

Oxygen Sensing using Microrobots

Olgaç Ergeneman¹, George Chatzipirpiridis¹, Femke B. Gelderblom¹, Juho Pokki¹, Salvador Pané¹, Marta Marín Suárez del Toro^{1,2}, Jorge F. Fernández Sánchez², Georgios A. Sotiriou³, and B. J. Nelson¹

Abstract—We present a luminescence oxygen sensor incorporated in a wireless intraocular microrobot for minimally-invasive diagnosis. This microrobot can be accurately controlled in the intraocular cavity by applying magnetic fields. The microrobot consists of a magnetic body susceptible to magnetic fields and a sensor coating. This coating embodies Pt(II) octaethylporphine (PtOEP) dyes as the luminescence material and polystyrene as a supporting matrix, and it can be wirelessly excited and read out by optical means. The sensor works based on quenching of luminescence in the presence of oxygen. The excitation and emission spectrum, response time, and oxygen sensitivity of the sensor were characterized using a spectrometer. A custom device was designed and built to use this sensor for intraocular measurements with the microrobot. Due to the intrinsic nature of luminescence lifetimes, a frequency-domain lifetime measurement approach was employed. An alternative sensor implementation using poly(styrene-co-maleic anhydride) (PS-MA) and PtOEP was successfully demonstrated with nanospheres to increase sensor performance.

I. INTRODUCTION

To perform its fundamental function, the eye needs a sufficient supply of oxygen and nutrients. Inadequate oxygen supply (i.e., retinal hypoxia) is correlated with major eye diseases including diabetic retinopathy, glaucoma, retinopathy of prematurity, age-related macular degeneration, and retinal vein occlusions [1]. The influence of oxygen on these diseases is not well understood and in vivo oxygen measurements are essential for better diagnosis and treatment. Measuring the oxygen tensions both in aqueous humor and vitreous humor, and particularly in the preretinal area, is of great interest in ophthalmological research.

In [2], the concept and prototype of a minimally-invasive wireless optical sensor device to measure intraocular dissolved oxygen concentration was presented. The proposed device consists of a luminescence sensor film that is integrated on a magnetically controlled ferromagnetic sphere with a diameter of 3.25 mm. The device can be inserted through a small incision in the sclera. Closed-loop position control within the vitreous humor can then be accomplished via applied magnetic fields and visual tracking through the pupil. The precise magnetic control of wireless microrobots

¹O. Ergeneman, G. Chatzipirpiridis, F. Gelderblom, J. Pokki, Marta Marín Suárez Del Toro, S. Pané, and B. J. Nelson are with Multiscale Robotics Laboratory, Institute of Robotics and Intelligent Systems, ETH Zurich, 8092, Zurich, Switzerland vidalp@ethz.ch

²M. Marín Suárez del Toro and J. F. Fernández Sánchez are with the Department of Analytical Chemistry, University of Granada, E-18071, Granada, Spain jffernan@ugr.es

³G. Sotiriou and S. E. Pratsinis are with Particle Technology Laboratory, ETH Zurich, 8092, Zurich, Switzerland

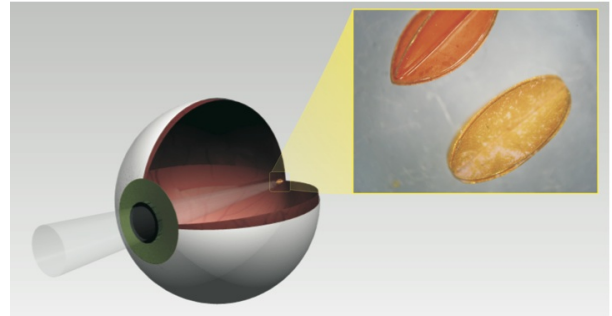


Fig. 1. The oxygen sensing microrobots can be used in the posterior segment of the eye. The inset photo shows a microrobot coated with the PS film with PtOEP dyes (top) and a microrobot with PS-ME nanospheres with PtOEP (bottom). Both types of microrobots were first coated with gold. The length of the microrobots is 2 mm.

was demonstrated in [3], and in [4] localization of intraocular microdevices using a single camera was presented.

