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Fluorimetric oxygen sensor for *in vitro* cell models

H. Välimäki[a]*, J. Kreutzer[a], J. Verho[a], K. Tappura[b], J. Lekkala[a]

[a]*Tampere University of Technology, Korkeakoulunkatu 3, FI-33720 Tampere, Finland*
[b]*VTT Technical Research Centre of Finland Ltd, Tekniikankatu 1, FI-33720 Tampere, Finland*

Abstract

A phase fluorimetric sensor targeted for the monitoring of dissolved oxygen concentration in microfluidic *in vitro* cell models is presented. The sensing surface of the sensor consists of oxygen sensitive fluorescent dyes (PtOEPK) embedded in a thin polystyrene film. The simulated fluorescence emission characteristics show highly anisotropic distribution, and an efficient optical read-out based on a parabolic lens is presented. Experimental results show that the applied sensing scheme allows one to use thin films (< 500 nm), dilute dye-polymer ratios (0.025%), low power LED excitation (< 1 mW), a simple phase locked photodiode read-out and yet achieve over 40 dB signal-to-noise ratio at 10 Hz data rate at physiologically relevant oxygen concentrations. These features are important in *in vitro* cell studies, as the potential cytotoxicity of the dyes and the sensing method (i.e. production of singlet oxygen) are reduced with low dye content and excitation power. In addition, thin and dilute polystyrene films are highly transparent and facilitate optical microscopy.

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Keywords: oxygen sensor; cell models; fluorimetric sensors; PtOEPK; thin polystyrene films

1. Introduction

Accurate control of the oxygen tension is important in *in vitro* cell culturing and disease models. Ideally, one should be able to maintain a stable oxygen tension, similar to the one in the corresponding tissue *in vivo,* and generate timely changes in order to enhance differentiation or trigger other responses.

Monitoring the oxygen tension in microfluidic cell cultures is a challenging task. Volumes can be very low, the number of other instrumentation high, and the biocompatibility requirements extremely strict. Ideally, the sensing surface should not only be non-toxic but rather an attractive breeding ground for the cells. In addition, the sensing surface should allow the use of standard optical microscopy. These facts rule out many commercial sensors and invoke tailored, highly integrated designs.

Polystyrene (PS) possesses many desired properties for the oxygen sensing surface in cell cultures. It has good optical and mechanical properties, and is a widely used cell culture dish material. In addition, it is relatively gas

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permeable, and mixed when with a porphyrin dye like platinum(II) octaethylporphyrinketone (PtOEPK), very suitable for fluorimetric oxygen sensing at physiological oxygen ranges [1]. To facilitate optical microscopy and enhance the biocompatibility, thin sensing films with low dye concentration and low excitation power should be applied. These facts underline the need for an efficient fluorescence detection scheme, which can be complicated as fluorescent molecules close to a glass-water-interface typically possess a highly anisotropic emission distribution [2].

2. Optical read-out

In order to develop an efficient detection scheme for fluorimetric oxygen sensing, we calculated the angular emission irradiance distributions of fluorescent PS films of various thicknesses. To simulate the emission distribution of a film with thickness *d*, doped homogenously with fluorescent molecules, we followed the modeling methodology presented in [3,4,5] and calculated firstly the separate contributions of vertically and horizontally oriented electric dipoles placed at definite distances $z \in [0,d]$ from the polystyrene-glass interface and then summed up the contributions. Here, a random dipole orientation was assumed, so that the horizontal orientation was weighted by two (two degrees of freedom). Fig. 1a shows the calculated results for five homogenously doped films with thicknesses between 100 nm and 500 nm. The curves show that the actual distribution depends on films thickness, but in all cases the main part is towards the glass, and the peak irradiance is found above the critical angle between glass and water (dashed line). Fig. 1b shows the simulated relative irradiation (i.e. scaled by the total power) into glass space (blue), the contribution of trapped light i.e. the part of the emission that cannot be detected with non-contact optics (red), and the amount that a non-contact lens with numerical aperture of NA = 0.45, placed underneath the glass plate, can maximally collect (green). It is instructive to realize that with all the simulated film thickness values, only about 1/7 of the glass-space radiation (or about 1/10 of the total power) can reach the detector when such a typical sensing scheme is applied.

