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Comparison of Label-Free ACh Image Sensors Based on CCD and LAPS

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Abstract:
Semiconductor-based chemical image sensors, like the pH-image sensor based on a charge-coupled device (CCD) or the light-addressable potentiometric sensor (LAPS), are becoming a powerful tool for label-free imaging of biological phenomena. We have proposed a polyion-based enzymatic membrane to develop an acetylcholine (ACh)-image sensor for neural cell activity observations. In this study, a CCD-type ACh image sensor and a LAPS-type ACh image sensor were fabricated and the prospect of both sensors was clarified by making a comparison of their basic characteristics.

Key words: charge-coupled device, light-addressable potentiometric sensor, label-free imaging, acetylcholine, polyion complex membrane

Introduction

Chemical image sensors, like the pH-image sensor based on a charge-coupled device (CCD) or the light-addressable potentiometric sensor (LAPS) are able to determine the pH value and its dynamic changes on the sensor surface in a spatial-resolved manner. Both sensors are semiconductor-based chemical sensors with an electrolyte/insulator/semiconductor (EIS)-layered structure. In case of the CCD-type set-up, each CCD spot is measuring the local pH value [1]. The CCDs and the signal processing circuits are fabricated by complementary metal-oxide-semiconductor (CMOS) technologies. For LAPS, a single EIS-structured sensor chip is used. By illuminating a local part of the semiconductor with a modulated light source, a photocurrent will be generated that depends on the local pH value of the sensor on that illuminated area [2,3]. In the present work, both sensor types should be compared in case of an acetylcholine (ACh)-image sensor. Therefore, the enzyme acetylcholine esterase (AChE) was immobilized on each sensor surface. Due to the ACh-AChE enzyme reaction, hydrogen ions will be generated that can be detected by the particular pH-sensing surface. The final goal of this experiment is to investigate and image the ACh release in the synapse of neurons [4].

Principle

The principle of the proposed ACh-image sensors is based on the enzymatic reaction of ACh-AChE, specifically the oxidation of ACh according to the following reaction:

$$\mathrm{ACh} + \mathrm{H_2O} \xrightarrow{\mathrm{AChE}} \mathrm{choline} + \mathrm{CH_3COO^-} + \mathrm{H^+} \quad (1)$$

The use of a polyion-complex enzyme membrane is one of the comprehensive methods to immobilize the enzyme on a substrate [5]. AChE is attached in the membrane by the electrostatic force of the polyanion (poly(L-lysine) hydrobromide) with a negative charge, and the polycation (poly(sodium-4-styrenesulfonate)) with a positive charge (see Fig. 1). The electrostatic force is achieved by the adjustment of number of charges into poly(L-lysine) hydrobromide and poly(sodium-4-styrenesulfonate) equivalently. This method has the advantage that the enzyme could be immobilized adjacent to the sensor at a high density

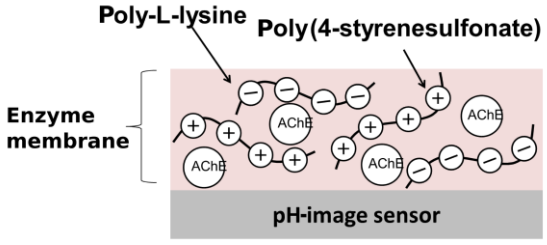

Fig. 1: Polyion-complex enzyme membrane including AChE on a pH-image sensor.

(~µm). The proposed ACh-image sensor was fabricated by immobilizing the enzyme AChE on a pH-image sensor as shown in Fig. 1. AChE converts ACh to choline and acetic acid, when ACh was hydrolyzed.

Due to the ACh-AChE enzymatic reaction, hydrogen ions will be released, which cause a pH change. This pH change results in a change of the surface potential Φ_s at the electrolyte/insulator interface according to the site-binding model [6,7]. This surface potential can be read out in a spatial-resolved manner by various sensor principles such as the CCD-type pH-image sensor or the LAPS.

