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Oxygen imaging in microfluidic devices with optical sensors applying color cameras

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Abstract

Luminescent sensors with improved performance for monitoring dissolved oxygen in microfluidic devices were developed. Our brightness enhanced sensing films with reduced film thickness showed higher signals than standard sensors and responded in real-time.
Sensor layers were integrated into microfluidic chips and readout off-chip on a fluorescence microscope with lifetime imaging or ratiometric imaging using the color channels of a CCD camera. Cell respirometry of model culture systems was monitored with this sensor setup.

Keywords: Oxygen; Imaging; Lab-on-a-Chip; microfluidic; optical sensors optical; fluorescence microscope; 2D-imaging; light-harvesting; FLIM; FRIM; CCD-camera; respirometric activity

1. Introduction

Microfluidic systems are often applied in life science research as they allow well controllable and reproducible investigations on a cellular level. Oxygen is one of the key parameters in cellular studies. For example one can obtain important information on the metabolic activity by measuring respiration rates [1]. Moreover many cells require aerobic or anaerobic conditions in bioreactors, thus oxygen control and monitoring is desirable [2]. However, few publications report on oxygen measurements in microfluidic devices [3-5].

Additionally, it is not only interesting to measure averaged oxygen levels at one point of a microbioreactor. Space resolved detection can for example give information on oxygen gradients in biofilms or on heterogeneous oxygen distribution inside a microfluidic system caused by a variable gas permeability of the surrounding matter [5].

Due to reduced analysis volumes in microfluidic bioreactors, sensing oxygen is a difficult task. For example electrochemical sensors, based on amperometric detection of oxygen, exhibit some drawbacks due to the difficult miniaturization of the reference electrode and consumption of the analyte [4].

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For this reason there is an increasing interest in the integration of optical sensors into microfluidic devices. Due to their sensitivity and selectivity, especially luminescence based sensors represent flexible tools to obtain analytical information in miniaturized systems [6]. Moreover they are scalable to smaller dimensions, rather low-priced and allow 2-dimensional measurements when combined with microscopic optics and appropriate cameras.

For our studies oxygen-sensitive films, based on collisional luminescence quenching, were characterized and integrated into microfluidic chips. The use of light harvesting [7], leading to increased brightness, enables application of sensor layers with reduced filmthickness. Readout was accomplished off-chip on an epifluorescence microscope with lifetime imaging or ratiometric imaging using the color channels of a CCD camera. Furthermore these systems were used to monitor cell respirometry.

2. Materials and methods

2.1. Chemicals and Materials

The following chemicals and materials were used: polystyrene (PS, MW 250 000; Acros Organics), trichloromethane (Carl Roth GmbH), 3-(5-chloro-2-benzoxazolyl)-7-(diethylamino)-2H-1-Benzopyran-2-one (Macrolex Yellow (MY); Simon und Werner GmbH), platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorphenyl)-porphyrin (PtTFPP; Frontier Scientific) TiO_2 P170 (ultrafine, Kemira, Finnland), nitrogen and synthetic air (Air Liquide) and microscope slides (76 x 26 mm; Carl Roth GmbH).

2.2. Preparation of oxygen-sensing layers and integration into microfluidic chips

Respective amounts of MY, PtTFPP, TiO_2 and PS were dissolved or dispersed in $CHCl_3$. These mixtures were placed in an ultrasonic bath for ten minutes to ensure finely dispersed TiO_2-particles. Sensor layers on microscope slides were obtained using a standard knife coating device.

These glass slides served as substrates for the microfluidic chips which were derived from the Austrian Institute of Technology GmbH (AIT), Health and Environment, Nano Systems (Ertl et al). Fluidic channels were formed in a PDMS (polydimethylsiloxane) layer as an imprint of a silicon master mold. Then fluidic was bonded to the coated sensor substrates.

2.3. Measurement setup and principles

Plain luminescence intensity images are usually unsuitable for directly measuring oxygen concentrations. Intensity fluctuations origin from inhomogeneities of the light source, of the sensitivity of the detection system or of the dye distribution within the sensor layer and have to be corrected. In this work two referencing schemes were used: ratiometric imaging and lifetime imaging by rapid lifetime determination (RLD) (figure 1).

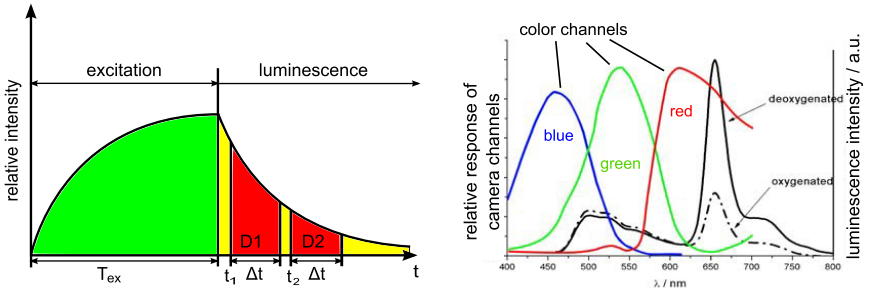

Fig. 1: Principles of (left) rapid lifetime determination and (right) ratiometric imaging.

