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


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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.

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I n t r o d u c t i o n

The accurate and rapid measurement of physiological oxygen transport is vital for understanding the dynamics of spatially and temporally separated metabolisms as 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 optrode 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 optrode 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 novel development 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 die and cycling, seeds exposed to hyperoxia, and soil profiles of roots during die and cycling. These experiments were found 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 × 10 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 pentadifluorophenyl 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/striped 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 ($t_{90}$) of the sensor was calculated by averaging the 95% steady state temporal response after placing
the optrode in a calibration test solution. To determine sensor hyster-esis, sensor response was first measured in one of the calibration solutions until steady state response was obtained, and then imme-diately inserted into one of the other calibration solutions. Finally, the sensor was returned to the original calibration solution until a steady state response was obtained. The hysteresis was deter-mined by calculating the percent change in steady state signal for the sensor before and after the calibration solution was changed. After calibration, the lateral positioning of the InOS was adjusted using the stepper motor until the maximum emission intensity was obtained for each channel.

To test the effect of salinity on optrode performance, solutions were prepared at various osmotic strengths (700 mM, 525 mM, 350 mM, and 175 mM); seawater has a salinity of 700 mM. After measuring phase angle in the oxygen saturated samples, 10 mg/mL of sodium bisulfite was added to scavenge oxygen and phase angle was measured for each sample. The optrode was thoroughly rinsed between measurements to reduce artifacts caused by bisul-fite salts trapped in the dye matrix. To determine the effect of temperature on optrode performance, phase angle was measured in DI water at various temperatures (22–12 °C; temperature was adjusted by addition of ice while stirring the solution). Solutions were de-aerated by adding 10 mg/mL of sodium bisulfite to scavenge oxygen. A thermocouple was used to monitor temperature in all samples.

Spatial profiling of marine microbialites

Microbialites are carbonate deposits made by the metabolic activity of marine microbial mat communities. Several characteris-tics of the Highborne Cay microbialites make them ideal models for study. These microbialites are: (i) highly abundant and cover approximately 2.5 km², (ii) undergo rapid growth and accretion (1–2 cm yr⁻¹), (iii) are easily collected from the shallow intertidal zone, and (iv) continue to produce carbonate structures under laboratory conditions. Spatially resolved oxygen concentration was monitored throughout the diel cycle of the living microbialites in a field station located on the island of Highborne Cay, The Bahamas. The microbialites of Highborne Cay are located in the intertidal zone where there is extensive wave activity, sand abrasion, and high levels of UV radiation. Microbialite samples were collected at sunrise and returned to the field station. Micro optrodes were mounted to a manual micro-manipulator fixed to a weighted plate. Optrodes were positioned at the mat surface, and concentration was recorded for 1 min. Sensors were then penetrated into the mat and concentra-tion recorded every 100 μm to a total depth of 8 mm. Profiles were collected (in triplicate) for three different microbialites (total n = 9). Previous researchers have demonstrated that microbialites may be penetrated with O₂ microsensors.

Profiling oxygen gradients in soils

A five-day study was conducted for monitoring oxygen in soil using a photo period of 17 h. The experiments were conducted in a controlled plant growth facility (Conviron Inc., Winnipeg, Mani-toba, Canada). Air temperature inside the chamber was cycled between 30 °C and 22 °C during day and night, respectively. During the photoperiod, photosynthetic photon flux was maintained at 450 μmol/m²/s at canopy height (measured using a photosynthetically active radiation (PAR) light meter). The chambers were kept at 55% relative humidity (RH); monitored using a RH/Temperature Monitor (Fisher Scientific, Waltham, MA). Pots (dimensions: 20 cm × 16 cm; diameter × depth) were filled with 4-L of potting soil (Scotts Miracle-Gro Co., Marysville, OH). Pots were placed in a shallow pan and sub-irrigated every 48 h. Oil plant (Brassica napus L. cv. Westar) seeds were planted at a depth of 5.08 cm. Canola (B.
napus L. cv. Westar) seeds were provided by Dr. Lanfang Levine who received from a canola oil manufacturing company in Canada.

After 60 days of continuous growth, two soil bores were made with a cylindrical ruler to obtain the appropriate depth (one 8 cm deep and one 12 cm deep). Micro optodes were inserted into the soil bores and oxygen was continuously monitored for five days. All sensors were calibrated before and after each individual experiment and sensor hysteresis was calculated. All measurements were made in triplicate.

