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BIOANALYTICAL HISTORY REPORT

Biosensing with surface plasmon resonance — how it all started

Bo Liedberg, Claes Nylander* & Ingemar Lundström

Laboratory of Applied Physics, Department of Physics and Measurement Technology, Linköping University, S–581 83 Linköping, Sweden

Abstract: A subjective description is given of how the development of surface plasmon resonance for immunosensing began. The main differences between the initial experiments and a commercially available instrumentation are pointed out. For the practical use of surface oriented methods for biosensing it is noted that the arrangements around the optical system itself, such as the sensing chip or sample cell, are most important. It is concluded that the instrumentation developed can be used not only for immunosensing but also for "real time biospecific interaction analysis" in general. It is pointed out that the use of surface plasmon resonance for detection is only one possibility and that many new (optical) methods for real time biospecific interaction analysis have been and will be developed.

INTRODUCTION

To our knowledge the use of surface plasmon resonance for biosensing purposes was first demonstrated in 1983 by Liedberg *et al.*, although at this time it had already been used for several years to study organized organic mono- and multilayers on metal surfaces (Pockard *et al.*, 1978; Swalen *et al.*, 1980). A practical and commonly used method by which to excite the surface plasmon was initially suggested by Kretschmann (1971). In this method light falls through a glass (prism) under total reflection conditions and onto a metal film evaporated onto the glass. At the beginning of the 1980s our group demonstrated that surface plasmon resonance in the Kretschmann configuration is well suited for both gas and biomolecular sensing purposes (Nylander *et al.*, 1982/83; Liedberg *et al.*, 1983). As a result of these initial observations Pharmacia of Sweden became interested in using surface plasmon resonance as an instrumentation to be developed for the study of interactions between biomolecules. A project was initiated at Pharmacia in 1984 and in 1986 a separate company, Pharmacia Biosensor, was formed for the development of new biosensor technology. These developments resulted in the launch of BIAcore in 1990 and BIAlite in 1994. Such instrumentation can, for example, be used for real time biospecific interaction analysis without the use of labelled molecules. In the present communication we would like to describe the development of surface plasmon resonance for biosensing purposes by discussing work carried out in Sweden over the past decade or so.

* Sensitor AB, Box 76, S–581 02 Linköping, Sweden.

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HOW IT ALL BEGAN

At the physics department in Linköping University we were asked to develop laboratory exercises for the undergraduate students. One idea was to build a simple set-up to demonstrate the phenomenon of surface plasmon resonance. We had at that time developed a quartz crystal microbalance sensor for anaesthetic gases (halogenated hydrocarbons) based on a silicone oil as the sensing layer (Kindlund and Lundström, 1982/83). As part of this research we sought to discover whether the refractive index changes that must occur in the silicone oil on the adsorption of the anaesthetic molecules could give rise to appreciable shifts of the surface plasmon resonance angle. The study showed that as a measurable parameter surface plasmon resonance performed quite well compared with a commercial instrumentation based on the quartz crystal microbalance (Nylander *et al.* 1982/83).

At the Laboratory of Applied Physics we used ellipsometry to study the adsorption of organic molecules on solid surfaces. Furthermore we had already used such techniques both for biosensing purposes (Arwin and Lundström, 1987; 1988) and for detailed studies of macromolecular interactions at surfaces (Jönsson *et al.*, 1982; Elwing *et al.*, 1987). With this background knowledge we decided to try immunosensing with surface plasmon resonance; this resulted in a paper which was published in 1983 (Liedberg *et al.*). Before we describe this initial experiment, a very brief description of the physics of surface plasmon resonance will be given.

SURFACE PLASMON RESONANCE

A surface plasmon is a charge density wave occurring at the interface between a metal and a dielectricum. A surface plasmon can be excited by light as demonstrated in Fig. 1a. At the surface plasmon resonance angle the energy and momentum (along the interface) coincide for the incident photon and the charge density wave. The photon energy is then transferred to the charge density wave. This phenomenon can be observed as a sharp dip in the reflected light intensity (Fig. 1b). The dielectric function of the metal should have a (large) negative real part at the chosen wavelength of the light. Surface plasmon resonance (SPR) occurs therefore in the visible region in so-called free electron-like metals such as silver and gold. Furthermore, the thickness of the metal film should be a fraction of the wavelength.