An efficient way to collect the trapped light is to apply a parabolic lens in optical contact with the substrate [3, 6]. We applied here the arrangement shown Fig. 2a, where the parabolic lens is truncated so that the focal point of the lens is at the glass-water interface, which results to a collimated output from the fluorescent molecules close to the focal point. The lens with focal length of f = 2.78 mm and upper and lower diameters of 13.00 and 24.90 mm, respectively, can collect the emission radiated into angels between 48.1° and 81.2 °. As Fig. 2b shows, this range fits especially nicely with film thicknesses of 100 nm, 400 nm and 500 nm. Note that the same lens is also utilized for the total internal reflection excitation, which both focuses the excitation to the focal point and significantly reduces the power radiated into the cell chamber.

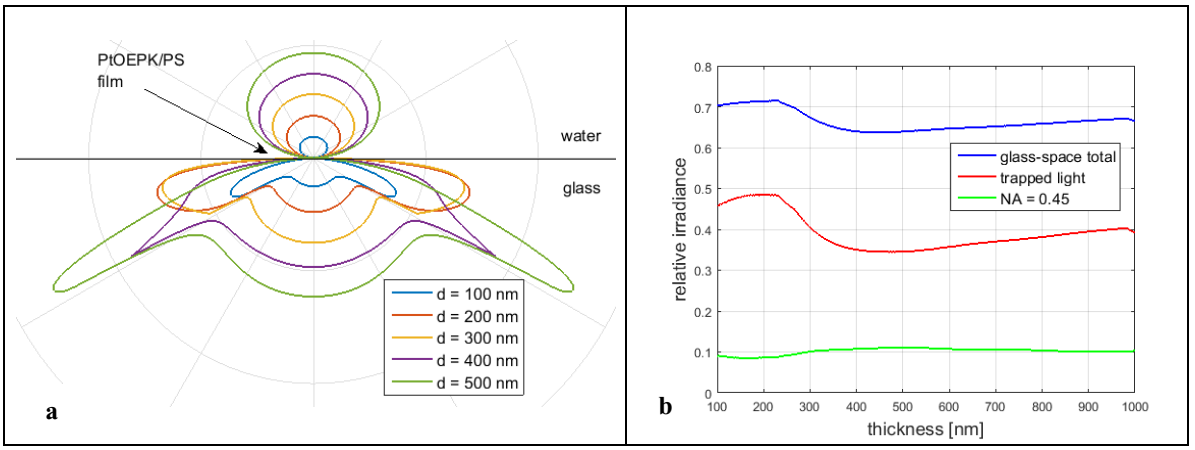

Fig. 1. (a) The angular emission distribution of polystyrene films with varying thickness *d*, doped with fluorescent dyes emitting at 750 nm; (b) the relative irradiance towards glass-space (blue), the amount of trapped light (red) and the collection efficiency of a lens with NA = 0.45 (green).

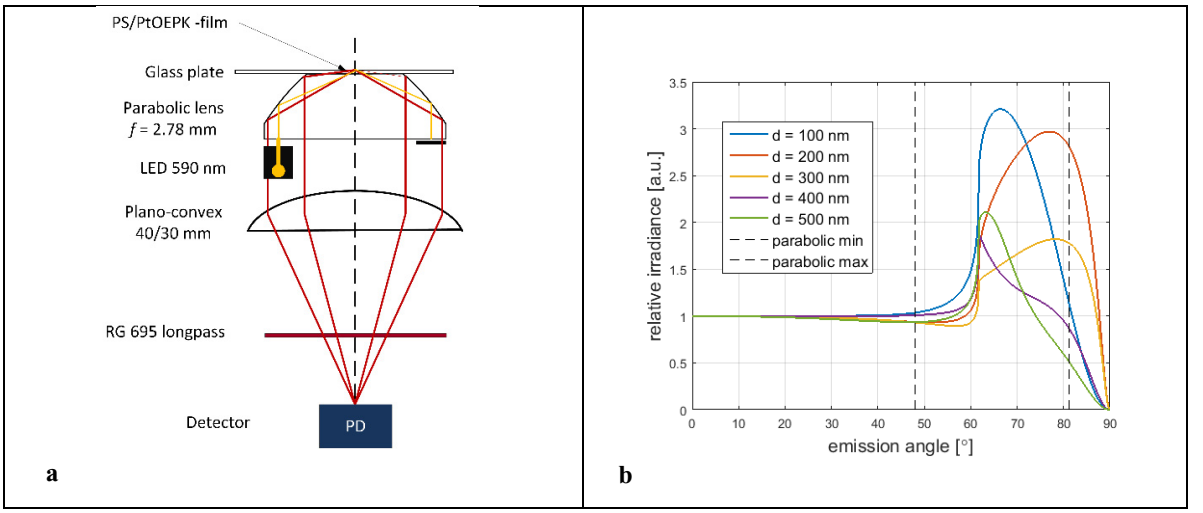

Fig. 2. (a) The optical set-up based on a truncated parabolic lens; (b) The relative emission irradiance (scaled by power emitted directly downward) vs emission angle