Ratiometric imaging is based on calculating the ratio of the red channel (PtTFPP-emission; oxygen-sensitive signal) to the green channel (MY-emission; reference signal) of a color camera. In lifetime imaging applying RLD the luminescent probe is excited by a light pulse and emission is measured at two different measuring windows at different delay times after the excitation pulse. Luminescence lifetime (τ) is calculated using the following formula (for definition of parameters see Figure 1).

$$\tau = \frac{t_1 - t_2}{\ln(D_1 / D_2)} \qquad (1)$$

Images were obtained using an epifluorescence microscope (Zeiss Axiovert 25 CFL), equipped with a 50 W mercury arc lamp for luminescence excitation. For luminescence lifetime imaging a blue ultrabright light emitting diode (Luxeon, 470 nm, 5 W) was used as triggered light source. A monochrome CCD camera (Sensimod, PCO, Kehlheim, Germany) and a color CCD camera (AVT Marlin F-201C) were connected to the microscope.

3. Results and Discussion

3.1. Characterisation of sensor layers

Sensor materials were optimized for the respective detection system. For lifetime imaging sensor films containing 4% (w/w) MY, 0.5% (w/w) PtTFPP and 30% (w/w) TiO_2 coated from a 2% (w/w) PS-solution (approximately 1µm film thickness) showed best results. Ratiometric imaging using the color channels of a CCD color camera was carried out using sensor layers with 1% (w/w) MY and 0.5% (w/w) PtTFPP coated from a 2% (w/w) PS solution. Figure 2 shows the calibration plots of the sensor layers. Both Stern-Volmer plots show a slightly nonlinear developing and were fitted using the simplified two-side model [8].

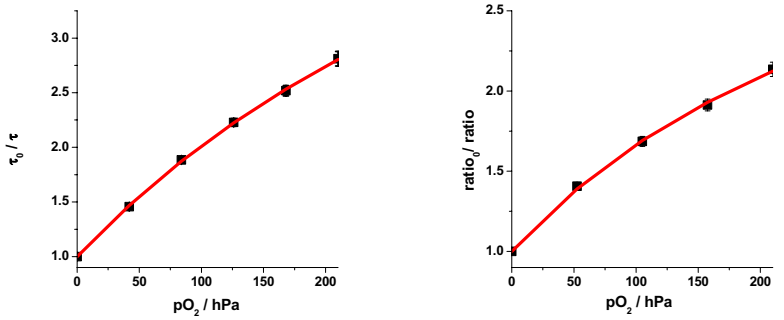

Fig. 2: Calibration plots of (left) lifetime imaging and (right) ratiometric imaging. The lower ascending slope of the ratiometric calibration plot is due to an overlap of green and red channel of the color CCD camera.

3.2. Application of optical sensors in microfluidic devices

The oxygen-sensitive sensor layers were integrated into microfluidic devices. Oxygen concentration inside the microfluidic channel was readout from the outside with the gateable monochrome camera as well as with the color camera. These microfluidic systems were inoculated with microorganisms and monitoring of cell respirometry was demonstrated. Figure 4 shows an overlay of transmitted light image and lifetime image of *Candida albicans* in a microfluidic chip with an integrated sensor layer.

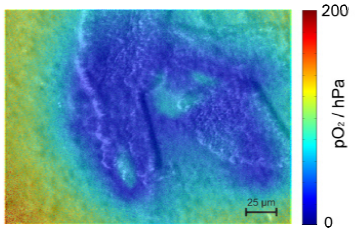

Fig. 3: Overlay of transmitted light image and image of partial pressure of oxygen in a microfluidic chip with an integrated sensor layer. The chip was inoculated with *Candida albicans*. Lowest oxygen levels were observed at high densities of microorganisms.

4. Conclusion and further outlook

The presented sensor films using the concept of light harvesting were integrated into microfluidic devices. Highly homogeneous lifetime and ratiometric images of these sensors were obtained using the illustrated referencing schemes. Additionally, laterally varying oxygen concentrations were observed when monitoring cell respirometry of model cell cultures in microfluidic systems.

For the future our research may focus on applying other sensor designs in microfluidic chips. It is possible to transfer the presented sensing principle to nanosensor particles and simply add them to the medium. Moreover we want to investigate the integration of sensors for pH, carbon dioxide or glucose into microfluidic devices.

Acknowledgements

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