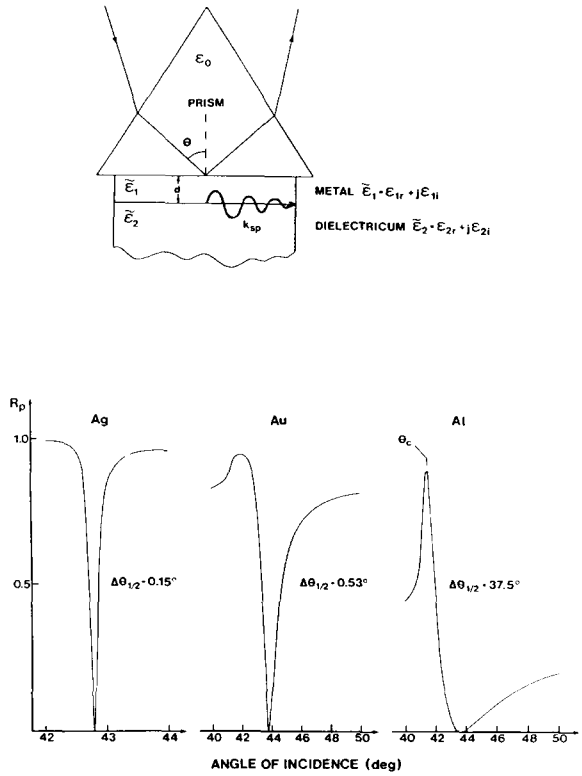


Fig. 1. (a) Schematic drawing of the experimental set-up used in our initial experiments. The light source was a He-Ne laser (632.8 nm) and the detector a photodiode. (b) Calculated reflectance curves for three metals in air. Metal thicknesses 56 nm (Ag); 48.5 nm (Au), and 8.5 nm (Al).

Outside the metal there exists an evanescent electric field, a field that decays exponentially with distance from the metal surface with a decay length of the order of 0.2 to 0.3 of the wavelength of light. This evanescent field interacts with the close vicinity of the metal. Changes in the optical properties of this region will therefore influence the (SPR) angle, which is the basis of the use of SPR for (bio-)sensing purposes.

THE INITIAL IMMUNOSENSING EXPERIMENT

A silver film evaporated onto a microscope slide was used as the sensing surface, and a goniometer

arrangement with a photodiode as a light detector was used to measure the position of the resonance angle. An antigen (an immunoglobulin in this case) was spontaneously adsorbed on the silver surface. The subsequent binding of an antibody (a-IgG) was detected as shown in Fig. 2. In order to monitor the SPR-angle shift more directly the change in photocurrent at a given resonance angle was detected upon injection of the antibodies (Fig. 3a). From the (maximum) slope of such curves a calibration curve was constructed (Fig. 3b). The sample cell used consisted of a channel (approx. 2 mm) formed between the sensing surface and a second glass slide. With this setup it was possible to determine a-IgG concentrations down to about 0.2 μg/ml.

The simplicity of the experimental set-up and the reasonable sensitivities obtained using our non-optimized equipment made SPR an interesting candidate for the basis of a practical immunosensor.

PHARMACIA BIOSENSOR, BIAcore AND BIAlite

At the beginning of the 1980s Pharmacia became interested in the possibilities of biosensor technology and a decision was made to investigate the possibility of developing methods for the direct detection (without labels) of biomolecular interactions. In 1984 a project investigating the use of surface plasmon resonance for biosensing purposes was initiated. Researchers were employed from both the Laboratory of Applied Physics and the Swedish Defense Research Institute in Umeå, where studies of biomolecular interactions were also undertaken (Jönsson *et al.*, 1985). In 1986 a company called Pharmacia Biosensor was formed to develop, produce and market a product for real time biospecific interaction analysis. A large effort was made to provide an instrumentation that would be easy to use—a regenerable sensing chip to which biomolecules could be coupled using known coupling chemistries. Furthermore an efficient liquid handling and a small sample cell were developed to make kinetic analysis possible. The first products were launched in 1990; namely the instrument BIAcore and sensing chips that had a dextran layer on top of the metal surface as a sensing matrix. This development has been described by Jönsson *et al.* (1991) and Jönsson and Malmqvist (1992). Recently a new instrument, BIAlite, has been introduced, which has the same analytical performance as BIAcore, but where the sample handling is manual and not computer-controlled as in a BIAcore.

