Integrated Optical Mach-Zehnder Interferometer as Simazine Immunoprobe

B. Drapp, J. Piehler, A. Brecht and G. Gauglitz
Institute for Physical and Theoretical Chemistry, University of Tübingen
Auf der Morgenstelle 8, D-72076 Tübingen, Germany

B. J. Luff, J. S. Wilkinson, Optoelectronics Research Centre, University of Southampton, Highfield, Southampton SO17 1BJ,UK

J. Ingenhoff, IOT Entwicklungsgesellschaft für Integrierte Optik Technologie mbH
Bruchsaler-Straße 22, D-68753 Waghäusel-Kirrlach, Germany

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Abstract

An indirect immunoassay was carried out on integrated optical interferometer devices. We investigated a common integrated optical Mach-Zehnder interferometer and we present a newly developed interferometer device. On the new device a Mach-Zehnder interferometer structure was combined with a 3x3 coupler on a single chip. The additional coupler structure works as an optical preprocessing for signal improvement. Having a working immunoprobe the device surface had to be chemically modified. A characterisation of the devices was done with increasing amounts of antibodies. The calibration of the devices was carried out in an indirect competitive test scheme. A complete test cycle consists of the incubation of antibodies and a regeneration step is presented. The surface chemistry applied allows the regeneration of the transducer surface for the multiple use of the interferometer device. Simazine concentrations were detected in the sub ppb range.

Introduction

The concept of integrated optical devices originally has been introduced for the use in telecommunication and signal processing. A variety of manufacturing processes were developed or already established including ion-exchange and silica-on-silicon. At present, integrated optical devices become increasingly important as optical sensors.

Evanescent field based sensor systems were applied with very promising results to the field of chemical sensing [1], [2]. As the refractive index of organic or biological materials is different

from aqueous media, such devices can also be used as bioaffinity sensor which responds to the binding of biological material [3]. Immunoanalytical techniques use antibodies to selelectively bind and detect an analyte from a sample. The target of our work is to detect the binding of an antibody at the surface without use of any label in real time.

For sensor purposes two different types of devices are common. Thin film waveguides and strip waveguides. Film waveguides are more easily fabricated. However strip waveguides are used in the field of telecommunications and the technology for pigtailing such waveguides is at hand. Thus we investigated the potential of strip waveguides. Therefore we used two types of interferometer based devices with surface strip waveguides.

The device we used for our first measurements was the well known integrated optical Mach-Zehnder interferometer (see fig.1). The device is covered with a patterned buffer layer. The Mach-Zehnder interferometer structure is used to convert the changes in bulk refractive index in intensity effects. The Mach-Zehnder device is explained in detail in [4] and the outcoupled intensity can be described by eq. 1.

$$\frac{I(\Delta\varphi)}{I_0} = \frac{1}{2}\left(1 + \cos\left(l \cdot k_o \cdot \Delta n_{eff}\right)\right) \qquad (1)$$

Where I_0 denotes the incoupled intensity, $I(\Delta\varphi)$ the measured intensity, l the length of the measuring window, Δn_{eff} the difference between the effective refractive indices in the measuring and the reference arm, and $k_0 = 2\pi/\lambda$ where λ means the wavelength. The outcoupled intensity of the interferometer is modulated by the cosinus function which effects that the sensitivity also depends on the starting point of the intensity curve itselfs. The best response will be obtained by starting at the turning point of the intensity curve.

The new device we designed is schematically drawn in fig. 2. The working principle of the device can be explained by the combination of the interferometer structure and a 3x3 coupler structure. The first part of the device is the interferometer structure. As on the Mach-Zehnder device in fig. 1 one of the interferometer arms can be influenced by changes in bulk refractive index. In the second part of the device the coupler structure gives three different interference signals. The 3x3 coupler can be explained by the phenomenon of optical tunneling and the overlapping of the evanescent field of the guided modes. With an carefully optimized coupler structure the phase decoding of the guided modes coming out from the interferometer part is possible. Thus the coupler structure with its three outcoming signal ports allows to find an

optimal staring point on the intensity curve. The possibility of referencing the signal ports is another goal of the combined structures. The coupler in fig. 2 was generally used to reference the intensity at port 1. Thus the influence of instabilities in light incoupling or light source noise can be reduced.

