Optical sensor based on fluorescent quenching and pulsed blue LED excitation for long-term monitoring of dissolved oxygen in NASA space bioreactors

Frank G. Gao
Wyle Life Sciences
1290 Hercules Drive
Suite 120, Mail Stop BT-37
Houston, Texas 77058

James M. Fay
Texas Tech Medical School
Lubbock, Texas 79409

Grace Mathew
Wyle Life Sciences
1290 Hercules Drive
Suite 120, Mail Stop BT-37
Houston, Texas 77058

Antony S. Jeevarajan
Melody M. Anderson
National Aeronautic and Space Administration
Lyndon B. Johnson Space Center
Cellular Biotechnology Program
2101 NASA Road 1
Houston, Texas 77058

Abstract. There is a need to monitor the concentration of dissolved oxygen (DO) present in the culture medium for NASA's space cell biology experiments, as well as in earth-based cell cultures. Continuous measurement of DO concentration in the cell culture medium in perfused bioreactors requires that the oxygen sensor provide adequate sensitivity and low toxicity to the cells, as well as maintain calibration over several weeks. Although there are a number of sensors for dissolved oxygen on the market and under development elsewhere, very few meet these stringent conditions. An in-house optical oxygen sensor (HOXY) based on dynamic fluorescent quenching of Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride and a pulsed blue LED light source was developed in our laboratory to address these requirements. The sensing element consisted of the fluorescent dye embedded in a silicone matrix and coated onto a glass capillary. Photobleaching was minimized by a pulsed LED light source. The total noise in the sensor output is 2% and the sensor dynamic range is 0 to 200 mm Hg. The resolution of the sensor is 0.1 mm Hg at 50 mm Hg, and 0.25 mm Hg at 130 mm Hg, while the accuracy is 5%. The LED-based oxygen sensor exhibited stable performance and low drift, making it compatible for space-flight bioreactor systems.
 [DOI: 10.1117/1.2062427]

Keywords: sensors; fluorescence; cells; biology; photodiodes; optical devices.

Paper 05046R received Feb. 18, 2005; revised manuscript received May 4, 2005; accepted for publication May 18, 2005; published online Oct. 4, 2005.

1 Introduction

For research conducted in microgravity conditions aboard the Space Shuttle or International Space Station, it is desirable to maintain an optimal cell culture environment to fully utilize the allotted experiment duration. One of the critical parameters during cell culture is the dissolved oxygen (DO) concentration in the culture medium. While point measurements (manual sampling and measurements) are routine in both ground-based labs and space flight, continuous, noninvasive measurement of DO is desirable. For long-term, continuous oxygen measurements, a sensor must operate under sterile conditions, maintain calibration for weeks or months, and should not interfere with the cell culture (toxicity, etc.).

To date, the majority of oxygen sensors are based on electrochemical Clark-type[1] electrodes. Amperometric oxygen sensors can suffer from signal drift over time, which can compromise the validity of long-term measurements. Fluorescent sensors do not typically have such limitations.[2] The first oxygen sensors based on fluorescence quenching were introduced by Bergman in 1968[2] and Lubbers and Opitz in 1975.[3] Over the years numerous reports have demonstrated the use of oxygen sensing in biology and medicine.[4–12]

Luminescent molecules, typically based on Ru (II) complexes, are used to probe oxygen concentration. These dyes are characterized by relatively high quantum yields and long luminescence lifetimes, with fluorescence originating from the metal-ligand charge transfer band to the ground state. Gas permeable matrices such as silicone are employed to immobilize the dye and prevent it from entering the culture medium or human subject. The blue-excited red fluorescence is quenched by oxygen, making these molecules reversible, nondestructive reporters for oxygen.

The oxygen quenching is predicted by the Stern-Volmer equation, and changes in intensity or lifetime of the fluorescence can be monitored. Generally, the oxygen optical sensor has a sensing range of 0 to 1000 mm Hg, a sensitivity close to or larger than 0.1 mm Hg, a rapid response time from 1 to 10 s, a probe temperature range of −80 to 110 °C, and a probe lifetime of about 1 yr.

