Optical sensing: recognition elements and devices

Guenter Gauglitz


Event: SPIE Security + Defence, 2012, Edinburgh, United Kingdom
Optical Sensing: recognition elements and devices

Günter Gauglitz*
Eberhard Karls University Tuebingen, Institute for Physical and Theoretical Chemistry,
Auf der Morgenstelle 18, 72076 Tuebingen, Germany

ABSTRACT

The requirements in chemical and biochemical sensing with respect to recognition elements, avoiding non-specific interactions, and high loading of the surface for detection of low concentrations as well as optimized detection systems are discussed. Among the many detection principles the optical techniques are classified. Methods using labeled compounds like Total Internal Reflection Fluorescence (TIRF) and direct optical methods like micro reflectometry or refractometry are discussed in comparison. Reflectometric Interference Spectroscopy (RIfS) is presented as a robust simple method for biosensing. As applications, trace analysis of endocrine disruptors in water, hormones in food, detection of viruses and bacteria in food and clinical diagnostics are discussed.

Keywords: Biosensors, Recognition elements, surface characterization, TIRF, RIfS, direct optical detection, environment, healthcare

1. INTRODUCTION

Optical sensing at low limits of quantification and good selectivity requires highly optimized recognition elements as biomolecules or molecular imprinted polymers as well as well sophisticated detection technologies. The quality of chemical and biochemical sensors depends on well optimized research result in both areas. In addition, in dependence on the chosen application, the best suited assay format has to be selected. The detection of molecular interaction as the basis of all sensing principles can take place in homogeneous phase only in case either scattering is changed during the interaction process or at least one of the interacting partners is labeled (usually by a fluorescent marker)\(^1\). Normally interaction takes place at the heterogeneous phase of the transducer where the recognition element is immobilized. However, at these conditions certain requirements for the interaction at the transducer have to be considered like avoiding of non-specific binding, improved specific binding, high loading with recognition elements, and high stability of the surface and recognition layer stability to allow regeneration\(^1,2\).

Various possibilities exist to modify the surface to reduce non-specific binding and improve loading with recognition elements. These are usually based on streptavidin-biotin layers, hydrogels like dextrane or polyethylene glycols. These “shielding layers” can be activated for different reactive groups like carboxy-, amino- or thiol-groups in dependence on optimal binding to various recognition elements\(^3\). In the past, antigens, peptides, and DNA-strands had been the main recognition elements immobilized onto the dextrane or polyethylene glycol layers. In recent years antibodies or proteins are also immobilized. In addition, scaffolds\(^{4}\), aptameres\(^{5}\) or even molecular imprinted polymers\(^{6}\) are used with good success.

Optical detection principles can be classified according to monitoring labelled and non-labelled interaction partners, thus, applying fluorescence or direct optical detection techniques. The latter case can be further subdivided into evanescent field techniques (micro-refractometry) and micro-reflectometry based techniques\(^7\). For fluorescence based sensors and instrumentation for direct optical sensing, a wide variety of transduction principles exist. It should be mentioned that development of optical components is well advanced. Therefore sensing problems in most cases depend on the biopolymer layer improving reduction of non-specific interaction and increasing loading as well as on the chosen assay format. Furthermore, problems caused by micro fluidics should not be underestimated.

Accordingly, in this paper some properties and consequences of assay format, biopolymer shielding layer, choice of recognition elements in combination with selected transduction principles are discussed for some application in the area of environmental, food, and healthcare sensing.

*Tel.: +497071-2976927,Fax.: +497071-295772, gg@ipc.uni-tuebingen.de
2. METHODOLOGY

2.1 Assay types

For the measurement of biomolecular activity the signal quality can depend on the assay used. As mentioned, normally the assays at the heterogeneous phase open a wider range of possible assays. For this reason in biosensing these assays are mostly used. In principle, fluorescence markers allow the measurements at lower limits of detection and have less problems with non-specific binding. Therefore they are used in many routine applications. However, they need more expenditure and cost for labeling which also can reduce bioactivity. Preferably the recognition element is immobilized to the biopolymer layer and the analyte can be directly detected. In case of fluorescence detection only self fluorescent analytes will work. For direct optical detection, the analyte normally has to be large in size or mass; otherwise changes in refractive index or physical thickness will be small (Figure 1A). This problem can be overcome by applying a competitive test format where the analyte competes with a labeled similar analyte. In dependence on relative amount of labeled analyte to non-labeled one the fluorescence signal will vary (Figure 1B).