**Canola seed oxygen**

To study the effect of ambient oxygen conditions on seed oxygen, two sets of canola (*B. napus* L. cv. Westar) plants were grown in controlled environmental growth chambers and monitored for seed respiration. One set was grown at the Space Life Sciences Lab, Kennedy Space Center, FL and the other set was grown in the Agricultural and Biological Engineering Department at the University of Florida in Gainesville, Florida. Seeds were imbibed and germinated in small 6 cm × 6 cm × 8.5 cm seedling pots. After the second true leaf was visible, seedlings were transplanted to 20 cm × 16 cm plastic pots with Fafard 3B mix (Sungro Horticulture, Agawam, MA) at a final planting density of 1 plant per pot. Plants were grown in a controlled environmental chamber maintained under the following conditions: a photoperiod of 14 h daytime/10 h night, day/night temperatures 23 °C and 18 °C respectively, 65% relative humidity, and 400 mmol/mmol air carbon dioxide concentration. During the daytime, the photosynthetic photon flux was maintained at 450 mmol m⁻² s⁻¹ at plant canopy level using a PAR meter. Supplemental carbon dioxide was provided using an external 100% carbon dioxide tank (Airgas, Inc). Watering/nutrient schedule for the plants was alternated between deionized water and a modified Hoagland's solution every other week. The watering schedule for young plants was 0.5L of solution each day until the plants were 20–25 cm tall. Once plants attained this height, they were irrigated with 1 L of DI water per day. Once the plants reached a height of 30 cm, the irrigation rate was increased to 2 L per day. Fresh Hoagland's solution (pH 5.7) was prepared every 2 weeks and stored in a sealed black container.

During all experiments, measurements were taken during the seed developmental stage of 28–34 days after pollination (DAP). The MUX system was used to rapidly assess seed oxygen levels in 28-day-old *B. napus* siliques exposed to hyperoxia and non-lethal freezing events. Seed oxygen was first measured in control plants, and then in siliques placed in 21 kPa oxygen. Sensors in metal needles were carefully inserted into interstitial space between the seeds in the silique while backlighting the seed silique with a bright white light (see Supplemental Fig. S1A). *In-situ* hyper oxygen treatment was carried out by slipping disposable syringe barrels (10 ml capacity) over the developing seed pods (total of 18 siliques per treatment) and attaching the Luer end of the syringe barrel to a manifold (see Supplemental Fig. S2). Siliques were flushed with oxygen for approximately 40 h prior to the oxygen measurement. Each silique was inserted inside an individual barrel, and flow rates were set to maintain either 21 kPa or 45 kPa oxygen. A mixture of pure oxygen and air was used to achieve the desired oxygen partial pressure. The flow rate on individual barrels was 200–300 mL/min. Phase angle was recorded within 5 min of inserting a sensor to avoid leakage. Measurements were taken for at least 3 seeds and the average oxygen concentration was calculated using the arithmetic mean of the measured phase angle in this initial 5 min interval.

**Statistics**

Each experiment was conducted in triplicate. Significance was determined using a paired t-test or analysis of variance in the open source programming language R with a 95% confidence level. All error bars represent standard deviation of the arithmetic mean.

**Results and discussion**

A conceptual schematic of the fiber optic assembly and dye structure (PiTFPP) are shown in the supplemental section (Fig S3). Prior to application in field studies, coated fibers were inserted into standard 26-gauge needle to facilitate penetration into tissues and to protect the sensing element (Fig 1a). For penetration into soil and lithifying microbial mats, coated fibers were inserted into a custom 26-gauge needle with 20 µm side ports purchased from Hamilton Co., (Reno, NV) (Fig 1b). The main components of the MUX included a linear stepper motor, motor encoders, power supply, cooling system, A/D hardware, an integrated optics system, and a lock in amplifier (Fig 1c). The integrated optical system (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 (Fig 1d). The InOS was positioned over an individual ST connector by the stepper motor, and emission was read from PiTFPP-functionalized fibers connected to the unit. A beam splitter/dichroic mirror, and band-pass 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). MUX software recorded phase angle, emission, intensity, and temperature for each sample.

Frequency modulated excitation (also known as phase modulation) was used to excite the luminophore. As reviewed by Ast et al. [3] and others, phase modulation has numerous advantages over pulsed excitation. For example, pulsed excitation requires a relatively long computing time, and correlation of the signal to oxygen concentration is not trivial. In pulsed excitation, any microheterogeneity of the dye on the sensor tip significantly alters the acquired decay curves. On the other hand, frequency modulated excitation is less dependent on the homogeneity of the dye within the membrane on the fiber tip due to direct monitoring of phase shifts associated with quenching (this is a result of improved resolution due to phase sensitive detection). Thus, coating of fibers with the dye is relatively simple and can even be carried out in the field (as demonstrated herein).

