Integrated optical applications based on the nonlinear optical propeties of the protein bacteriorhodopsin

Summary of the Ph.D. Thesis

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INTRODUCTION

Time is a crucial factor in several areas of life, however, it could be the most important in medical fields. One might simply consider the role of early diagnosis that could even save lives. Therefore, several research groups are engaged in the development of highly sensitive, selective, reliable, rapid and low-cost biosensors for point-of-care testing. Nowadays the most sensitive biosensors are mainly based on labelling techniques, such as fluorescent, radioactive or magnetic labelling. Due to these labels, even a single molecule of interest can be detected in the tested volume or on a surface. Despite the unquestionable advantages of these sensing methods, they also have drawbacks compared to label-free techniques. The chemical processes of labelling increase the complexity, time and cost of the analysis; moreover, the markers could have a non-negligible effect on the labelled molecules. To overcome these disadvantages, label-free sensing methods are intensively researched. Particularly, optical biosensors are investigated, and among them integrated optical solutions represent an important trend. Due to their high sensitivity, interference-based integrated optical biosensors are one of the best candidates. Unfortunately, as a consequence of this high sensitivity, inherent instability of the device due to slight changes of environmental parameters (e.g. temperature, humidity) also occurs. Fluctuations of humidity and temperature may result in intensity variations at the output of the interferometer, which prevent the precise and stable operation of the sensor.

One of the aims of my Ph.D. work was the realization of an integrated optical Mach-Zehnder interferometer based biosensor, in which the disturbing effect of the environment can be minimized, and a reliable and sensitive operation can be achieved.

Integrated optics also plays an important role in the field of information technology. Over the last decades, the increased demand for high-speed information access and data

processing requires faster and faster computers and data transmission. From the 1960s, electronics and computer technology have been going through an explosive evolution: the overall processing power of computers doubles in every 18 months. This development is described by the famous Moore's law, formulated by the Intel co-founder Gordon E. Moore. Moore's law states that the number of transistors per square inch on integrated circuits doubles about a year and a half. Integrated circuit manufacturers strive to reduce the size of integrated components as much as possible, in order to increase the processing power of a device of a given size. This continuously growing trend is valid even in our days, but most of the experts, including Gordon E. Moore himself, agree that the limit of the miniaturization will be reached soon. Therefore, alternative solutions are required to further increase computing speed in the long term. One possible solution may be provided by integrated optics.

In computers, transistor-based logic gates serve as the building blocks of digital logic circuits. A main goal of optical data processing research is the development of an integrated optical analogue to integrated electronic logic gates, where the logic values are represented by light intensities, utilizing the advantages of optics as high bandwidth, high switching speed, and low transmission loss. Mostly electro-optical configurations are realized, but in these devices electro-optical conversion is a strong limiting factor. Hence, in the last decade, attention increasingly turned to all-optical solutions. Some of the implementations are based on classical optical systems, but mostly integrated optical concepts could be found in the literature. Although the research of optical logic systems is still "in its infancy", the rapid raise in the number of publications over the past decade shows the increased interest towards this field.

One of the aims of my work was the realization of an all-optical logic gate, constructed from an integrated optical Mach–Zehnder interferometer as a passive structure, covered by a nonlinear optical material - bacteriorhodopsin (bR) - as the active component.

MATERIALS AND METHODS

Integrated optical Mach-Zehnder interferometer

The underlying passive structure of the devices presented in my dissertation is an integrated optical Mach-Zehnder interferometer. The operation principle is similar to the classical interferometer, but in this case the device is constructed as an integrated optical waveguide structure. The light entering the waveguide is divided into two beams, propagating independently in the reference and measuring arms of the interferometer, and then recombined again after traveling along a given optical path. When the optical path length is different in the measuring and reference arms, a phase difference occurs between the guided waves, which can be detected as a light intensity change at the output of the interferometer. In an integrated optical waveguide, the guided light penetrates into the surrounding medium (so-called evanescent field), therefore refractive index changes near to the surface could also be detected.

The integrated optical Mach-Zehnder interferometer was prepared by direct UV lithography into a NOA 81 (*Norland Product Inc.*) photopolymer layer spin coated on a microscope cover slide. The optical connection for in- and out-coupling was established by optimally positioned and fixed single-mode optical fibres (*S630-HP, Thorlabs*).

Bacteriorhodopsin

Bacteriorhodopsin is an integral membrane protein of the *Halobacterium salinarum*, which, upon light absorption, pumps protons from the cytoplasmic side to the extra-cellular side of the membrane. In the end of this transport process bacteriorhodopsin is reprotonated from the cytoplasm, and then returns to the ground state. The resulting transmembrane proton gradient is used for ATP synthesis by ATP synthase, providing the energy of the *Halobacterium* in oxygen- and nutrient-poor environment. This light-induced proton transport process is called the photocycle of the protein.