In case of the CCD-type set-up, the depth of the potential well in the semiconductor varied when it detects the change in the concentration of hydrogen ions generated by the ACh-AChE enzyme reaction in the sensing area (Si_3N_4), as shown in Fig. 2. The output signal of the sensor depends on the ACh concentration in the solution. The pH-image sensor chip was composed of a 32x32 pixel array and CMOS technique with horizontal and vertical shift registers. Each pixel was 130x130 µm^2, with a sensing area of approximately 40 µm^2.

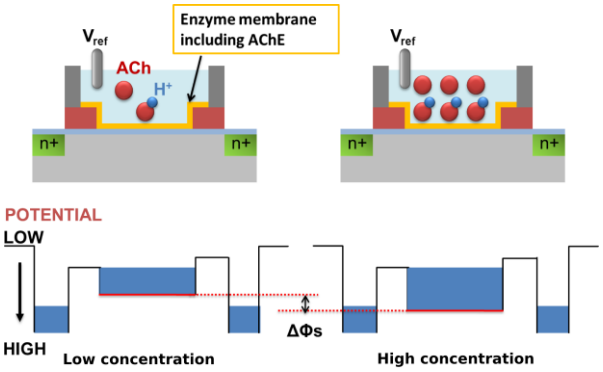

Fig. 2: Principle of the CCD-type ACh image sensor depicted for one measurement pixel.

In case of the LAPS-type set-up, a single EIS-structured sensor chip with a sensing layer of Ta_2O_5 was used. The bias voltage as well as the surface potential will affect the local width of the space-charge region on the insulator/semiconductor interface. A light pointer is used to shine modulated light into the semiconductor and realize the addressability, as shown in Fig. 3. Depending on the width of the space-charge region an external photocurrent will be generated. As light pointer a 4x4 infrared light-emitting diode (IR-LED) array is used for rear-side illumination resulting in measurement spots with a radius of about 1.5 mm. The surface potential is calculated by the measured photocurrent amplitude and a previously determined photocurrent/bias voltage curve and is given as sensor-output signal. More information about the applied LAPS set-up can be found elsewhere [2,3,7-11].

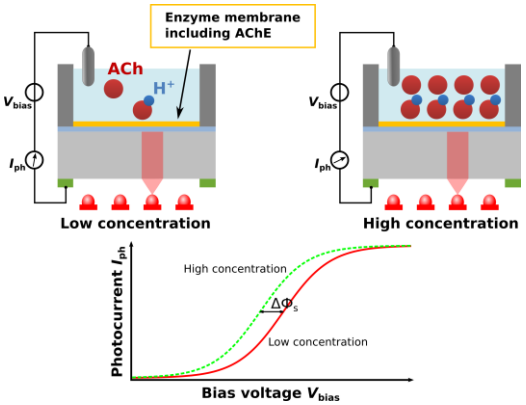

Fig. 3: Principle of the LAPS ACh image sensor.

Experimental

A CCD-type pH image sensor with 32x32 pixels and an active sensor size of 4x4 mm^2 [1] as well as an LAPS with a 4x4 IR-LED array and an active sensor size of 15x15 mm^2 [10] have been used. The enzyme AChE has been immobilized with the help of a polyion-based membrane. Three different aqueous solutions were prepared using 0.01 M phosphate buffer solution (PBS), as follows: poly(L-lysine) hydrobromide (60 mM in monomer units), AChE (100 kU/10 µl), and poly(sodium-4-styrenesulfonate) (75 mM in monomer units). The three solutions were mixed, and placed on the CCD-type and LAPS-type image sensor, respectively (60 µl and 1.08 ml). After that, the sensors were allowed to dry at room temperature for one day.