Most optical oxygen sensors reported in the literature used optical fibers and blue LEDs to excite and collect fluorescence.[13–23] Glass capillary[24–26] and radioluminescent (RL) light sources[25–28] have also been used. The RL light source consists of ^{147}Pm (a β-emitting radionuclide) and ZnS:Ag phosphor, producing 450-nm excitation light. The

Address all correspondence to Antony S. Jeevarajan, Tel: 281-483-4298; Fax: 281-483-2888; E-mail: antony.s.jeevarajan@nasa.gov

1083-3668/2005/10(5)/054005/6/$22.00 © 2005 SPIE

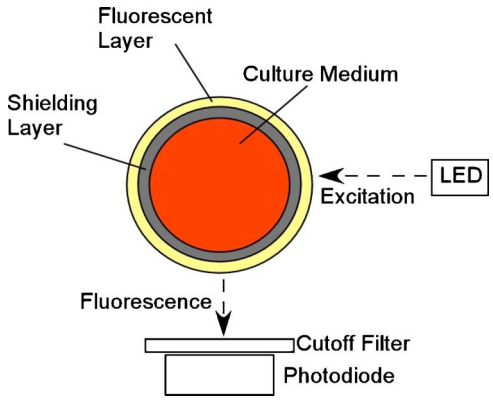


Fig. 1 End-on schematic view of the optical oxygen sensor. The LED fully illuminates the capillary tube; fluorescence is detected at 90 deg using a photodiode.

main advantage for a RL source oxygen sensor is its compact size and no photobleaching of the dye (due to very low intensity of the light source). However, its application is limited due to the use of a β particle source.[27]

The numerous sensor systems available commercially and in academic laboratories do not currently meet the strict requirements for space flight. Commercial sensor systems were evaluated in our laboratory and exhibited photodegradation and toxicity to the cell cultures. Namely, a fiber-optic based DO sensor, an electrochemical sensor, and a radioluminescent-source DO sensor were all tested. Our main criteria for sensor evaluation were toxicity to the cell culture and long-term stability. All of the sensors tested were biocompatible; however, none of the sensors had a stable output for longer than a week. We believe that dye photobleaching is the primary cause of bleaching in the fiber-optic optical sensor, while fouling of the electrode may be primarily responsible for the degradation of the electrochemical device. The RL source was expected to have a stable, low-intensity output for continuous monitoring and was the first candidate chosen for cell run testing. The results of that cell run proved the sensor to be unstable over long periods of time. The cause of this instability is unknown. In order to achieve continuous monitoring of dissolved oxygen in NASA bioreactors, we developed an in-house optical oxygen sensor based on fluorescence quenching, using a pulsed blue LED source. The pulsed source minimizes photobleaching, a necessity for long-term ($>$30 days) cell culture experiments. In our preliminary experiments an oxygen sensor was tested continuously in phosphate-buffered saline (PBS) for 15 days and in air/nitrogen for 20 days, showing neither significant fluorescent dye bleaching nor leaching of the dye. The sensors were also tested in a perfused rotary bioreactor supporting a BHK-21 (baby hamster kidney) cell culture over one 28-day, one 43-day, and one 180-day cell runs.[29]

2 Experimental Section

2.1 *Sensor Configuration*

Figure 1 shows the schematic view of the development HOXY optical oxygen sensor. The highly luminescent molecule tris(4,7-diphenyl-1,10-phenanthroline)-ruthenium(II)Cl_2, represented as Ru(dpp)$_3Cl_2$, is employed as the probe dye. It has a relatively high quantum yield of luminescence (0.30, dimensionless) and long luminescence lifetime (natural lifetime=5.34 μs). The lifetime of the dye in the sensor element was not measured. The dye is encapsulated in a gas permeable, but ion impermeable silicone layer, and incorporated to the optical probe (a glass capillary) to form the sensing element. A blue LED centered at 470 nm is used as the excitation light source. Upon excitation, Ru(dpp)$_3Cl_2$ emits at 615 nm. In the presence of oxygen, the fluorescence is reversibly and quantitatively quenched. A photodiode, which is covered with a sheet of 575-nm long-pass optical filter, serves as the fluorescence detector (Fig. 1). There is significant emission from the LED at the detection wavelengths, but the offset is expected to be linear with LED output and was subtracted out during the calibration process. Additional details are provided in Ref. 29.

