelled molecules, is therefore finding uses also in molecular biology, medical research and biotechnology.

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