**Sensor calibration**

The absorption spectrum of the PiTFPP/TiO₂ polystyrene membrane was measured with a fiber optic spectrophotometer (see Fig S4). Absorption bands were observed at 400 nm, with weak satellite bands at 500 nm and 548 nm. This is similar to previous studies using PiTFPP [20,27], Papkovsky et al. [26] noted that the Soret is the origin of the second excited state and appears between 380 and 420 nm, while the Q bands are the electronic origin of the lowest-energy excited singlet state. When excited in the Soret band (400 nm) optodes displayed strong phosphorescence at 648 ± 4 nm at room temperature (see Fig S4), which is similar to the original reports by Papkovsky et al. [28] and Lee and Okura [27]. As predicted by the Stern–Volmer equation, the emission intensity was significantly lower in the presence of oxygen. The average KS values based on the data in see Fig S4 was 0.115 ± 0.003 kPa⁻¹. The lateral position of the InOS was adjusted using the computer-controlled stepper motors to properly align the objective over the fiber optic ST connector. This positioning was conducted at 0 kPa and 21 kPa. A representative plot of the lateral InOS position versus measured phase angle is depicted in Fig 2a. For each calibration, the location of the objective lens was changed by 0.005 cm until the maximum phase angle was measured. For the example in Fig 2a, the optimum lateral position was 1.02 cm from the base...
of the instrument. It is important that the objective is properly aligned over the fiber optic connector so that the maximum signal is detected.

Calibration of PtTFPP fiber optic sensors was linear between 0 and 21 kPa oxygen, and also between 21 and 32 kPa oxygen [20,22]. The average sensitivity of the micro optodes was $-2.69 \pm 0.31$ kPa$^{-1}$ oxygen between de-aerated (0 kPa oxygen) and deionized water (21 kPa oxygen) at 20 °C (Fig. 2b). These results were similar to values obtained using commercial hardware reported by Chatni and Porterfield [20] and McLamore et al. [22]. Sensors calibrated between 21 kPa and 32 kPa oxygen had an average sensitivity of $-0.91 \pm 0.13$ kPa$^{-1}$ oxygen (Fig. 2b). For supersaturated solutions, the optodes were less sensitive than the values between 0 and 21 kPa due to the rapid quenching of luminescence by oxygen as predicted by the Stern–Volmer principle. The average hysteresis in all calibration solutions was $+0.5 \pm 0.4\%$ change in baseline signal.

Prior to application in field studies, the MUX system was tested and compared to a commercial optrode system (MicroOxy from World Precision Instruments, Sarasota, FL). Each sensor was calibrated in DI under various temperature/salinity conditions. For all the conditions tested (12–24 °C and 175–700 mM-NaCl), there was no significant difference between the MUX system and the WPI system (based on ANOVA analysis). In general, temperature had a more pronounced effect on optrode performance than salinity within the range tested. The effect of temperature was approximately seven times less significant in de-aerated water compared to oxygen saturated water due to the relatively low phase shift at high oxygen concentrations (see Fig. S5). At high concentrations, this could serve to bias the output data if calibration does not account for temperature shifts. The temperature compensation system in the MUX was designed to monitor sample temperature with a thermostate and correct for this artifact. Alternatively, the artifact can be removed ad hoc using the data in Fig. S5 if the sample temperature is known. Conversely, there was no significant difference between the phase angle measured in de-aerated or oxygenated water for different salinity (increases in salinity caused a slight increase in phase angle for both test solutions, but the shift was similar for calibration solutions; see Fig. S5).

After calibration and proper InOS alignment, the MUX was demonstrated by measuring spatially resolved, real time oxygen concentration in marine biology and agricultural applications. These demonstrations represent challenging environments for minimally invasive oxygen sensing, as optrodes are used in seawater, plant tissue, and agricultural soils for experiments that span several days.