![Figure 1. Test formats: (A) direct test format (preferable with non-labeled partners), (B) competitive test format, (C) binding inhibition test format, (D) Sandwich test format](image)

A special case is the so-called binding inhibition assay in which the analyte or a derivative of the analyte is immobilized. The recognition partner is added in a pre-incubation step to the analyte solution. The resulting signal is inverse proportional to analyte concentration, since blocked recognition element cannot be detected at the surface for high analyte concentration (Figure 1C). Especially in clinical diagnostics, Sandwich assays are preferred (Figure 1D). After binding of the analyte to the immobilized recognition element, a secondary recognition partner. This assay increases selectivity and in some cases also lowers limit of detection.

2.2 Biopolymer layer against non-specific binding

Besides the classical biopolymer layers (see Figure 2A) to shield the surface against non-specific binding many other polymeric and biopolymeric structures are mentioned in literature. In some cases, even multilayer systems have to be considered, especially in samples with complex matrices like blood or milk.

Sometimes also photolinkers are used to form the layer system (see Figure 2B). In dependence of the analyte hydrophobicity or hydrophilic behavior of this “shielding” layer might control non-specific interaction in simple cases. Well characterized layers allow the necessary control of the properties.
2.3 Recognition elements

Besides the classical recognition elements like antibodies or DNA strands, peptides, aptamers or even peptides nucleic acids and locked nucleic acids are used. The idea is to use more stable elements to offer surfaces with recognition elements which can be used and stored for months and in rather aggressive sample conditions. Introduction of scaffolds aims in the same direction. Recent developments try to combine biomolecular selectivity with polymeric inertness as molecular imprinted polymers. During the polymerization process the template molecule (the analyte) is added. Afterwards this template is extracted and the polymeric structure serves as a biomimetic "antibody". Volume imprinting revealed some problems with accessibility of the internal recognition sites. The problem is overcome by emulsion resulting in nano-particles with a highly imprinted surface.

Figure 3. molecular imprinted polymers (MIPs)

Figure 4. Using carbo-nanotubes as recognition elements

Another possibility is the use of carbo-nanotubes immobilized onto the transducer. They can be functionalized to increase selectivity. They can be used to bind and characterize other nanoparticles. Binding curves and AFM measurements are given in Figure 4.
2.4 Transduction principles

A large number of optical transduction principles exist quantifying either the amount of fluorescence labeled interaction partner or directly properties of optical thickness of the interaction layer or changes of these, respectively. This optical thickness is the product of refractive index times the physical thickness of the interaction layer. Among the many methods in biosensing preferably Total internal Reflection Fluorescence (TIRF) is used. A guided laser beam in the waveguide excites fluorophores close to the surface if they interact with binding partners immobilized to the transducer surface in a micro flow cell. Laterally resolved the fluorescence signal can be monitored by a CCD camera.

![Figure 5. Principle of Total Internal Reflection Fluorescence (TIRF)](https://example.com/image5)

Higher laser excitation intensity can lower the limit of detection. However, photo-degradation can influence the quantification and normally hinders kinetic measurements. In contrast, direct optical detection has no problems with photostability, but has higher limits of detection.

![Figure 6. Principle of Reflectometric Interference Spectroscopy (RIFS)](https://example.com/image6)

Many optical methods can be used for direct optical sensing. Among the refractometric methods (evanescent field techniques) surface plasmon resonance is commercialized and Mach-Zehnder-Chips have been considered to be especially sensitive. However, all evanescent techniques need extreme thermo-stabilization. This restriction is not valid using Reflectometric Interference Spectroscopy (RIFS). It uses white light interference comparable with a Fabry-Pérot arrangement. As demonstrated in Figure 6 incident light is partially reflected at the interfaces of the sensitive layer (rays $l_{21(1)}$ and $l_{21(2)}$).
and \( I_{R3(0)} \) or \( I_{R3'(0)} \) in dependence on changes in physical thickness of this layer (focus on changes in physical thickness). The interference depends on wavelength and optical thickness. Therefore, a modulation is achieved time resolved. Measuring time resolved allows determination of binding curves and thus gives information on kinetic and thermodynamic data and thus gives information on kinetics and thermodynamics of the evaluated system.

Figure 7 (left) shows the spectral RiFS set-up combining a flow injection system, the flow cell, the fiber for illumination and carrying the reflected light. It turns out that good calculation of the optical conditions allows the reduction of the reflectometric measurement from a spectral one to the measurement of changes in reflected intensity at just one single wavelength. This simple set-up has been commercialized and has proven to give reliable results in some bioanalytical applications. This device is robust, can be driven by battery, and is portable with the dimensions 25x15x8 cm.

