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A multiplexing fiber optic microsensor system for monitoring spatially resolved oxygen patterns

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ABSTRACT

Single channel luminescent oxygen-quenched optrodes and micro optrodes have been commercially available for over a decade. However, many field experiments in biological research require multiple oxygen sensors to collect large spatial datasets, or to monitor real time oxygen transport in various regions of interest. This paper demonstrates the design, validation, and application of a fiber optic oxygen microsensor system that is designed to conduct real-time measurements of multiple samples in field studies. The ten channel system was validated in laboratory conditions and then used to monitor spatially resolved, real time oxygen concentration in marine microbial mats, agricultural soil, and developing seeds. Sensor stability, drift, sensitivity, and response time were similar to a single channel commercial technology. The effects of temperature and salinity were analyzed and compared to a commercial micro optrode system (there was no statistical difference in performance between the two systems). In addition to the multiplexing capability, an advantage of the system developed here is the ability to map oxygen gradients in three dimensions. The multiplexing system is a minimally invasive tool for in vivo monitoring of form-function relationships with sub-millimeter spatial resolution.

Introduction

The accurate and rapid measurement of physiological oxygen transport is vital for understanding the dynamics of spatially and temporally separated metabolismas well as unique functions involved with stress signalling in cells and tissues. A number of recent reviews describe application of available techniques for measuring oxygen in plant physiology research [1–3] and other biological applications [4]. These techniques include, but are not limited to: polarography, electron paramagnetic resonance oximetry, photoacoustic spectroscopy, anthraquinone amperometry, lab on chip devices, self-referencing microsensors, nanosensors, fluorescent microassays, and planar foils. These technologies have been used in biomedical, environmental and agricultural studies of oxygen transport where there is no need for high spatial resolution [5–9]. With a few exceptions, these technologies were limited to *in vitro* laboratory studies.

Most field studies have used either polarographic sensors [8–19] or fiber optic sensors [20–25]. For example, Rolletscheck et al. [2] and Paterson et al. [15] used electrochemical (Clark type) microsensors to measure oxygen levels in field studies of seed physiology and microbial mat physiology, respectively. Although these microsensors did provide high spatial resolution and rapid measurement, a major drawback of Clark electrodes is the consumption of oxygen at the sensor tip, which is known to cause anomalous readings. Another common problem with Clark microelectrodes is “antenna noise” which significantly reduces the signal-to-noise ratio. Furthermore, to date there are no reports of a multiplexing Clark microelectrode system that can be used in field studies, which limits application to small systems that can be profiled with a single sensor.

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Over the last few decades, a number of optical sensors have been developed to solve some of the inherent challenges of Clark electrodes (namely problems with oxygen consumption and miniaturization). Early pioneers such as Papkovsky et al. [26] first demonstrated oxygen sensing with platinum porphyrin dyes, which was later followed up by Lee and Okura [27] and others. Many excellent reviews discuss the advantages of optical oxygen sensors (optrodes) over polarographic sensors [3,4,28]. Optrodes exhibit improved sensitivity/selectivity, no oxygen consumption at the sensor tip, rapid response times (≈milliseconds), lack of antenna noise, and the ability to measure oxygen in liquid and/or air. The most common type of oxygen optrodes are fabricated by immobilizing a luminescent dye on the tip of a fiber optic cable. Most applications have been in the medical field, where a number of advancements have been made regarding biocompatibility, durability, and reliability [29].

For most modern optrodic systems, frequency-modulated excitation is used to measure quenching of luminescent lifetime by oxygen. Lifetime mode optrodes are preferred over intensity-based optrodes due to less noise and drift, as well as an elimination of calibration shifts associated with photobleaching [20,22]. Optrode performance can be enhanced by including photocatalytic nanomaterials (*e.g.*, silica nanospheres, titanium dioxide, barium sulfate, metal oxides, *etc.*) in the sensing membrane to improve sensitivity [30]. To further improve optrode technology, reference-free systems have recently been developed by Chatni and Porterfield [20].

To date, commercial systems developed for optrodic sensing of oxygen are single channel systems. Some of these systems (such as MicroOxy by World Precision Instruments, Inc.) are portable and can be used in field studies. Although single optrode technologies are vital for basic field studies, many biological experiments require real time, simultaneous monitoring in multiple locations (instead of at a single point). Recently, planar sensor foils have emerged as a technology to fill this gap [31,32]. A luminescent planar foil adheres to the sample via a thin film of deionized water between the sample and the foil. A camera acquires fluorescent data that is easily converted into two dimensional oxygen concentrations along the surface of the foil. Planar sensor foils have high resolution for 2D mapping, but the technique is limited to measuring oxygen levels on surfaces, and thus cannot be used to profile in three dimensions. While technologies such as planar foils or single optrode systems have some application in agricultural and environmental research, there is a need for field-capable microsensor technologies with the capability of supporting three dimensional oxygen mapping and/or simultaneous measurement in multiple locations.