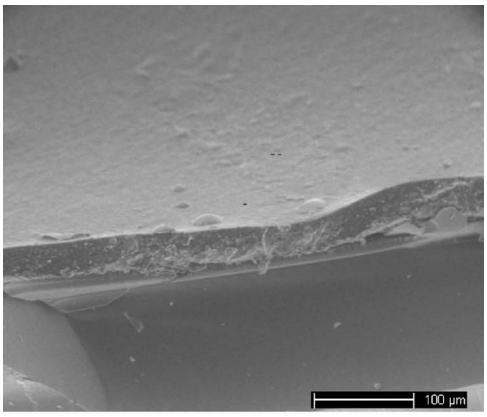
2.2 *Chemicals and Sensing Element*

Commercially available Ru(dpp)$_3Cl_2$ (GFS Chemicals) was recrystallized once in ethyl alcohol (Aldrich, HPLC grade) and dried at 95°C. Milli-Q (18 MΩ) purified water was used for all purposes. To prepare the sensing element, 3.8 mg Ru(dpp)$_3Cl_2$, 4.0 g Superflex Clear RTV Silicone Adhesive Sealant (Loctite, distributed by Lab Safety Supply, Inc.), 0.48 g TiO_2 (Aldrich), and 9 mL dichloromethane (Aldrich, 99.9%, HPLC grade) were weighed into a 20-mL precleaned amber glass vial, tightly closed, and mixed well with a shaker. The black shielding solution was prepared by mixing 0.42 g graphite (Aldrich), 4.2 g 3145 RTV silicone sealant (Dow Corning, MIL-A-46146), and 10 mL Toluene (GFS Chemicals, 99.7%) in a 20-mL precleaned amber glass vial. The vial was tightly closed and mixed well with a shaker. A transparent glass capillary tube (3 mm OD, 1.6 mm ID) was cut into 2.6-cm-length pieces. They were first cleaned with Alconox detergent solution by boiling for 15 min, and rinsed with water. The glass capillary tubes were then further cleaned by sonicating them at 80°C in 80:20 (w/w) H_2O/ethanolamine (Aldrich, 99+%) for 15 min, rinsed with water for several times, and then dried at 95°C.

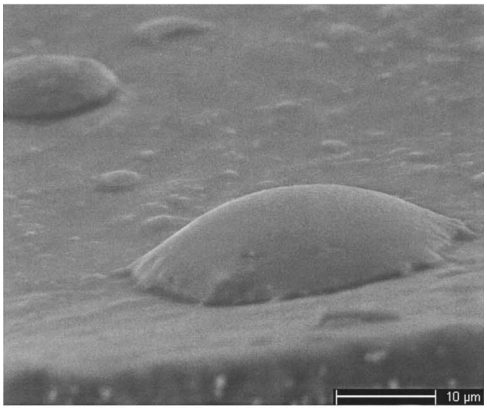
The sensing membrane was constructed inside the glass capillary tube by flowing dry air through the capillary for 5 min. The fluorescent dye solution was then pushed through the capillary tube using a syringe. Dry air was then forced through the capillary for 5 min to dry the solution and form a thin, uniform layer. Air was forced through the capillary using a small air pump. A special mounting jig was fashioned to hold the sensor vertical while air flowed downward through the capillary. The second layer of black shielding membrane was formed in a similar manner. The finished sensing elements were placed in a covered dish for 1.5 h to allow further solidification. The sensors were then boiled in water for 20 min to reinforce the sensor layer adhesion to the capillary. Sensing elements were stored away from light for 24 h to cure before use. The sensor was subsequently sterilized by autoclaving at 110°C for 15 min.