Experimental

Devices

The integrated optical devices both the Mach-Zehnder and the coupler device and its waveguide structures were designed and manufactured by IOT (Entwicklungsgesellschaft für integrierte Optik-Technologie, Waghäusel). The waveguides were produced by changing the refractive index in contrast to the glass substrate BGG36 with a refractive index of 1.6009 at 588 nm. The changes in refractive index were achieved by dipping the masked device in a 10% Ag ion salt melt, reaching a Δn of 0.075.

To observe a change of intensity due to a phase shift between the two interferometer arms only one of both arms is permitted to be in contact with the analyte. This is achieved by covering the chip with a protecting layer except the so called measuring window (see fig. 1 and fig. 2). The Mach Zehnder device was covered with a SiO_2 layer made by an PICVD process. The coupler device was covered with a teflon layer. On both devices the measuring window is 15 mm in length.

Surface chemistry

Using an indirect test scheme the hapten has to be bound at the transducer surface. This was reached in several steps. After the activation of the device surface by silanisation an aminodextran was coupled and modified by atrazine caproic acid. The covalent coupling of atrazine modified dextran layer on a glass type surface is described in detail in [5].

The experimental set-up

Measurements with the Mach-Zehnder device were made with the set-up schematically drawn in fig. 3. A semiconductor laser diode operating at 785 nm was used as a light source.
A polarizer supplies a linearly polarised light beam in the TM-mode The polarised light was incoupled into the waveguide with a microscope objective (10 x 0.22). The out-coupled light was guided to a photo diode by a multimode fiber. The microscope objective, the integrated optical device and the multimode fiber were fixed on micropositioning mounts.
In principle the experimental set-up for the measurements with the coupler was quite similar to the set-up of the Mach-Zehnder device. The light incoupling was also done in an end-fire construction with a microscope objective. The outcoming light from the three outgoing coupler waveguides was imaged with a microscope objective on three seperate photo diodes. For each signal port the lock-in amplifying method was used. The outcoming signal from port 1 (see fig. 2) was referenced with the total intensity output.
In both experimental set-ups the integrated optical devices were integrated in a flow system and the data acquistion was done with a 12 bit A/D card and a personal computer.

Results and discussion

Characterisation of the devices

In a first step the non specific binding was tested by the incubation of ovalbumin (1mg/ml) whereby no signifigant change in intensity and phase shift was observed see fig. 4 and fig. 5
The intensity modulation due to the incubation of antibodies in a small concentration of 5µg/ml gives information about the initial surface coverage with atrazine. To compare the response of both the Mach-Zehnder and the coupler device the phase shifts were calculated from the measured intensities (fig. 4 and fig. 6) and plotted in fig. 5 and fig. 7. For a small antibody concentration of 5 µg/ml (fig. 5) the phase shift linearly increased with time. So the binding of the antibodies is only limited by diffusion which indicates a high loading of antigen at the transducer surface. Atrazine is immobilized at the transducer surface in exass. Therefore even at maximum antibody coverage, binding sites remain unoccupied. As the diffusion limited binding rate is independent from the amount of atrazine imobilized, the phase shifts obtained from both the Mach-Zehnder and the coupler device during test cycles are comparable.