During the photocycle, the protein undergoes different conformational states: BR_{568} (ground state), J_{625}, K_{610}, L_{540}, M_{412}, N_{550}, O_{630}, where the lower index corresponds to the absorption maxima of the intermediate state in nanometres. The absorption difference between two intermediate states also implies a corresponding refractive index change, according to the Kramers-Kronig relations. The largest refractive index change - approximately $4 \cdot 10^{-3}$ - occurs between the BR and M states. The different conformational transitions take place on different time scales spanning from milliseconds (e.g. N-O transition) to picoseconds (e.g. BR-I transition). Beside the ground state, some of the intermediate states are also photoexcitable: the photocycle can be shortcut by applying a second excitation of proper wavelength, which is close to the absorption maximum of the corresponding intermediate state. A further advantage of bacteriorhodopsin to be exploited in integrated optical applications is the long-term stability and activity of the dried and gel-embedded forms of the protein.

Due to the above-described reversible light-induced refractive index change, bacteriorhodopsin is a promising candidate to be used as an active optical material in the integrated optical devices investigated in my Ph.D. thesis.

To form a thin (*30-35 μm*), homogeneous bacteriorhodopsin film on top of the arms of the interferometer, dried droplets of purple membrane fragment suspension were applied.

Experimental setup

During the performed experiments, different light sources were applied. For exciting the bacteriorhodopsin layer, a variable intensity light source was used. In the case of the biosensor measurements, it was the lamp of the observing microscope (*Zeiss Axiovert200*), while in the case of the logic gate experiments, the light of a diode laser (λ=532 nm) served as exciting light. In continuous-wave measurements, a beam of a CW diode laser (λ=674 nm, P=10 mW) was coupled into the interferometer, while in the pump-probe logic gate experiments, short laser pulses were applied. The probe pulses (τ =3.4 ns, λ=671 nm) were provided by an optical parametric oscillator driven by the third harmonic (λ=355 nm) of a Surelite II-10 Nd:YAG laser. As pump beam, the second harmonic beam (τ =5 ns, λ=532 nm) of the Nd:YAG laser was used to excite the bR layer.

The output intensity of the Mach-Zehnder interferometer was measured by a photomultiplier (H5783-01, Hamamatsu) and the excitation intensity was detected by a photodiode. The signals were recorded by a digital storage oscilloscope (WaveRunner 6100A, LeCroy).

For the biosensor experiments, PDMS microfluidic channels were prepared by soft lithography. First, the mould was made from SU-8 photopolymer using photolithography, then a liquid-degassed PDMS (Polydimethylsiloxane) prepolymer was poured over the master. The cured PDMS replica was then peeled from the master, and inlet-outlet holes were formed. Thereafter the PDMS was sealed precisely above the arms of the Mach-Zehnder interferometer. Silicone tubing was connected to the inlets and outlets of the device, and a

syringe pump was used to circulate the solutions in the microfluidic channels during the functionalization process and the measurements.

RESULTS

BIOSENSOR

1. Biosensor based on integrated optical Mach-Zehnder interferometer

1.1. Tuning the operating point of the biosensor using a bacteriorhodopsin layer

It was demonstrated that the excitation of a bacteriorhodopsin adlayer deposited on one arm of an integrated optical Mach-Zehnder interferometer based biosensor enables the optimal adjustment of the operating point. The key step was the proper adjustment of the phase shift of the propagating modes in the Mach-Zehnder interferometer. This was realized by the light-induced refractive index change of the bacteriorhodopsin film. Depending on the intensity and duration of the excitation, the ratio of the ground and intermediate states is altered in the bR layer, resulting in a refractive index change (as described previously), and the modified optical path length leads to a phase shift in that arm. Consequently, the phase shift between the arms can be tuned by the excitation of the asymmetrically placed bacteriorhodopsin layer.

The sensitivity of the biosensor was investigated in the following way: a rectangular probe illumination (laser diode, λ=532 nm) excited the bR adlayer at different operating point settings, and the output light intensity of the interferometer was monitored. The measurements proved that the sensitivity of the device highly depends on the actual setting of the operating

point. This way it is possible to maximize the sensitivity during the operation of the sensor. Furthermore, the adjustability of the operating point enables the minimization of the inherent instability of the Mach-Zehnder interferometer due to environmental changes, which is extremely useful in real-world applications.

1.2. Antibody detection using the integrated optical biosensor

I participated in immunological test experiments, what were carried out to demonstrate the applicability of the biosensor. From the applied probe solution, a biotinylated anti mouse immunoglobulin adlayer was formed on the measuring arm (functionalized with IgG2a mouse monoclonal antibodies), resulting in an optical path difference between the arms, what was detected as an output intensity change of the interferometer. During the experiments, the applied solutions were exchanged in PDMS cuvettes attached above the arms of the Mach-Zehnder interferometer. The experiments proved that the device is sensitive enough to detect the development of a monomolecular layer due to a specific antigen-antibody reaction.