2.3 *Sensing Element Morphology*

In order to characterize the surface of the Ru(II) complex film in the capillary, scanning electron microscope (SEM) images



(a)



(b)

Fig. 2 (a) SEM image showing cross section of sensing film (scale bar is 100 μm). (b) Magnified image of sensing film and its surface features (scale bar 10 μm).

were obtained for the sensing element and shielding layers. Figure 2(a) shows the sensing element film coated into the glass capillary, without the shielding layer. The capillaries were mechanically fractured and placed into a SEM in environmental (wet) imaging mode. The imaging chamber pressure was approximately 1.5 Torr. The sensor film was measured to be 33.7 ± 0.4 μm, and is shown to be uniform over the field of view. Figure 2(b) shows a magnification of some of the dome-like features seen in Fig. 2(a). The identity of the features is not known, but the overall surface morphology of the film is smooth. SEM images of the black shielding layer were identical to the sensing element, and are not shown. Since the two layers are primarily composed of the same material (silicone), the film thickness of the shielding layer could not be accurately measured.

2.4 *Sensor Hardware and Electronic Circuit*

The entire sensor was built in a shielded aluminum box with a dimension of $2.3 \times 2.4 \times 2.5$ in. The box is divided into two separable parts—a base plate and top cover plate. The main electronic board, photodiode, LED supporter, and cable adaptor are housed in the base plate. The blue LED is secured to the base plate by two small bolts. The LED is placed perpendicular to the sensing element and at the same height (Fig. 1). Two rubber seals, one between the base plate and the top plate and another between the cover and the base plate, are used to prevent moisture from entering the sensor. Ambient light did not enter the sensor once the system was closed. The advantage of designing the sensing element plate separated from the base plate is that the sensor can be sterilized independently of the sensor electronics.

Circuits for the ±9-V dc power supply, signal amplifier, blue LED power supply, and LED pulse control are built into the main PCB board. A connector interfaces the main board and the photodiode board. The +5-V dc from a National Instruments data acquisition board (NIDAQ PCI 6052E) is converted to +12-V dc by a dc-dc converter (Pico5A12D); the ±12-V power is further stabilized by two voltage regulators (78L09 and 79L09) to achieve ±9-V dc, which supplied the power for the photodiode (with built-in preamplifier), amplifier, and blue LED (Lumex).

A Hamamatsu S5591 photodiode with built-in preamplifier is used for the light detector. A feedback resistor external to the photodiode case controls the gain for the photodiode. An external capacitor is used as a filter. All three components, photodiode, resistor, and capacitor, are placed in an independent board, and the ±9-V power source and signal output are connected to the main board. The amplification circuit uses an optional amplifier (AD548JN), with a 500-kΩ trim potentiometer for gain control. The voltage output is connected to a NI DAQ board for data acquisition. A blue LED is used as the excitation light source for the fluorescent sensing element. Its output (on/off) and the pulse width are controlled via an electronic switch (MAX323CPA) through a digital I/O channel by the software.

2.5 *Software*

For long-term DO monitoring using an optical oxygen sensor based on dynamic fluorescence quenching, bleaching of the fluorescent dye due to long-term continuous irradiation can be a problem. Therefore, software written in LabVIEW (National Instruments, version 6i, Austin, TX) was developed to run the HOXY sensor in pulse mode to solve that problem. The advantage of using the LED in pulsed mode is that the photodegradation that occurs during continuous operation is avoided. For example, if we plan to use the sensor to continuously measure DO for 30 days (that is 720 h, or 43,200 min), at a sampling rate of 1 sample/min, and the LED pulse width is 100 ms (meaning that each minute the blue LED will be turned on for only 100 ms), the total irradiation time in 30 days is only 4320 s, or 72 min. Bleaching of dye in 72 min is insignificant and will not influence the results.