### Spatial profiling of marine microbialites

Microbialites are carbonate build-ups that are derived from the trapping, binding, and mineral precipitation activities of photosynthetic microbial mat communities [35]. These microbial ecosystems have a long geologic record dominating the Earth for billions of years [36]. Microbialites are found globally, however, the microbialites of Highborne Cay, The Bahamas have been the subject of several recent studies regarding the biogeochemistry, molecular biology, and microbial diversity of the community [37–44]. The upper layers of microbial mats (0–3 mm) contain a photosynthetic layer, below which a transition zone and an anoxic zone exist [15] (Fig. 3a). Photosynthetic activity by cyanobacteria in the upper layers is known to be very high. As a result of this activity, the oxygen concentration during daylight hours can exceed 650 µM; similarly, the local pH can reach values higher than 10 during the day [45]. The microbialites of Highborne Cay were examined at two key time points; at noon, which is the peak of photosynthesis in the microbialite communities and; at midnight, the peak of heterotrophic activity.

Reproducible spatial patterns of steady state oxygen concentration were observed in the microbialites at both time points (Fig. 3b). These oxygen gradients are similar to the patterns described for stromatolitic mats where Clark electrodes were used [18]. At noon, the data indicated that supersaturated conditions existed within the photosynthetic cyanobacterial layer found in the upper regions of the mat with an average oxygen concentration of 456.3 ± 128.4 µM. Below a depth of 3 mm, local steady state oxygen concentrations decreased linearly with depth at a rate of 55.0 µM–oxygen mm$^{-1}$. Below this transition zone (i.e., deeper than 6 mm), an anoxic zone existed where average oxygen concentration was 2.3 ± 3.3 µM. These spatial patterns correlate with known biogeochemical profiles observed by previous researchers [15,44–46]. Microbialite metabolic processes strongly alter local geochemical signatures, and there is a wealth of data describing the patterns associated with microbialite activity and ion gradients [40]. Monitoring spatial oxygen profiles under field conditions allows real-time bioenergetic activity of the mats to be determined in situ. These types of field measurements are vital for gaining...
During the insightful process, Fig. 76 describes the concept of biogeochemical profile in stromatolites. A fiber optic microsensor is shown near the lithifying biofilm approximately 6–7 mm beneath the surface. Lithifying biofilms appear regularly through the mat and can be on the surface. (b) Oxygen profiles collected from microbialites of Highborne Cay, The Bahamas during June, 2012. Distinctive oxygen profiles were observed during noon and midnight.

**Profiling oxygen gradients in soils**

Three fiber optic sensors were used to continuously measure oxygen in well watered soil during growth of 60-day-old *B. napus*. Real time oxygen concentration data was collected at three different soil depths during five days of successive monitoring (Fig. 4a). Results demonstrated that the MUX was clearly capable of detecting changes in local oxygen as an indicator of soil metabolic activity. The average time required for establishment of steady state oxygen during day-night transition (13 ± 4.5 min) was significantly longer than night-day transition (3 ± 1.5 min) (Fig. 4b). For all data, as depth increased, the oxygen concentration decreased. The average oxygen concentration for all sensors during simulated day and night conditions are shown in Fig. 4c. During daytime, oxygen concentration decreased in a linear fashion with depth (shown in Eq. (1)). During nighttime conditions, oxygen concentration decreased, but the trend was not as linear ($R^2 = 0.86$) when compared to daytime conditions.

$$\text{Oxygen} = -0.5 \times (\text{depth}) + 22\text{kPa} (R^2 = 0.97)$$

Measurement of real-time $O_2$ profiles in soils provides essential information on root physiology. Conditions such as water logging and root hypoxia can be detrimental to oxygen transport. If excessive water is present in the soil, the flow of oxygen to plant roots significantly decreases. Prolonged exposure to water logged soil can cause plant death as well. For plants without aerenchyma, soil flooding or waterlogging causes rapid oxygen depletion and $CO_2$ buildup in soils, which is especially deleterious. In the United States in 2011, natural flooding caused approximately $33.1$ billion in losses due to irreversible agricultural crop damages [47]. Low oxygen availability during soil flooding (i.e., waterlogging) adversely affects root metabolism, often leading to a drop in cytosolic pH, low adenylate energy charge, and a decline in protein synthesis. Viro-lainen et al. [48] report that low oxygen concentration near roots leads to an increase in mitochondrial matrix Ca$^{2+}$ concentration and the release of cytochrome c from mitochondria; which promotes programmed cell death in most systems. Furthermore, flooding impedes proper transport of nutrients and gases between roots and the rhizosphere, which can also lead to plant death. Plants have a multitude of stress response pathways for adapting to low soil oxygen [49]. To date, most of our knowledge on flooding tolerance derives from laboratory experiments under controlled conditions. The MUX facilitates field studies to determine how similar laboratory conditions mimic those actually present in agronomic fields and natural environments. This work should lead to identifying plants that acclimate to short-term flooding or water logging in different soil types and environments.