In this work, the sensors presented in [2] were further miniturized and oxygen sensing using microrobots was demonstrated. A microrobot which is coated with a luminescence sensing film was analyzed and a setup that can be used in the magnetic control system presented in [3] was prepared. Pt(II) octaethylporphine (PtOEP) dyes were used in the sensor due to their long lifetime and visible excitation and emission spectra. Polystyrene (PS) was chosen for the supporting matrix as it is transparent in the visible spectrum and highly permeable to dissolved oxygen. Magnetic microrobots were first coated with gold by electroless plating for biocompatibility and then dip-coated with the oxygen sensitive luminescence film. The excitation and emission properties were characterized as well as the sensing kinetics and oxygen sensitivity of the coated microrobots. Furthermore, poly(styrene-co-maleic anhydride) (PS-MA) nanospheres containing PtOEP were synthesized and used in sensors. Instead of applying a thin coating of PS with the PtOEP, nanospheres were deposited on the surface. This method is demonstrated on gold-coated chips and was compared to sensors based on dip-coated films. This method is also applied to coat microrobots. A microrobot coated with the luminescence film and a microrobot coated with luminescence nanospheres are shown in Fig. 1

II. LUMINESCENCE OXYGEN SENSOR

Photoluminescence is the emission of photons from a material in response to absorption of photons. The intensity and the lifetime of emission can be decreased by a variety of processes referred to as luminescence quenching. Optical

luminescence oxygen sensors work based on quenching of luminescence in the presence of oxygen, which acts as the quencher; the decrease in luminescence is related to the concentration of oxygen. A number of devices using this principle have been demonstrated and the basic principles of different methods can be found in [5]. In [6], novel luminescence oxygen sensing films were evaluated. A variety of oxygen sensors have been developed, e.g. Clark electrode sensors. Luminescence based oxygen sensors are attractive, because they provide wireless readout, fast response, high accuracy, and they do not consume oxygen. They also do not require reference electrodes or stirring, and they can be used as disposable sensors. These sensors do not interfere with magnetic fields which is crucial for a magnetic microrobotic system.

Luminescence sensing can be performed in different ways. A lifetime measurement approach was chosen for this work, since lifetime is an intrinsic property. The lifetime of emission decreases in the presence of oxygen as a result of the quenching process. Hence, oxygen concentration can be obtained from the lifetime of emission. There are two methods that are used for measuring luminescence lifetimes: time-domain measurements and frequency-domain measurements [5]. In time-domain measurements, the sample is excited with light pulses, and the intensity signal that changes as a function of time is measured and analyzed. The time in which the intensity decays to e^{-1} (36.8%) of the initial value is the lifetime. In frequency-domain measurements the sample is excited with a periodic signal that consequently causes a modulated luminescence emission at the identical frequency. Because of the lifetime of emission, the emission signal has a phase shift (i.e., time delay) with respect to the excitation signal. The input excitation signal is used as a reference to establish a zero-phase position and the lifetime is obtained by measuring the phase shift between the excitation signal and the emission signal. The intensity of light collected by the photodetector is extremely sensitive to extrinsic conditions, which are difficult to control in such a wireless sensor application. While the sensor is steered in the ocular cavity, the optical path distance from the light source to the sensor and back to the photo detector changes. The total amount of light collected by the sensor changes depending on the orientation and location of the sensor as well. Furthermore, there are other parameters that affect the photodetector output such as dye concentration, optical surface quality, fluctuations of the excitation source, photo-bleaching, and incidence angle. However, these changes in measurement conditions do not affect the lifetime of emission. Owing to their intrinsic nature, the time-domain lifetime measurement approach is used to characterize with the spectrometer, and frequency-domain lifetime measurement approach is used in the proposed system.