The performance of the commercial instrumentation (for immunosensing) is summarized in Fig. 4. The comparison between Fig. 3 and 4 speaks for itself. The commercial development has increased the sensitivity and accuracy of SPR for biosensing purposes by several orders of magnitude. However, as described later, the most interesting application may not be to determine

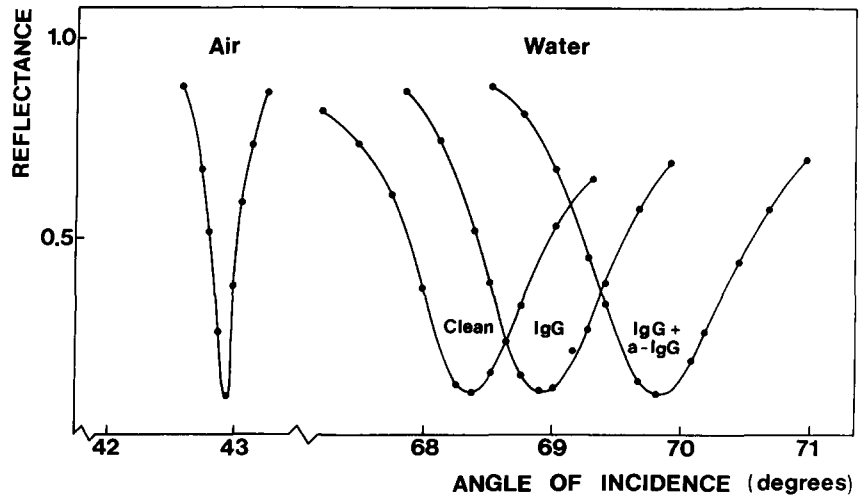


Fig. 2. Experimental results showing the adsorption of a protein molecule, an antigen (IgG) and the subsequent binding of its antibody (anti-IgG). The experiments were performed on a silver film, 60 nm thick (from Liedberg et al., *1983).*

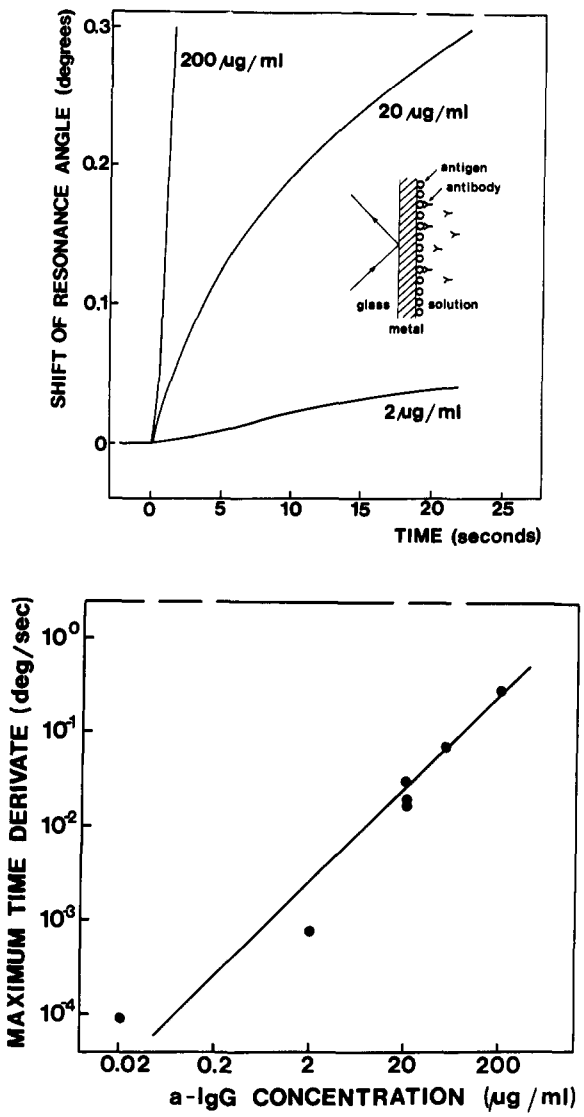


Fig. 3. (a) Kinetics of the antibody binding to a layer of spontaneously adsorbed antigens at different antibody concentrations measured as the change in reflected light at a given angle of incidence close to the resonance angle. (b) Calibration curve constructed for the maximum derivative of the curves in (a) (from Liedberg et al., *1983).*

concentrations but to follow biospecific interactions in real time.