This paper reports on the development of a multiplexing oxygen micro-optrode (MUX) system designed for field studies. The 10-channel system was tested using a variety of applications in marine biology and agriculture. The applications include monitoring of oxygen in: developing seeds, lithifying microbial mats during diel cycling, seeds exposed to hyperoxia, and soil profiles of roots during diel cycling. These experiments were chosen to demonstrate the ability of the MUX to operate in a wide variety of field conditions without any additional modifications; from seawater to developing plant tissues.

Materials and methods

Sensor fabrication

Fiber optic microsensors were prepared using previously published methods [20,22,33]. Briefly, 2 m long × 140 μm thick multi-mode fiber optic cables (Thor Lab Inc, Newton, NJ) were cut in half using a FBC-007 diamond blade fiber cleaver (Corning, Inc. Corning, NY). Fibers were examined using a dissecting microscope to ensure the cleaving was flat and there were no cracks. On the cleaved end, approximately 5 cm of the outer PVC jacket and 3 mm of the polymer cladding were removed using micro-surgical blades and tweezers under a dissecting microscope (World Precision Instruments, Sarasota, FL). The tip of the optical fiber was coated with a solution containing polystyrene, chloroform, titanium dioxide and an oxygen-quenched luminescent dye. The dye used for these experiments was platinum tetrakis pentafluorophenyl porphine (PtTFPP) (Frontier scientific, Inc., Logan, UT). To prepare approximately 20 fibers, 96 mg of polystyrene beads (Sigma–Aldrich, St. Louis, MO) were vortex mixed (Vortex Genie, Bohemia, NY) with 1.15 g chloroform (Fisher Scientific, Waltham, MA) for 30 min in a sealed glass vial. Titanium dioxide (45 mg; Fisher Scientific) and PtTFPP (5 mg) were mixed into the solution and vortex mixed for 30 s. The solution was sealed immediately to avoid evaporation of the chloroform.

To coat optical fibers, a cleaved/stripped fiber optic cable was positioned under a dissecting microscope using manual linear actuators. The fiber optic cable was positioned in the focal plane together with a glass capillary dipped in the dye mixture; the fiber was inserted into the dye cocktail for approximately 1 s. The sensing membrane was inspected for uniformity under the dissecting microscope, and fibers with a dye membrane thicker than ≈40 μm were discarded. Coated fibers were inserted into needles to facilitate the penetration into tissues and brittle materials (see Fig. 1).

Working principle

The optical system is based on the frequency-modulated excitation of PtTFPP with a 400 nm LED, transmission of emission signal through the fiber optic core, and conversion of this signal at 645 nm to a voltage using a photomultiplier tube and lock in amplifier. The main components of the MUX included a linear stepper motor, motor encoders, power supply, cooling system, A/D hardware, and an integrated optics system (InOS). The InOS contained an LED for excitation, a dichroic mirror, band-pass filters (B390 for blue and O-56 for red from Hoya Corp., Santa Clara, CA), a 10× objective (0.25 NA), and a photomultiplier tube (see Fig. 1).

Frequency-modulated excitation (*e.g.*, sinusoidally modulated light at a frequency of 5 kHz) was used to excite the dye (peak to peak current was 50 mA). Emitted phosphorescence (at 645 nm) was monitored with a photomultiplier tube with lock in amplifier. Phase shifts between excitation and phosphorescence light were correlated to oxygen concentration using the Stern–Volmer principle. To account for interference from the red fluorescence of chlorophyll in plant tissues, a dual frequency technique was used based on Schmälzlin et al. [34]. This *ad hoc* signal filtering technique omits background signals with relatively short time delays.

Prior to sensor calibration, the InOS was positioned over an individual ST fiber optic connector by the stepper motor. Emission was recorded from PtTFPP-functionalized fibers connected to the unit. A beam splitter/dichroic mirror, and bandpass filters within the InOS facilitated measurement of emission at 645 nm. The focal length, from ST connector to objective lens, was constant for all channels (1.2 cm).

Sensor calibration

Sensor calibration was performed using known concentrations of dissolved oxygen in deionized water, growth media, or seawater. Solutions were prepared by nitrogen purging (0 kPa), exposure to air (21 kPa), or oxygenation (32 kPa) [22]. Where noted, sodium bisulfite (1 mg/mL) was used to scavenge oxygen during proof of concept testing. Response time (t_{95}) of the sensor was calculated by averaging the 95% steady state temporal response after placing