III. EXPERIMENTAL

A. Preparation of the Film Sensor

Microrobots were made of various magnetic materials. In Fig. 1 two assembled CoNi microrobots are shown. For

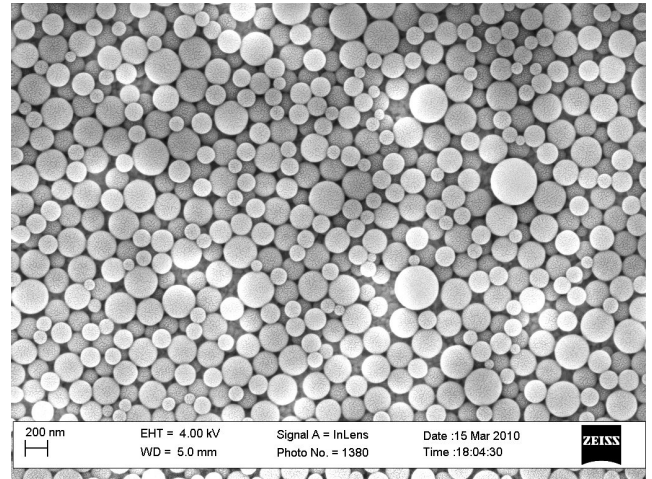


Fig. 2. SEM image of nanospheres of PS-MA containing PtOEP deposited on a gold layer.

biocompatibility and surface functionalization they are first coated with a thin layer of gold by electroless deposition. The PtOEP was purchased from Frontier Scientific, UT, USA. To prepare the luminescence film, 3 mg of PtOEP and 197 mg of PS were dissolved in 2 ml of chloroform by stirring. The microrobots are dip-coated and stored 2 hours, allowing evaporation of chloroform. Gold coated silicon chips with 10 mm² were also spin-coated with the prepared solution for characterization.

B. Preparation of the Nanospheres Sensor

The porphyrine-functionalized poly(styrene-co-maleic anhydride) (PS-MA) nanospheres were prepared with a method reported in [7]. PS-MA and PtOEP dye were dissolved in tetrahydrofuran (THF). The resulting solution was added dropwise into water under vigorous agitation and a dispersion was formed. Nitrogen was bubbled in order to accelerate the evaporation of THF. This forms an aqueous suspension of PS-MA nanospheres with PtOEP. The microrobot or gold-coated chips were first cleaned by immersing in acetone, IPA and piranha solution, consecutively, 10 minutes each. To increase the wettability of the gold substrate, they were kept in a hydrophilic thiol solution (MPS) at 50°C for one hour [8]. Lastly the suspension of nanospheres was pipetted onto microrobots or chips. The nanospheres spread over the entire surface producing a uniform coating of nanospheres. Fig. 2 shows the SEM image of the nanospheres on the gold surface. The nanospheres vary in size, but almost all of them are smaller than 500 nm. This provides a sensor with a high surface area, which is generally desirable for fast response times and higher sensitivity.

C. Characterization Setup

A Cary Eclipse fluorescence spectrophotometer (Varian Inc., CA, USA) was used to characterize the luminescence of the developed sensors. Excitation scan, emission scan, kinetics, and lifetime measurements were performed using this equipment. A custom flow cell which is compatible

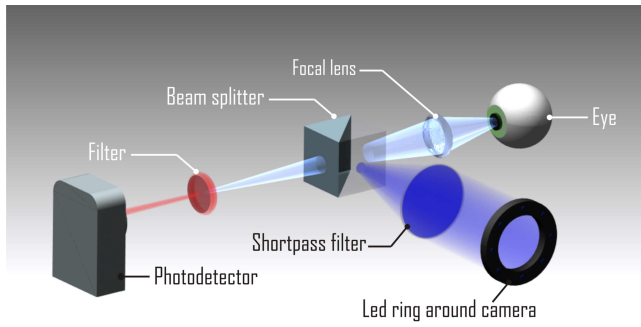


Fig. 3. Setup for detecting oxygen

with the spectrophotometer was built and used in all experiments. Oxygen and nitrogen was mixed at different ratios using two gas flow controllers (Bronkhorst High-Tech B.V., Netherlands) and applied to the cell. A total gas flow of 500 ml/min was maintained in all gas measurements. Before the experiments, the flow controllers were calibrated using a flow sensor.

The dissolved oxygen (DO) measurements were also performed using the same chamber circulating water instead of gas. The oxygen concentration in water is changed by bubbling nitrogen or oxygen in a container. A commercial DO sensor (Oxi 340i, WTW GmbH) was used to monitor the DO concentration in a second chamber in order to avoid possible interference by gas bubbles. The water circulated using a pump and the fluid flow rate was kept constant.