There are of course many differences between the commercial instrumentation and our initial experimental set-up. A few of the most important ones are briefly described below (see also Fig. 5).

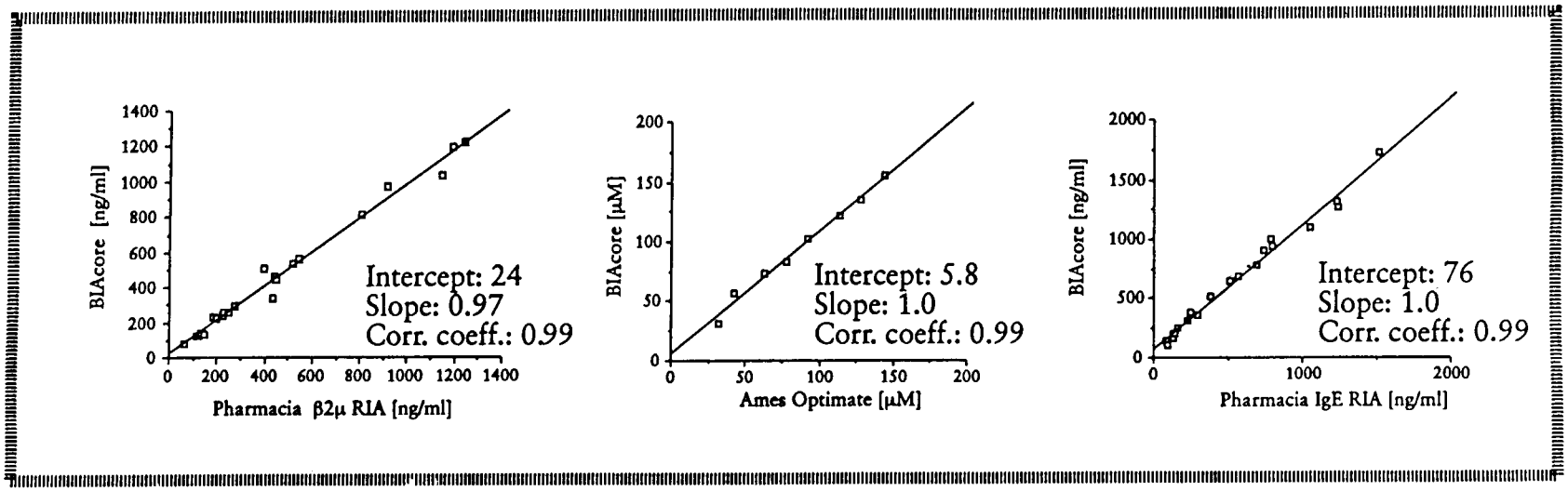
INITIAL EXPERIMENT VERSUS DEVELOPED INSTRUMENTATION

Sensing surface

Pharmacia Biosensor uses a hydrogel, a carboxymethylated dextran layer, on the surface of a gold film as the matrix for biomolecular interactions. The dilute dextran layer, about 100 nm thick in swollen form, utilizes the evanescent field in an efficient way and provides the sensing surface with carboxyl groups to which biomolecules can be coupled using known techniques (Jönsson *et al.*, 1991). Since the dextran layer contains 97 to 98% water, interaction between the covalently bound molecule and the other molecule in an interaction pair will take place in a more native surrounding than interactions that would otherwise take place directly on the metal surface. Furthermore, it is possible to provide an extended matrix, even if it is dilute, with more coupling sites per unit area than a bare surface and thereby increase the sensitivity of the method (Löfås *et al.*, 1991; Liedberg *et al.*, 1993). The matrix is also regenerable, for example, through the use of a buffer of low pH which desorbs all but the covalently bound biomolecules from the matrix. The dextran molecules are attached to the gold surface by a linker layer consisting of alkane thiols. This use of self assembled monolayers appears to be one of the first commercial applications of such layers spontaneously formed on gold (Löfås and Johansson, 1990).