Using a diode array instead the photodiode and measuring just at one wavelength, a highly parallelized set-up can be build. A prototype is available and was successfully used in screening approaches discussed in the application chapter.

Modern electronic multi-layer techniques allow the fabrication of miniaturized detectors, which have wavelength dependent selectivity and can be used as pseudo spectral diodes. The chip on the left (see Figure 8) is a disposable. It can be used as a ten channel parallel sensing transduction head. On the right the layer system is enlarged.

3. APPLICATIONS

Optical sensors have demonstrated high quality. For this reason, many applications exist in the fields of environmental monitoring, food control, and healthcare diagnosis. Some of these are given in the following. Examples of fluorescence based detection and direct optical detection are discussed.

3.1 Monitoring environment

Pollutants in water cause health problems. In many cases even modern waste water plants cannot reduce or destroy such compounds. First interest was attracted by pesticides as pollutants in ground, river or coast water. Besides measurements with RiFS\textsuperscript{20} the fluorescence based TIRF transduction proved to be advantageous with respect to limits of detections.
(nano gram per liter range). In projects funded by the European Union multi-analyte detection was possible (for some compounds at few nano grams per liter)\textsuperscript{21}. The AWACSS System has proven its possibilities in a field test (results given in Figure 9)\textsuperscript{22}. Furthermore such instrumentation allows the monitoring of endocrine disruptors (EDCs)\textsuperscript{23}. Reflectometry can be used to measure metals like Cadmium using a nuclear receptor as a sensitive element\textsuperscript{24}.

![Figure 9. Results of field tests for estrone and bisphenol A compared to HPLC-DAD reference measurements (according to cit.\textsuperscript{22}).](image)

### 3.2 Biotechnology

Optical sensing is considered to be a system, which can be used for remote monitoring under harsh conditions during biotechnological processes. Normally, typical biosensors lack stability and inertness against these harsh conditions (pH, solvents, temperature, metabolites). For this reason, the optical transduction was combined with robust recognition elements based on molecularly imprinted polymers. Using MIP-nanospheres diffusion usual barriers are overcome, sensitivity is increased, response times are reduced, and regeneration becomes possible\textsuperscript{13}. Figure 10 shows measurements of MIPs with the template molecule (protected aminoacid) and the corresponding calibration curve.

![Figure 10. RIIS measurements with imprinted and non-imprinted polymers at different concentrations together with the calibration curves.](image)
3.3 Quality control in food

Besides point-of-care diagnosis and environmental analysis, the detection of contaminants in food has gained increasing importance in the last years. To protect the consumer’s health, the European Community has set maximum residue limits for some contaminants like for example veterinary drugs. It is of great importance to develop reliable tools for detecting those contaminants before the food reaches the consumer. The most common analytical techniques used to determine those contaminants in food are gas or liquid chromatography combined with mass spectrometry. Nevertheless, the detection with biosensors offers great advantages such as being cost-effective, quick to perform, sensitive and easy-to-handle. Measurements in complex food matrices like milk require the investigation of matrix effects before immunoassays against contaminants can be developed (Figure 11). RIfS has been used to investigate matrix effects of whole pasteurized bovine milk. As model system the hormone testosterone has been chosen because this immunoassay has been well characterized in buffer. By minimizing matrix effects and establishing a suitable evaluation method, reliable quantitative measurements could be obtained, resulting in a sensor calibration. The gained knowledge can be adapted on the establishment of new biosensor immunoassays for different contaminants which can be found in bovine milk.

![Figure 11. RIfS measurements of testosterone in milk](image)

3.4 Healthcare

Two assay formats, binding inhibition and sandwich assay format, are reported for detection of C-reactive protein (CRP) in human serum. Both assays were characterised and compared with respect to their suitability and adaption into a complete sensor system. An automated, optical biosensor system, based on TIRF, was used to carry out a full threefold calibration in each case. Due to the resulting working ranges, from 0.044 – 2.9 mg L⁻¹ and 0.13 – 22.9 mg L⁻¹ respectively, the assays also qualify to detect high-sensitivity CRP (C-reactive protein).