D. Intraocular Sensing Setup

A custom setup was designed and built for wireless oxygen concentration measurements considering the anatomy of the eye and the control system described in [3]. A UV LED and a shortpass filter were used as the excitation source, and a Si photodetector (PD-100A, Thorlabs GmbH) with a longpass filter were used for the read out. Using a beamsplitter (Edmund Optics) two separate optical paths were generated: one for the detecting system and the other one for the excitation system and tracking camera. An indirect non-contact ophthalmoscopy lens is needed in front of the eye for localization and tracking of the microrobot in a real scenario, and hence was placed at the end of the setup. Fig. 3 shows the illustration of the measurement setup. A lock-in amplifier (HF2LI, Zurich Instruments, Switzerland) was used for the readout. Its internal signal generator modulated the excitation circuit of the led and acted as the reference signal for the detection of the photodetector signal. A bandwidth of 48 mHz was used for the detection of phase change as a function of oxygen concentration. By this method, effective noise cancellation was obtained.

IV. RESULTS

A. Sensor Film Characterization

First, the excitation and emission characteristics were obtained for the PS sensor film containing PtOEP. The peak

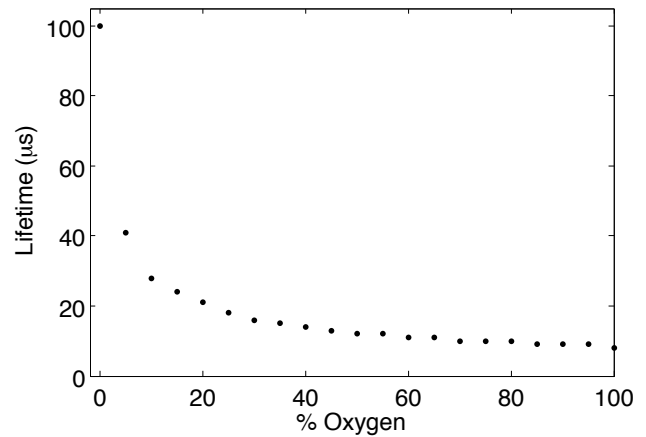


Fig. 4. The lifetime of emission of a coated microrobot in response to different ratios of oxygen to nitrogen. The total flow rate was 500 ml/min.

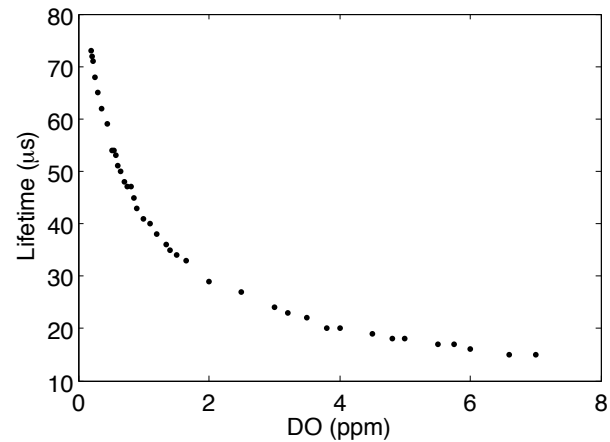


Fig. 5. The lifetime of emission of the coated microrobot at different dissolved oxygen concentrations in water. The oxygen concentration was obtained by the commercial oxygen sensor.

emission wavelength was found to be 645 nm. Excitation wavelengths between 300 nm to 400 nm produced high emission intensity. Next, using the flow cell described, the oxygen sensitivity of the sensor was measured in gas and in water. Fig. 4 shows the lifetime of emission of a coated microrobot in response to different ratios of oxygen to nitrogen under a constant flow of 500 ml/min. In Fig. 5, lifetime of emission of the same microrobot is shown in water with a flow rate of 3.15 l/min at different dissolved oxygen concentrations observed by the commercial oxygen sensor. An unquenched emission lifetime of 100 μ s was observed. A Stern-Volmer constant K_{sv} of 24.14 bar^{-1} was found in gas and 17.75 bar^{-1} in water using Lehrer's model. Last, the response time of the sensor was obtained going from 100 % nitrogen gas to 100 % oxygen gas and back to 100 % nitrogen gas. The fall time of the sensor was determined to be approximately 30 seconds and the rise time approximately three minutes, as seen in Fig. 6(a).