Sample cell and microfluidic system

A very important development concerns the liquid handling and sample delivery to the sensing surface. By using micromachining methods an integrated microfluidic cartridge was fabricated, which contains sample and buffer loops, and which together with the sensing chip automatically forms a sample cell 50 μm high and 0.5 mm wide with a volume of *60 nl*. This enables very efficient and accurate delivery of the analyte to the sensing surface making (non-diffusion limited) kinetic studies possible (Sjölander and Urbaniczky, 1991). The microfluidic cartridge is made of silicone rubber. The valves controlling the liquid flow are operated by a small air pressure.



Conditions	β2-microglobulin	Theophylline	IgE
Range:	20-1000 ng/ml (1.7-83 nM)	4.5-34 μg/ml (25-190 μM)	100-2500 ng/ml (0.6-14 nM)
Sample:	serum	serum	serum
Analysis:	8 min	5 min	15 min
Precision:	≤ 5 % CV	≤ 7 % CV	≤ 5 % CV

Fig. 4. Comparison between assays developed for BIAcore and other commercially available assays. With BIAcore, using a sandwich assay, it is possible to determine a concentration of about 40 pM (courtesy of Pharmacia Biosensor).

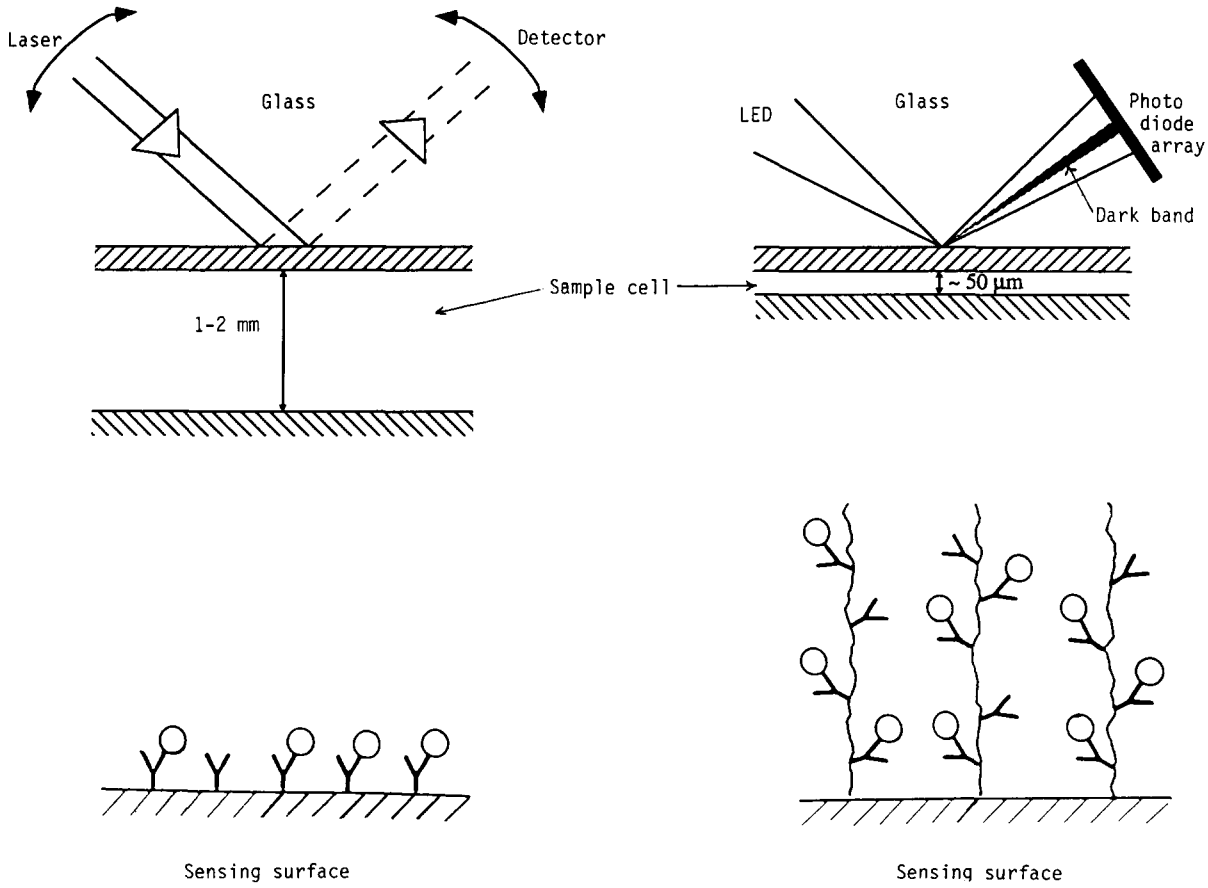


Fig. 5. Schematic illustration of the main physical differences between the initial immunosensing experiments (left) and the commercial instrumentations, BIAcore and BIAlite (right). A further description is given in the text.