When it comes to the fluorimetric oxygen sensing, fluorescent life-time based methods – being insensitive to variations in excitation power, dye concentration, photo bleaching and other gain-type factors – are preferable to direct irradiance-based methods [7]. By modulating the excitation amplitude and measuring the phase between the excitation and emission signals one can make a life-time based oxygen sensing scheme that is well described by the Stern-Volmer equation [1]

$$\frac{\phi_0}{\phi} = 1 + K_{sv} pO_2 \tag{1}$$

where ϕ_0 and ϕ are the measured phase values in the absence and presence of oxygen, respectively; K_{sv} is Stern-Volmer constant and pO_2 is the oxygen partial pressure.

3. Materials and methods

Polystyrene pellets (m_w = 200 000, from Sigma) were dissolved in toluene (4 % w/w), and platinum(II) octaethylporphyrinketone (PtOEPK, from Frontier Scientific) were added to solution in variable proportions to PS (from 0.025 % to 0.4 % w/w). Glass plates (49 x 49 x1 mm) were cleaned, and PS/PtOEPK films were fabricated by spin-coating at 3000 rpm for 60 s under clean room conditions. This resulted in films with the average thickness of 480 nm (measured with Bruker Dektak XT contact profilometer).

The lenses, filters and LED (LED591E, 590 nm, 2 mW) needed in the set-up shown in Fig. 2a were from Thorlabs. The parabolic lens was manufactured by Jenoptik and made of polystyrene, which caused some additional aberrations at the glass plate/polystyrene lens interface that are neglected in the analysis. Optical contact between the glass plate and the parabolic lens was assured with immersion oil (n = 1.51).

The oxygen-induced changes in the fluorescence lifetime were measured by a quadrature synchronous detection with a tailored hardware. The excitation LED was driven using 2kHz square wave current, and the fluorescent emission was detected with a photodiode (S1226-18BQ). The synchronous detector had a bandwidth of 23 Hz and

its I/Q outputs were sampled to produce phase angle measurements at a data rate of 10 Hz.

4. Results and discussion

The sensor performance with three PtOEPK/PS films having the same average thickness of d ~ 480 nm but different dye/polymer ratios, was investigated. A self-made gas chamber was filled with a gas containing a variable concentration of O_2 at room temperature and atmospheric pressure, and the sensor phase responses were recorded. Fig. 3 shows the corresponding Stern-Volmer plots, generated according to equation (1), for three different PtOEPK/PS ratios. At the physiologically interesting oxygen concentrations, the sensor possesses linear Stern-Volmer characteristics, although a minor deviation can be seen with the lowest PtOEPK/PS at high oxygen concentrations. Sensor sensitivities as well as noise characteristics are summarized in table 1.

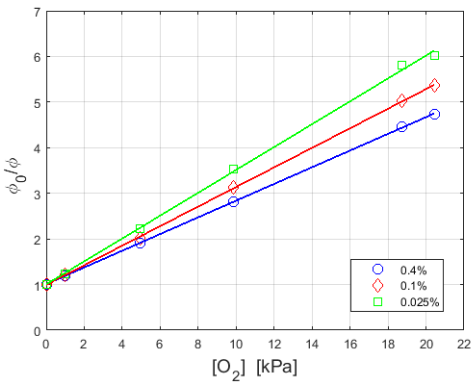

Fig. 3. Stern-Volmer plots of with three different PtOEPK/polystyrene ratios.

Table 1. Oxygen sensor characteristics with different PtOEPK/polystyrene -ratios.

PtOEPK/PS [%]	K_{SV} [kPa^{-1}]	Sensitivity [°/kPa] (at pO_2 = 5.0 kPa)	Phase noise [° rms] (at pO_2 = 5.0 kPa at 10 Hz data rate)	pO_2 noise [kPa rms] (at pO_2 = 5.0 kPa at 10 Hz data rate)
0.4	0.181	-1.91	0.0076	0.0039
0.1	0.211	-1.87	0.019	0.010
0.025	0.247	-1.68	0.050	0.030

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