B. Measurements with Nanospheres

The emission and excitation characteristics for the PS nanospheres containing PtOEP were very similar to the PS

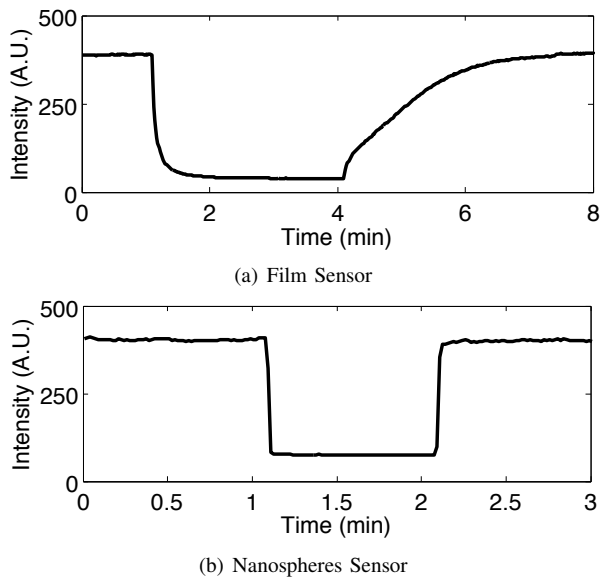


Fig. 6. Response time of the film sensor and the nanospheres sensor. The high intensity state is 100% nitrogen and the low intensity state is 100% oxygen flow.

film containing PtOEP. The main difference was observed for the response time. Both the rise and fall time were found to be less than three seconds (Fig. 6(b)). This is most probably due to the increased surface area caused by the nanospheres.

C. Measurements using Lock-in Amplifier

Lastly, the film coated microrobot sensor was used with the custom-built setup. The same flow chamber was used with this setup. To mimic the optical properties of the eye, another lens was placed after the ophthalmoscopy lens in front of the flow chamber. Using the commercial sensor the oxygen concentration was observed and the phase change was acquired from the lock-in amplifier. Fig. 7 shows the response of the sensor in the custom-built ophthalmic setup under different dissolved oxygen concentrations. A curve similar to the the curve obtained from the spectrophotometer was obtained, indicating that DO concentration can be measured with the custom setup and the microrobot.

V. CONCLUSIONS AND FUTURE WORK

A wireless micro oxygen sensor was developed using a magnetic microrobot and PS film with PtOEP dye. A custom setup to excite and readout this sensor was designed and implemented. Oxygen sensing using this microrobot and setup was demonstrated. This sensor can be precisely controlled in the ocular cavity by applying magnetic fields as described in [3]. Minimally-invasive oxygen concentration measurements can be performed using these systems. Future work will focus on using the readout system together with the control and tracking systems.

An alternative sensor coating using nanospheres of PS-MA containing PtOEP was also demonstrated and improvements were obtained in response time due to the increased surface area. Microrobots were successfully coated with these nanospheres. In the future, oxygen sensitivity of the

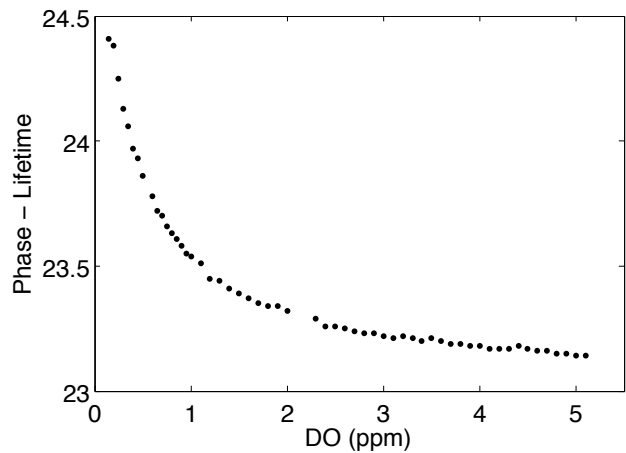


Fig. 7. The response of the microrobot sensor under different oxygen concentrations using the custom ophthalmic setup with the lock-in detection.

nanospheres will be explored and compared to the film sensors. The nanospheres will be further characterized (i.e., size distribution) and their effect on the sensor performance will be explored.

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