Optical detection system

In the commercial instrumentation a convergent light beam together with a photodiode array is used to accurately determine the surface plasmon resonance minimum position. A computer algorithm calculates the exact position of the minimum to a fraction of a single diode. The accuracy of the optical system corresponds to about 0.0001° shift of the resonance angle. A light emitting diode (λ = 760 nm) is used as the light source. Another important improvement is the use of a polymer layer between a cylindrical prism and the glass side of the sensing chip for refractive index matching instead of the immersion oil used in our initial experiment.

APPLICATIONS

The possible applications of surface plasmon resonance for biosensing have also been developed extensively since the first demonstration of immunosensing. One interesting possibility is to obtain kinetic information as shown in Fig. 6. There exist today more than a hundred publications describing applications for real time biospecific interaction analysis in several areas of molecular biology, medicine and environmental science. We exemplify some of these applications by a few recent references given in Table 1.

The possibility of real time biospecific interaction analysis has proven to be time saving in many investigations of biomolecules and their interactions. It has also provided experimental results on biomolecular interactions, which would otherwise have been very difficult or even impossible to obtain.

CONCLUSIONS

During the last ten years surface plasmon resonance has developed into a useful technique

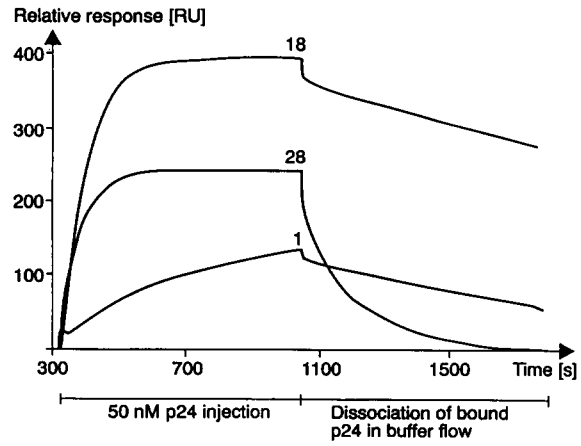


Fig. 6. Examples of kinetic binding curves obtained with BIAcore. In this experiment the binding of an antigen (HIV-1 protein p24) to three different antibodies immobilized (by a capturing antibody) in the matrix and the subsequent dissociation of the antigen were studied. For screening purposes the information obtained from such plots is often sufficient. From the curves, however, affinity and rate constants may also be calculated (after Karlsson et al., *1991).*

TABLE 1 Some recent references to applications for BIAcore.

Topics	*References*
Antibody characterization	Zeder-Lutz *et al.* (1993) Gruen *et al.* (1994) Malmqvist (1994)
Receptor ligand interactions	Panayotou *et al.* (1993) Schuster *et al.* (1993) Calakos *et al.* (1994)
Nucleic acids	Wood (1993)
Environmental monitoring	Minunni & Mascini (1993)
Chromatography	Nice *et al.* (1994)

for immunosensing and biospecific interaction analysis. It is clear, however, that details such as the sensing surface and the microfluidic system play a very important role in BIAcore and BIAlite.

Surface plasmon resonance is only one of the (optical) methods that can be used for detection purposes and the study of interactions between biomolecules. Several other detection methods have also been introduced for biospecific interaction analysis and at least two other commercial instrumentations are marketed today: IAsys, from Fisons, and BIOS-1, from ASI. IAsys is based on a resonant mirror sensor device, a waveguiding structure on top of a prism (Buckle *et al.*, 1993; Cush *et al.*, 1993; Davies and Pollard-Knight, 1993). BIOS-1 utilizes an input grating coupler and an optical waveguide (Tiefenthaler and Lukosz, 1989; Nellen and Lukosz, 1991; Tiefenthaler, 1992 and 1993; Bier and Schmid, 1993, Ramsden and Schneider, 1993). A commentary on the commercial technologies (BIA, BIOS, IAsys) has been published by Hodgson (1994).