Fast, cheap and reliable analytical methods in the domain of diagnostics are more and more in demand. This is not only the case in human diagnostics, but also in veterinary diagnostics. For example, to prevent the transfer of infections between animals and humans (zoonoses), control of food originating from animals is necessary. Furthermore, veterinary diagnostics plays an important role in surveillance of live stock, as early detection of infected animals can avoid larger economical damages. To analyse salmonella infections, different biosensor surfaces for antibody detection were tested and optimized. Therefore, the antigen, a lipopolysaccharide, was immobilized onto the surface and the antigen-antibody interaction was monitored in a time-resolved manner by Reflectometric Interference Spectroscopy (RIfS). The new assay including the surface chemistry and assay procedure was set up and fully characterized. The calibration curve is presented in Figure 12.

Other biosensor have to be mentioned: Instead of creatinine the small serum protein cystatin C is a more reliable marker for the detection of renal failure. Using RIfS 0.53 - 1.02 mg L⁻¹ can be reliably detected which is in the normal clinical range of healthy individuals.

One has been developed for the detection of β2GP-I autoantibodies. β2GP-I autoantibodies are highly relevant markers for diagnosis of antiphospholipid syndrome. β2GP-I proteins were therefore immobilized on the sensor surface in a biomimetic assay. An adapted regeneration procedure allows many measurements without recalibrating.
Reflectometry allows the measurement of interaction between recognition elements and viruses. This was proven for quantification of the H1N1 virus by a direct assay without labeling\textsuperscript{32}.

The multisport parallel determination of a large number of binding events (up to 700) becomes possible to use RIfS in the single wavelength mode. The CCD camera is used to monitor kinetic interactions at different spots (laterally resolved rather than a spectrum). An example is given in Figure 13 where different peptides (complementary sequence to celiac disease) are immobilized to the surface of a chip. Best suited peptide sequence and binding rate constants can be determined\textsuperscript{33}.

![Figure 12. Binding signals for different peptides (left): selected spots on the transducer (right)](image)

Figure 12. RIfS measurements of salmonella, (left): Assay format, (right) calibration curve

Reflectometry allows the measurement of interaction between recognition elements and viruses. This was proven for quantification of the H1N1 virus by a direct assay without labeling\textsuperscript{32}.

The multisport parallel determination of a large number of binding events (up to 700) becomes possible to use RIfS in the single wavelength mode. The CCD camera is used to monitor kinetic interactions at different spots (laterally resolved rather than a spectrum). An example is given in Figure 13 where different peptides (complementary sequence to celiac disease) are immobilized to the surface of a chip. Best suited peptide sequence and binding rate constants can be determined\textsuperscript{33}.

![Figure 12. Binding signals for different peptides (left): selected spots on the transducer (right)](image)
4. CONCLUSIONS

In principle, optical transduction methods are based on a variety of high quality optical detection principles. Fluorescence is leading in routine applications, for measuring at extremely low limits of detection, and allows read-out of large and miniaturized arrays. In recent years direct optical detection techniques gain increasing interest. Refractometry as well as reflectometry measures the product of refractive index and optical thickness and its changes, respectively. Nevertheless, all the evanescent field techniques preferably determine the changes in refractive index and are dependent on good thermo-stabilization, accordingly. Both principles can use direct assay formats, avoid cost for labeling and photodegradation of the marker on the interaction partner. The advantage of reflectometry is the good independence on temperature, since changes in refractive index by temperature are widely compensated by changes in volume. Furthermore, reflectometry does not depend on penetration depth of evanescent field into the observed medium. Accordingly, problems of measuring layers thicker than 100 nm are reduced and in addition independent on layer thickness (evanescent field decays). For this reason, reflectometric techniques have gained interest for some bio-applications (especially measuring cell interactions or interactions at membranes). Parallelization to arrays is possible. All these aspects are demonstrated by the applications.

5. ACKNOWLEDGEMENTS

The author thanks his coworkers O. Bleher, M. Ehni, M. Ewald, P. Fechner, D. Furin, U. Hilbig, F. Kolarov, A. LeBlanc, G. Proll, F. Pröll, S. Rau, B. Schwarz, N. Schweizer, J. Widmaier for supplied data. The authors group thanks for funding by EU projects INSTANT (NMP.2011.1.3-1/280550) and CARE-MAN (NMP-CT-2006-017333), bmbf-project “Pepper and Salt” (16SV5049), Landesstiftung BWL (P-LS-Meth (19)-2) and the BMWI/AiF-projects “Tierdiagnostik” (16061 N) and “Acutlab” (16696N)

6. REFERENCES

[27] Rau, S., Gauglitz, G., Reflectometric interference spectroscopy (RIfS) as a new tool to measure in the complex matrix milk at low analyte concentration, Anal Bioanal Chem, 402(1), 529-536 (2012)