**Measuring oilseed (Brassica napus) oxygen levels**

During normal development of many oil seeds (such as soybean, canola, and flax), embryos develop from a green photosynthetic tissue to a mature, desiccated and dormant form which is devoid of chlorophyll [50]. In canola, various environmental conditions can lead to green seed at maturity, resulting in low quality oil that is expensive to process [51,52]. For instance, an untimely brief non-lethal freezing/frost results in green seeds at maturity. No amount of time or crop management is able to mitigate the green seed problem, which presents a great economic loss due to the green tint and bad taste in the final Canola oil product. As described by Xin et al. [53], micro-optrodes are an invaluable tool for monitoring seed physiology. While the approach by Xin et al. is excellent for laboratory testing, this is the first demonstration of a multiplexing optrode field system for monitoring seed oxygen.

Fig. 5a shows a photograph of a micro-optrode inserted into a Brassica seed. The average oxygen concentration in the three conditions was not statistically different between 45 kPa and 21 kPa point ($p = 0.92$) or between control and 21 kPa point ($p = 0.72$). Fig. 5b and c shows a plot of average oxygen concentration in seeds of their silicic acid was exposed to elevated external oxygen. Fig. 6 shows the oxygen concentration within seeds that experienced 3 hr non-lethal freezing at −4 °C. In cold treated (−4 °C) plants, Seed oxygen was higher than that in control plants (5.6 ± 0.23 kPa vs 1.78 ± 0.52 kPa; $p = 0.005$). The result unambiguously demonstrated not only the suitability of the sensor system for oily seeds, but also that the seed oxygen was affected by its surrounding oxygen tension.

There has been a great deal of research in this area to understand the mechanism of green seed formation and subsequently devise agricultural mitigation strategies. Several hypotheses have been proposed, but to date, the biochemical processes leading to green seed formation are still elusive. Bonham-Smith et al. [54] showed that embryo abscisic acid (ABA) concentration declines precipitously (compared to the non-stressed control) following a non-lethal freezing event, with the greatest change occurring in
seeds stressed at a time that provoked the greatest retention of chlorophyll at maturity (32 DAP). During the past few years, a collaboration between Dr. Mary Musgrave and Dr. Lanfang Levine found that cold stress leads to an increase in oxygen levels in the siliques space between seeds (unpublished data). It is also known that the gaseous environment (oxygen tension in this case) around the seed affects ABA accumulation within seeds and has a cascading effect on other chemical pathways. Drs. Musgrave and Levine hypothesized that the altered gaseous microenvironment around the seed during sensitive developmental stages is the immediate stressor changing the normal seed developmental course. In order to prove this hypothesis, it is imperative to know the oxygen concentration within seeds that have undergone the stress. To meet this need, the MUX system was successfully used to monitor *B. napus* seed O2 levels during external hyper oxygen tension and cold stress. While the details of these results are still under investigation, the important point here is that the MUX clearly demonstrates the ability to provide minimally invasive oxygen measurements which can be combined with other molecular techniques to investigate the biological mechanisms associated with green seed formation (and other similar developmental problems in seeds).

**Conclusion**

A multiplexing fiber optic oxygen microsensor system was designed and used to conduct real time experiments relative to marine biology and agricultural research. The hardware and design of the system are described such that the device can be re-created by any lab with basic optical equipment and programming skills. The ten channel system was used to monitor form-function relationships in marine microbialites, agricultural soils, and developing oil seeds. The multiplexing system provides the unique capability of minimally invasive, accurate monitoring of oxygen with sub-millimeter spatial resolution. This important tool meets a critical technology need for biologists performing field experiments.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2014.01.094.

References

Biographies

Prachee Chaturvedi is a PhD candidate in biological engineering at the University of Florida. Her research work is focused on sensor/biosensor fabrication for environmental, agricultural and medical applications.

Bernard Hauser is an Associate Professor at the University of Florida in the Department of Biology. Dr. Hauser is an expert in plant developmental biology with a particular emphasis on seed physiology and seed abortion.

Jamie Foster is an Associate Professor at the University of Florida Space Life Science lab. Her research lab is focused on understanding the microbial metabolic potential of modern marine microbialites.

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