In conclusion it can be stated that real time biospecific interaction analysis without the use of labelled molecules provides a new way to study the interaction between biomolecules with applications ranging from immunosensing to fundamental studies of receptor–ligand interactions.

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REFERENCES

Arwin, H. & Lundström, I. (1988). Surface oriented optical methods for biomedical analysis. *Methods in Enzymology*, **137**, part D, 366–381.

Arwin, H., Welin, S. & Lundström, I. (1988). Reflectance method for immunoassay on solid surfaces. In: *Nonisotropic Immunoassay*, T. T. Ngo (Ed.) Plenum Press, New York, pp. 313–330.

Buckle, P. E., Davies, R. J., Kinning, T., Yeung, D., Edwards, P. R., Pollard-Knight, D. & Lowe, C. R. (1993). The resonant mirror–novel optical sensor for direct sensing of biomolecular interactions. 2. Applications. *Biosensors & Bioelectronics*, **8**, 355–363.

Calakos, N., Bennet, M. K., Petersson, K. E. & Scheller, R. H. (1994). Protein–Protein interactions mediating the specificity of intracellular vescular trafficking. *Science*, **263**, 1146–1149.

Cush, R., Cronin, J. M., Stewart, W. J., Maule, C. H., Molloy, J. & Goddard, N. J. (1993). The resonant mirror – a novel optical biosensor for direct sensing of biomolecular interactions. 1. Principle of operation and associated instrumentation. *Biosensors & Bioelectronics*, **8**, 347–353.

Davies, R. J. & Pollard-Knight, D. (1993). An optical biosensor system for molecular interaction studies. *American Biotechnology Laboratory*, July.

Elwing, H., Askendal, A., Ivarsson, B., Nilsson, U., Welin, S. & Lundström, I. (1987). Protein adsorption on solid surfaces: physical studies and biological model reactions. In: *Proteins at Interfaces*. ACS Symposium Series, Vol. 343, pp. 469–488.

Gruen, L. C., McKimm-Breschkin, J. L., Coldwell, J. B. & Nice, E. C. (1994). Affinity ranking of influenza neuraminidase mutants with nomoclonal antibodies using an optical biosensor. Comparison with ELSIA and slot blot assay. *Journal of Immunological Methods*, **168**, 91–100.

Hodgson, J. (1994). Light, angles, action. Instruments for label-free, real-time monitoring of intermolecular interaction. *Bio/Technology*, **12**, 31–35.

Johansson, B., Löfås, S. & Lindquist G. (1991). Immobilization of proteins to carboxy methyl dextran-modified gold surfaces for biospecific analysis in surface plasmon resonance sensors. *Anal. Biochem.* **198**, 268–277.

Jönsson, U., Malmqvist, M. & Rönnberg, I. (1985). Adsorption of immunoglobulin G, protein A and fibronectin in the submonolayer region evaluated by a combined study of ellipsometry and radiotracer techniques. *J. Colloid Interface Sci.*, **103**, 360–372.

Jönsson, U., Fägerstam, L., Ivarsson, B., Johnsson, B., Karlsson, R., Lundh, K., Löfås, S., Persson, B., Roos, H., Rönnberg, I., Sjölander, S., Stenberg, E., Ståhlberg, R., Urbaniczky, C., Östlin, H. & Malmqvist, M. (1991). Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. *Bio Techniques*, **11**, 620–627.

Jönsson, U. & Malmqvist, M. (1992). Real time biospecific interaction analysis. The intergration of surface plasmon resonance detection, general biospecific interface chemistry and microfluidics into one analytical system. *Advances in Biosensors*, **2**, 291–336.

Karlsson, R., Michaelsson, A. & Mattsson, L. (1991). Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J. Immunol. Methods*, **145**, 229–240.

Kindlund, A. & Lundström, I. (1982/83). Physical studies of quartz crystal sorption detectors. *Sensors and Actuators*, **3**, 63–77.

Kretschmann, E. (1971). Die Bestimmung optischer Konstanten von Metallen durch Anregung von Oberflächenplasmaschwingungen. *Z. Phys.*, **241**, 313–324.