The HOXY software combines the oxygen sensor control and bioreactor control, forming a virtual instrument (VI). The software has a simple graphical interface that allows control over the various experiment parameters, such as blue LED pulse/continuous mode, LED pulse width, sampling rate, ADC (analog/digital converter) scan rate, number of scans, manual infusion/auto infusion and its schedule, and calibration equation. Data for up to four different channels (sensors) can be simultaneously acquired and logged. This software has been successfully used to operate the HOXY oxygen sensor in long-term stability tests and continuous measurement of media DO in bioreactors supporting cell cultures lasting for 180 days. The major functions of the HOXY software are

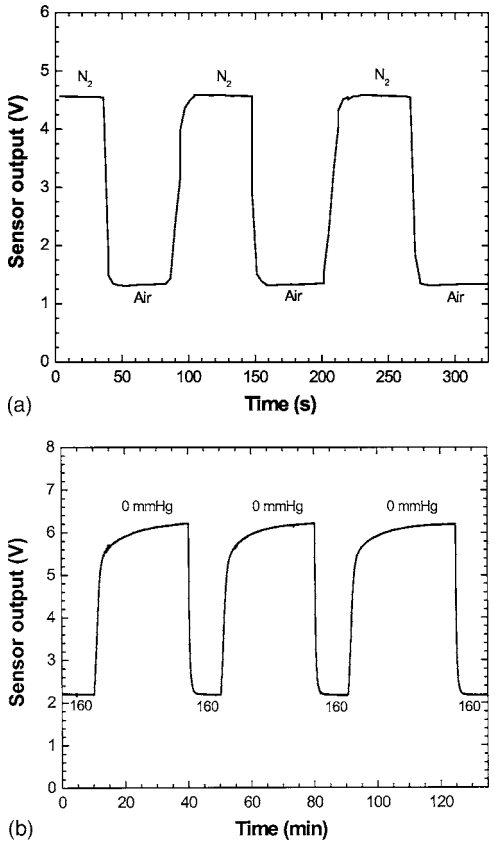


Fig. 3 (a) Oxygen sensor signal-time trace in nitrogen/air and (b) signal-time trace in PBS solution with the DO alternated between 0 to 160 mm Hg.

data collection and display, LED pulse control (frequency 0.017 Hz and pulse width 100 ms), conversion of photodiode voltage to oxygen concentration via a calibration function, and control cell culture infusion and perfusion.

3 Results and Discussion

3.1 *Oxygen Sensor Response*

Figure 3(a) shows the response of the HOXY sensor when nitrogen and air are alternately flown through the sensor. The sensor output signal is 240% higher in nitrogen than in air, indicating that the HOXY sensitivity is adequate for the range of DO concentrations observed in cell culture media. Figure 3(b) shows a similar response curve in PBS solution with the DO alternated between 0 to 160 mm Hg. The typical oxygen concentration range in mammalian cell cultures observed in our laboratory is 0 to 100 mm Hg. In solution, the response time τ (time required to reach 90% of the steady state) from 0 mm Hg oxygen level to 160 mm Hg oxygen level is about 42 s, and from 160 mm Hg to a low level is about 390 s. The response times are given the time scale for mammalian cell consumption of oxygen in perfused bioreactor systems.

3.2 *Calibration of the Oxygen Sensor*

Calibration of the HOXY oxygen sensor in PBS solution was performed by periodically increasing the oxygen ratio of the mixture of air and nitrogen that continuously purged the solution flowing through the sensor. The dissolved oxygen concentrations of each step were measured by a Ciba-Corning blood gas analyzer (BGA Model 248). The calibration curve fitted by a biexponential equation is shown in Fig. 4. The calibration equation for the HOXY oxygen sensor is given by the following:

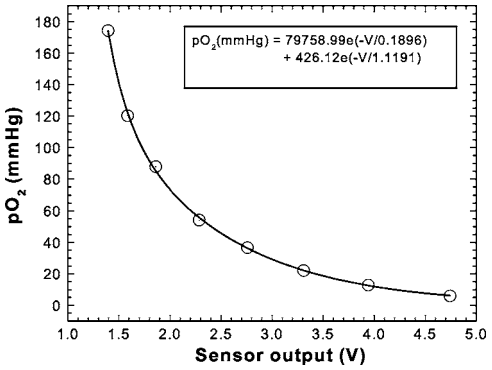


Fig. 4 Oxygen sensor calibration curve.

$$pO_2(\text{mm Hg}) = Ae^{(-V/B)} + Ce^{(-V/D)}$$

where V is the sensor voltage output, and A, B, C, and D are constants determined in the curve fitting process. The biexponential was used as it increased measurement precision. The calibration curve given above was used in all our experiments. Other equations for fitting the sensor calibration curve might also be feasible.