Calibration of the devices

Different concentrations of antigen were used to calibrate the integrated optical device. We used a modified indirect competitive test scheme as described explicitely in [5]. The anti-simazine antibodies were incubated with samples of varying simazine content. Afterwards the sample was brought to the interferometer and the binding of unoccupied antibodies was monitored. A complete test cycle consists of the incubation and a regeneration step with pepsin, acetonitrile and PBS (phosphate buffered saline). For the measurements with the Mach-Zehnder device an antibody (anti simazine IgG) concentration of 2 µg/ml was used. In fig. 8 the measured intensity during a complete test cycle is plotted versus the time. The rms value of the baseline is given at $5.3 \cdot 10^{-4}$. From the measured intensity the phase shift and the slope of phase shift were calculated. The slope of the phase shifts are presented in fig. 9.

To check the coupler device and its performance detecting changes in bulk refractive index different aqueous sugar solutions were applied. The detection limit for changes in bulk refractive index was found at $\Delta n = 1.5 \cdot 10^{-6}$. Similar measurements with the Mach-Zehnder device pointed out a detection limit at $\Delta n = 3.48 \cdot 10^{-5}$. Subsequently the calibration experiments with the coupler device were done with an antibody (anti simazine Fab) concentration of 0.33 µg/ml. A further improvement was reached by using anti-simazine Fabs instead of anti-simazine IgG. The influence of antibody concentration to the detection limit of an immunoassay is explicitely discussed in [6]. Using anti-simazine Fabs instead of anti-simazine IgG the relation between the midpoint of test and receptor concentration can be descripted by eq.2

$$c_{0,MoT} = \frac{1}{K} + \frac{c_{0,receptor}}{2} \tag{2}$$

wherby K denotes the affinity constant, $c_{0,MoT}$ the concentration of the midpoint of test and $c_{0,receptor}$ means the Fab concentration.

The test cycle we applied to calibrate the coupler device was the same as in the previous calibration of the Mach-Zehnder device. The different simazine concentrations implicated different changes in the meassured intensity at the coupler output (see fig. 10). From the measured and referenced coupler output the phase shift generated by the interferometer

structure was calculated. The slope of the phase shifts are presented in fig. 11. Simazine concentrations in the sub ppb range were detected.

Conclusion

An indirect competitive immunoassay for simazine was carried out on an integrated optical Mach-Zehnder interferometer. The specific antigen antibody reaction was detected in real time and without labelling. The incubation of ovalbumin in a concentration of 1 mg/ml showed that the non specific binding was minimized by the dextran layer. The covalent surface chemistry also allowed regeneration with pepsin and acetonitrile. After the regeneration step the interferometer was suitable for a further test cycle. The complete test cycle including the regeneration was done in less than 30 minutes. The new interferometer device with an additional 3x3 coupler structure improved the signal to noise ratio. The improvement of both the transducer and the biochemicals lead to detect simazine concentrations in the sub ppb range. Thus the integrated optical interferometer device was succesfully applied as an immunoprobe of simazine in water.

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Figure captions

Fig. 1: The integrated optical Mach-Zehnder interferometer with ion exchanged waveguides and a patterned buffer layer

Fig. 2: The new interferometer device with both the Mach-Zehnder and a 3x3 coupler structure on a single chip.

Fig. 3: The experimental set-up

Fig. 4: Intensity modulation due to the incubation of ovalbumin (1mg/ml) and different concentrations of antibodies.

Fig. 5: Phase shifts of the Mach-Zehnder device due to different concentrations of anti-simazine IgG.

Fig. 6: Characterisation of the coupler device by the incubation of antibodies.

Fig. 7: Phase shifts of the coupler device due to the incubation of ovalbumin (1mg/ml) and 50 µg/ml anti-simazine Fab.

Fig. 8: Intensity modulation during a complete test cycle. Sample with 0.1 ppb simazine pre-incubated in 2 µg/ml anti-simazine IgG and regeneration step.

Fig. 9: Relative slope of the phase shifts due to different simazine concentrations in 2 µg/ml Fab on the Mach-Zehnder device.

Fig. 10: Changes in outcoupled intensity at port 1 of the coupler device due to varying simazine concentrations in 0.33 µg/ml anti-simazine Fab.

Fig. 11: Calibration curve of simazine with 0.33 µg/ml anti-simazine Fab.