Liedberg, B., Nylander C. & Lundström, I. (1983). Surface plasmon resonance for gas detection and biosensing. *Sensors and Actuators*, **4**, 299–304.

Liedberg, B., Lundström, I. & Stenberg, E. (1993). Physics of biosensing with an extended coupling matrix and surface plasmon resonance. *Sensors and Actuators B*, **11**, 63–72.

Löfås, S. & Johansson, B. (1990). A novel hydrogel matrix on gold surfaces in surface plasmon resonance sensors for fast and efficient covalent immobilization of ligands. *J. Chem. Soc. Chem. Commun.*, 1526–1528.

Löfås, S., Malmqvist, M., Rönnberg, I., Stenberg, E., Liedberg, B. & Lundström, I. (1991). Bioanalysis with surface plasmon resonance. *Sensors and Actuators B*, **5**, 79–84.

Malmqvist, M. (1994). Kinetic analysis of engineering antibody antigen interactions. *Journal of Molecular Recognition*, in press.

Minunni, M. & Mascini, M. (1993). Detection of pesticide in drinking water using real-time biospecific interaction analysis (BIA). *Analytical Letters*, **26(7)**, 1441–1460.

Nellen, Ph. M. & Lukosz, W. (1991). Model experiments with integrated optical input grating couplers as direct immuno sensors, *Biosensors & Bioelectronics*, **6**, 517–525.

Nice, E., Lackmann, M., Smyth, F., Fabri, L. & Burgess, A. W. (1994). Synergies between micropreparative high-performance liquid chromatography and an instrumental optical biosensor. *Journal of Chromatography A*, **660**, 169–185.

Nylander, C., Liedberg, B. & Lind, T. (1982/83). Gas detection by means of surface plasmon resonance. *Sensors and Actuators*, **3**, 79–88.

Panayotou, G., Gish, G., End, P., Truong, O., Gout, L., Chand, R., Fry, M. J., Hiles, I., Pawson, T. & Waterfield, M. D. (1993). Interactions between SH2 domains and tyrosine-phosphorylated platelet-derived growth factor β-receptor sequences: analysis of kinetic parameters by a novel biosensor-based approach. *Molecular and Cellular Biology*, **13**, 3567–3576.

Pockrand, I., Swalen, J. D., Gordon, J. G. & Philpott, M. R. (1978). Surface plasmon spectroscopy of organic monolayer assemblies. *Surface Sci.*, **74**, 237–244.

Ramsden, J. J. & Schneider, P. (1993). Membrane insertion and antibody recognition of a glycosylphosphatidylinositol-anchored protein: an optical study. *Biochemistry*, **32**, 523–529.

Schuster, S. C., Swanson, R. V., Alex, L. A., Bourret, R. B. & Simon, M. I. (1993). Assembly and

function of a quateternary signal transduction complex monitored by surface plasmon resonance. *Nature*, **365**, 343–347.

Sjölander, S. & Urbaniczky, C. (1991). Integrated fluid handling system for biomolecular interaction analysis. *Anal. Chem.*, **63**, 2338–2345.

Swalen, J. D., Gordon, J. G., II, Philpott, M. R., Brillante, A., Pockrand, I. & Santo, R. (1980). Plasmon surface polariton dispersion by direction optical observation. *Am. J. Phys.*, **48**, 669–672.

Tiefenthaler, K. & Lukosz, W. (1989). Sensitivity of grating couplers as integrated optical chemical sensors. *J. Opt. Soc. Am.*, **B6**, 209–220.

Tiefenthaler, K. (1992). Integrated optical couplers as chemical waveguide sensors. *Advances in Biosensors*, **2**, 261–289.

Tiefenthaler, K. (1993). Grating couplers as label-free biochemical waveguide sensors. *Biosensors & Bioelectronics*, **8**, xxv–xxxvii.

Wood, W. J. (1993). DNA–DNA hybridization in real time using BIAcore. *Microchemical J.*, **47**, 330–337.

Zeder-Lutz, G., Altschuh, D., Geysen, H. M., Trifilieff, E., Sommermeyer, G. & Van Regenmortel, M. H. V. (1993). Monoclonal antipeptide antibodies: affinity and kinetic rate constants measured for the peptide and the dognate protein using a biosensor technology. *Molec. Immunol.*, **30**, 145–155.