3.3 *Oxygen Sensor Noise*

Figure 5 shows the data of the sensor output in PBS solution at 37°C during a period of 4 h. According to the signal fluctuation, random noise (N) can be determined to be about 3 mV. The noise fluctuation is attributed to the LED light source and photodiode detector noise. The drift (noise) is 2 mm Hg, with an accuracy (measured versus a blood gas analyzer) of 5 mm Hg. The resolution of the sensor ($2N$) is 0.15 mm Hg (at 50 mm Hg oxygen) and 0.375 mm Hg (at 130 mm Hg oxygen), respectively.

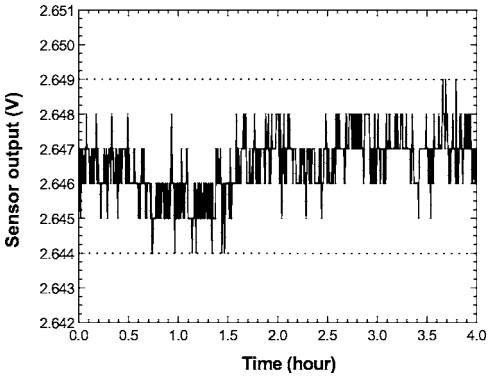


Fig. 5 HOXY oxygen sensor noise level measured in PBS in incubator at 37°C.

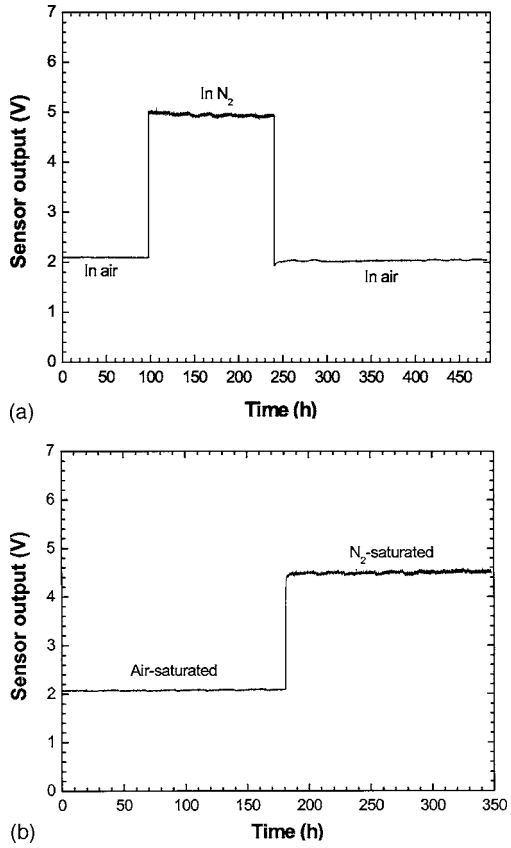


Fig. 6 (a) Long-term stability test of HOXY oxygen sensor in air/nitrogen and (b) in air/nitrogen-saturated PBS solution.

3.4 *Long-term Stability Test of the Sensor*

A HOXY oxygen sensor was employed to continuously measure the gaseous oxygen alternately in air or nitrogen at room temperature for a total period of 20 days, and the data is shown in Fig. 6(a). The drifting of the sensor voltage output in 20 days is only −2%, indicating that bleaching of the dye in this period is not significant.