2. Combining the integrated optical biosensor with a microfluidic system [T2]

The Mach-Zehnder interferometer based biosensor was combined with a microfluidic system. Microfluidic channels positioned above the arms of the interferometer allow for easier, more precise and automated functionalization of the surface of the sensor. After the proper functionalization of the measuring arm, using antibodies specific to the bacteria to be detected, the device was able to detect *Escherichia coli* bacteria at concentrations of $6.4 \cdot 10^6$ cfu/ml. Since this concentration is comparable to characteristic pathogenic concentrations in sputum and urine, my measurements proved that the device is appropriate for label-free detection of bacteria from such body fluids.

ALL-OPTICAL LOGIC GATES

3. Integrated optical logic gate working in binary mode [T3, T4]

An all-optical logic gate was constructed from an integrated optical Mach-Zehnder interferometer as a passive structure and a bacteriorhodopsin adlayer as an active element. The principle of all-optical logical operation is based on the all-optical light modulation utilizing the photo-induced refractive index change of bacteriorhodopsin. Based on my experiments, binary and ternary logical modes of operation were demonstrated depending on the operating point of the interferometer. The operating point was adjusted as described previously in the case of the biosensor. The input values of the logic device were represented by quasi-continuous laser beams exciting the bacteriorhodopsin layer, while the output was defined by the intensity level of the outcoupled measuring light. For the input values, the presence of excitation of the bacteriorhodopsin layer corresponded to the logic value 1, while the absence of it to 0. At the output (in binary mode), the logic value 1 was represented by maximum light intensity, while 0 corresponded to minimum intensity.

3.1. Inverter

First, the most basic logical operation, negation was realized to demonstrate the operation of the optical logic gate in binary mode. By the proper adjustment of the operating point, it was achieved that in case of the bacteriorhodopsin excitation ($X=1$) the output intensity of the interferometer was minimized ($Y=0$), while without exciting the bR ($X=0$) the output intensity was reset to the original level ($Y=1$). I proved experimentally that the device can operate as an inverter.

3.2. XOR logic gate

In addition to the inverter, an XOR (exclusive or) logic gate was realized in binary mode by the integrated optical interferometer. Contrary to the OR logical operation, the XOR operation results in *Y=1* logic value at the output only when the inputs are different. To achieve this, the operating point of the interferometer was tuned to minimize output intensity in the absence of excitation of the bacteriorhodopsin layer. In order to realize all possible combinations of input values, the bacteriorhodopsin layers on the two arms of the interferometer were excited both individually and simultaneously. My results showed that - in the case of the above settings - the integrated optical device operated as an XOR logic gate.

4. Ternary mode of the integrated optical logic gate [T3, T4]

Besides the above described binary mode, I also showed that the integrated optical logic gate is also capable of ternary operation. The operating point was adjusted to realize an output light intensity between the two extrema, thereby, upon the excitation of the bacteriorhodopsin, the output intensity could both increase and decrease relative to its initial value. Similarly to the binary mode, the input logic value 1 was represented by the excitation of the bacteriorhodopsin, while the logic value 0 corresponded to the absence of excitation. Unlike in binary mode, in the case of ternary logic, the output has three distinct logic values (*Y=-1, Y=0, Y=1)*.

4.1. Comparator

I demonstrated the operation of the logic device in ternary mode by constructing an integrated optical comparator. Similarly to the comparator used in electronics, this device is also capable of indicating the relation (larger, smaller, equal) of its two input values. In these experiments, every possible combination of input values were generated by the proper excitation of the

bacteriorhodopsin adlayer, and the corresponding output light intensity was measured. Based on the output intensity (output logic values) it could be determined which bR layer was excited, i.e. which logic input was larger, thus the device performed as a comparator.

4.2. Operation of the integrated optical comparator in pulsed mode, fast logical operation

The ternary mode operation of the integrated optical logic device in pulsed mode was demonstrated by pump-probe experiments. In this case the input and output logical values were represented by nanosecond laser pulses. During these experiments, similarly to the continuous mode, the change of the output intensity was monitored, while the pump pulse was exciting the bR film above either one or both arms of the interferometer (realizing all the possible input combinations). The results proved that the integrated optical logic device can be operated as a comparator in pulsed mode likewise continuous mode.

On the time scale of the nanosecond pump pulses, the dominant intermediate state in the bacteriorhodopsin layer is the K form, because the later intermediates accumulate on the microsecond time scale. Based on the pump-probe experiments, the switching time of the logic device was found to be 8 nanoseconds. This was the fastest operation of a protein-based integrated optical logic gate that has been demonstrated so far.

PUBLICATIONS

Refereed publications related to the thesis

[T1] A. Dér, S. Valkai, A. Mathesz, I. Andó, E. K. Wolff, P. Ormos
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