Results

Figure 4 shows a photograph of the two chosen sensor set-ups, that is the CCD-type enzymatic sensor (a) and the enzyme-based LAPS (b), respectively. In the experiments, the enzyme activity was varying from about 2 to 6 units/mm^2. The response of both sensor types towards different ACh concentrations from 1 to 100 mM was investigated as calibration measurement (Fig. 5), which underlines the expected dependence. The different behavior in the signal amplitude of the sensor-output signal might be explained due to i) the different amplification of the output signal of both sensor types and ii) the varying enzyme activity on top of the particular sensor surface. The LAPS showed a more stable adhesion of the polyion-based enzymatic membrane, which is probably to the absolutely flat sensor surface of the LAPS chip compared to the more “rougher” surface (caused by the photolithographic patterning) of the CCD-type sensor.

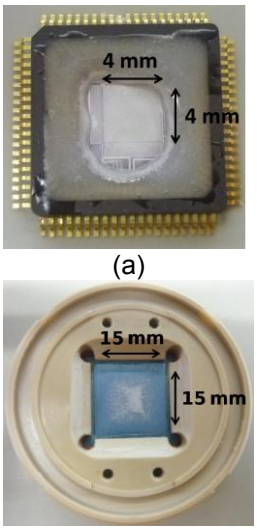

Fig. 4: Photographs of the ACh-image sensor based on (a) CCD and (b) LAPS.

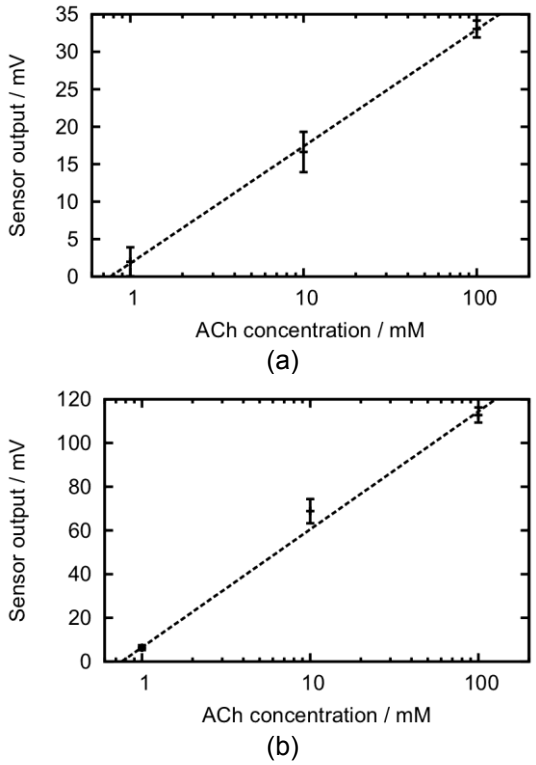

Fig. 5: Sensor response of the ACh sensors to different ACh concentrations with (a) CCD and (b) LAPS set-up, respectively.

In addition, real-time (i.e., dynamic video) investigations with a measurement time of 200 ms/frame have been performed with both sensor types. Here, the diffusion of a 200 mM ACh-containing droplet in PBS solution to the particular sensor surface and the subsequent catalytic conversion by the enzyme on top of the sensor surface has been monitored. The results demonstrated the ability of both sensor types to determine those changes spatially and time-resolved.

Conclusions

The CCD-type pH-image sensor and the LAPS are able to detect ACh with the help of the enzyme AChE, immobilized in a polyion-based membrane. Both sensor types determine the analyte ACh spatially and time-resolved. A comparison of the basic characteristics of both sensor types is summarized in Tab. 1. The fabrication of the CCD-type sensor is rather complex, but all necessary signal-processing circuits are integrated on the sensor chip in CMOS technology. On the other hand, the LAPS chips are easy to fabricate, whereas the driving of the light sources and the signal processing of the photocurrent have to be done externally from the LAPS chip. Due to the fact, that the measurements spots of the LAPS are defined by external light sources, an alignment to an additional micro-fluidic set-up might be possible.

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Tab.1: Properties of the ACh-image sensor for the CCD- and LAPS set-up.

	CCD-type ACh image sensor	LAPS-type ACh image sensor
Signal processing circuit	Integrated	External
Complexity of readout and driving	+	0
Addressability	Depending on fabrication	Depending on light sources
Adherence of ACh polyion-based membrane	-	+

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