Figure 6(b) shows the result of a similar long-term continuous test of the same oxygen sensor in PBS solution for a total period of 15 days; the PBS solution was alternatively saturated with air or nitrogen at room temperature. Drifting of the sensor voltage output in all 15 days is not detectable, meaning that leaching of the fluorescent dye is insignificant.

4 Conclusions

An in-house optical oxygen sensor (HOXY) using a pulsed blue LED as the excitation source showed good stability in a continuous 20-day test in air/nitrogen and another 15-day test in air/nitrogen saturated PBS solution. The drifting in sensor outputs is ±2%, indicating that bleaching and leaching of the fluorescent dye of the HOXY oxygen sensor is not significant. Using an initial calibration, the HOXY sensors successfully measured the dissolved oxygen concentration of GTSF-2 medium in a perfused rotary bioreactor supporting BHK-21 cell cultures. Software was successfully developed and tested for integrated control and data acquisition during long-term cell cultures.

Acknowledgment

We are grateful to Mr. Max Musso from Wyle Laboratories for his contributions in the hardware development. Mayra Nelman is gratefully acknowledged for providing technical operation of the SEM for imaging of the sensor films. We also thank Mr. Yuanhang Xu, Mr. James J. Sun, and Dr. Emma Hwang for their support during the long cell run periods. We thank the reviewers of this manuscript for their careful attention to detail. Financial support from NASA (NASA Contracts NAS9-97114 and NAS9-02078) is gratefully acknowledged.

References

1. L. C. Clark, "Electrochemical devices for chemical analysis," U.S. Patent No. 2,913,386 (1959).
2. I. Bergman, "Rapid-response atmospheric oxygen monitor based on fluorescence," *Nature (London)* **218**, 396 (1968).
3. D. W. Lübbers and N. Opitz, "The pCO_2/pO_2 optode: new probe for measurement of pCO_2 or pO_2 in fluids and gases," *Z. Naturforsch. C* **30C**, 532–533 (1975).
4. G. Holst, R. N. Glud, M. Kuhl, and I. Klimant, "A microoptode array for fine-scale measurement of oxygen distribution," *Sens. Actuators B* **38–39**, 122–129 (1997).
5. M. M. F. Choi and D. Xiao, "Linear calibration function of luminescence quenching-based optical sensor for trace oxygen analysis," *Analyst (Cambridge, U.K.)* **124**, 695–698 (1999).
6. C.-T. Lin, W. Böttcher, M. Chou, C. Creutz, and N. Sutin, "Mechanism of the quenching of the emission of substituted polypyridineruthenim (II) complexes by iron (III), chromium (III), and europium (III) ions," *J. Am. Chem. Soc.* **98**, 6536–6546 (1976).
7. O. S. Wolfbeis, M. J. P. Leiner, and H. E. Posch, "A new sensing material for optical oxygen measurement, with the indicator embedded in an aqueous phase," *Mikrochim. Acta* **III**, 359–366 (1986).
8. R. A. Wolthuis, D. McCrae, J. C. Hartl, E. Saaski, G. L. Mitchell, K. Garcin, and R. Willard, "Development of a medical fiber-optic oxygen sensor based on optical absorption change," *IEEE Trans. Biomed. Eng.* **39**, 185–193 (1992).
9. R. Palaniappan and T. A. Kumar, "Adsorptive differential-pulse voltammetric determination of trace amounts of ruthenium," *Analyst (Cambridge, U.K.)* **118**, 293–296 (1993).
10. S. Bambot, R. Holavanahali, J. R. Lakowicz, G. M. Carter, and G. Rao, "Optical oxygen sensor using fluorescence lifetime measurement," *Oxygen Transport to Tissue XVI*, M. C. Hogan, Ed., pp. 197–205, Plenum Press, New York (1994).
11. W. Trettnak, W. Gruber, F. Reininger, and I. Klimant, "Recent progress in optical oxygen sensor instrumentation," *Sens. Actuators B* **29**, 219–225 (1995).
12. I. Klimant and O. S. Wolfbeis, "Oxygen sensitive luminescent materials based on silicone-soluble ruthenium diimine complexes," *Anal. Chem.* **67**, 3160–3166 (1995).
13. M. K. Krihak and M. R. Shahriari, "Highly sensitive, all solid state fibre optic oxygen sensor based on the sol-gel coating technique," *Electron. Lett.* **32**, 240–242 (1996).
14. E. Toba, "Fiber optic fluorosensor for oxygen measurement," *Proc. IEEE 16th Conf. on Instrumentation and Measurement Technology*, Vol. 3, pp. 1426–1430, IEEE, Piscataway, NJ (1999).
15. J. F. Gouin, F. Baros, D. Birot, and J. C. André, "A fibre-optic oxygen sensor for oceanography," *Sens. Actuators B* **38–39**, 401–406 (1997).
16. S. H. Liao, Z. Xu, J. A. Izatt, and J. R. Alcala, "Real-time frequency domain temperature and oxygen sensor with a single optical fiber," *IEEE Trans. Biomed. Eng.* **44**, 1114–1121 (1997).
17. D. R. Walt, "Fiber optic imaging sensors," *Acc. Chem. Res.* **31**, 267–278 (1998).
18. M. E. Lippitsch, J. Pusterhofer, M. J. P. Leiner, and O. S. Wolfbeis, "Fibre-optic oxygen sensor with the fluorescence decay time as the information carrier," *Anal. Chim. Acta* **205**, 1–6 (1988).
19. B. P. H. Schaffar and O. S. Wolfbeis, "A fast responding fibre optic glucose biosensor based on an oxygen optrode," *Biosens. Bioelectron.* **5**, 137–148 (1990).
20. M. C. Moreno-Bondi, O. S. Wolfbeis, M. J. P. Leiner, and B. P. H. Schaffar, "Oxygen optrode for use in a fiber-optic glucose biosensor," *Anal. Chem.* **62**, 2377–2380 (1990).
21. D. B. Papkovsky, J. Olah, I. V. Troyanovsky, N. A. Sadovsky, V. D.

Rumyantseva, A. F. Mironov, A. I. Yaropolov, and A. P. Savitsky, "Phosphorescent polymer films for optical oxygen sensors," *Biosens. Bioelectron.* **7**, 199–206 (1991).
22. C. Preninger, I. Klimant, and O. S. Wolfbeis, "Optical fiber sensor for biological oxygen demand," *Anal. Chem.* **66**, 1841–1846 (1994).
23. B. H. Weigl, A. Holobar, W. Trettnak, I. Klimant, H. Kraus, P. O'Leary, and O. S. Wolfbeis, "Optical triple sensor for measuring pH, oxygen and carbon dioxide," *J. Biotechnol.* **32**, 127–138 (1994).
24. D. Kieslinger, S. Draxler, K. Trznadel, and M. E. Lippitsch, "Lifetime-based capillary waveguide sensor instrumentation," *Sens. Actuators B* **38–39**, 300–304 (1997).
25. H. Chuang and M. A. Arnold, "Linear calibration function for optical oxygen sensors based on quenching of ruthenium fluorescence," *Anal. Chim. Acta* **368**, 83–89 (1998).
26. H. Chuang and M. A. Arnold, "Radioluminescent sources for optical chemical sensors," *Pure Appl. Chem.* **71**, 803–810 (1999).
27. J. S. Reece, M. J. Miller, M. A. Arnold, C. Waterhouse, T. Delaplaine, I. Cohn, and T. Cannon, "Continuous oxygen monitoring of mammalian cell growth on Space Shuttle mission STS-93 with a novel radioluminescent oxygen sensor," *Appl. Biochem. Biotechnol.* **104**, 1–11 (2003).
28. H. Chuang and M. A. Arnold, "Radioluminescent light source for optical oxygen sensors," *Anal. Chem.* **69**, 1899–1903 (1997).
29. F. G. Gao, A. S. Jeevarajan, and M. M. Anderson, "Long-term continuous monitoring of dissolved oxygen in cell culture medium for perfused bioreactors using optical oxygen sensors," *Biotechnol. Bioeng.* **86**, 425–431 (2004).