## **Supporting Information:**

## Selected Applications of Methods for Optical Sensing and Imaging of Oxygen

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## S1. Sensing and Imaging of Oxygen in Blood and in the Vascular System

One has to differentiate between oxygen saturation ( $S_{O2}$ ) of blood (a parameter that does not exist for serum), and its oxygen partial pressure ( $pO_2$ ). Only the latter is treated here, as  $S_{O2}$  is not determined via sensors, but spectroscopically by a method called blood oxymetry. It is in widespread clinical use and reflects the ratio of (dark red) hemoglobin and (bright red) oxyhemoglobin which is determined by transcutaneous reflectometry (via the Kubelka-Munk equation; see the main part) at the tip of fingers or on ear lids. The partial pressure of oxygen in whole blood or serum is measured via sensors for oxygen, nowadays almost exclusively in whole blood and often along with pH and  $pCO_2$ . These are among the most often performed clinical tests, and respective instrumentation is available from various manufacturers, however practically all *in-vitro*.

Portable devices for *in-vitro* and single-shot blood analysis (such as the Opti<sup>®</sup> or Idexx<sup>®</sup> instruments) are commercially available and are making use of fluorescent sensor spots for oxygen, pH value, potassium, chloride, and other parameters (glucose included) placed in a disposable kit. The spots are illuminated with light of a blue LED, the oxygen sensor spot gives a red luminescence whose intensity is related to the  $pO_2$  of the blood sample above the sensor spot through the Stern-Volmer equation. Such sensors are used in ambulance cars, for example, to measure  $pO_2$  on the way to the hospital.

Rather than sensing oxygen in blood samples taken from a vein or artery, it appears to be highly attractive to measure oxygen transcutaneously. Optical methods are potentially superior because oxygen is not consumed. Measurements are performed at elevated skin temperatures, typically

between 41 and 43 °C in order to improve gas permeability. This approach has not yet resulted in respective instrumentation.

The challenge of on-line and *in-vivo* monitoring of  $pO_2$  (rather than analyzed blood after sampling) still is not adequately solved and represents one of the most challenging tasks in clinical diagnosis and in the surveillance of the critically ill. In one method, oxygen is monitored with the help of optical sensors placed in a flow cell integrated into an extracorporeal loop during cardiopulmonary bypass (CPB) operations. The first system to monitor oxygen, pH and CO<sub>2</sub> in the critically ill and during open-heart surgery appeared on the market in the mid-1980s (GasStat 200 and its successor models; from Cardiovascular Systems Inc.; now Terumo). The sensors are in contact with the bloodstream in an extracorporeal loop (such as in case of using artificial lungs). This device has been very successful ever since. Oxygen can be measured in the 20 - 500 Torr (2 -67 kPa) range. See: www.terumo-cvs.com/products/. The instrument still is in widespread use, for example in 70% of all critical care operations (including open heart surgery) in the US.

Ideally, fiber optic sensors are placed directly in the blood stream. In their milestone paper, Peterson et al. have adsorbed the OSP on hydrophobic particles and kept in position at the end of the fiber with a porous polyethylene tubing. The ratio of green fluorescence to scattered blue excitation light (which serves as an internal reference) is the optical information. A resolution of +/- 1 Torr up to 150 Torr oxygen is reported. From the user's (physicians) point of view, a continuous sensor for blood gases should have several attractive practical features: (1) While accuracy is considered important, accurate *trending* is imperative so to enable quick countermeasures to be undertaken in critical situations. (2) When arterial and venous gases are monitored, one can not only determine the true status of the patient by the venous gases but can also monitor the performance of the oxygenator by simply observing the arterial blood gas values. Should the oxygenator begin to fail, the drop in arterial  $pO_2$  is almost immediate. (3) By continuously observing pH it is possible to determine acidosis or alkalosis and respond with the appropriate treatment. Bearing in mind that these are real-time parameters with the option of temperature correction, it is possible to observe the effects of hydrothermia on the patient. The benefit of a continuous device is obviously tremendous and offers a unique margin of safety that did not exist previously.

The ultimate goal in continuous monitoring of blood gases and blood pH is, of course, a simple device that would allow on-line monitoring without the need for extracorporeal loops. The same company later reported on a catheter for measurement of pH,  $pO_2$ ,  $pCO_2$ , and temperature intravascularily which, however, was never commercialized. The main challenges in the design of such a sensor include miniaturization of sensors, in transmission, acquisition, and processing of low levels of signals, maintenance of mechanical integrity, and achieving a 99.9+ reliability when used *in-vivo*. The fiber bundle (three fibers, each 140  $\mu$ m thick) was introduced into the radial artery of a patient through a catheter. However, the "wall effect" (i.e., the  $pO_2$  near the wall of the vessel and its core is largely different. This prevented the commercialization and clinical use of this sensor. The second-best solution consists of a bundle of sensors placed in a flow-cell placed near the artery into which blood is pumped and analyzed (**Fig. S1**).

**Figure S1**. Fiber optic sensor bundle placed in a small flow-through chamber containing blood pumped out of the *arteria radialis*. It measures blood oxygen, pH, and carbon dioxide.



#### Table S1. Sensing and Imaging of Oxygen in Blood and in the Vascular System

Application keywords	Refs.
Oxygen determined on the surface of the isolated guinea pig heart using a sensor film	(1)
(pyrenebutyric acid in agarose particles and covered with black teflon); area $\sim 6$ mm;	

reference dye added to enable 2-wavelength referencing	
Fiber optic sensing of oxygen in the blood of an ewe; excellent agreement with data	(2)
obtained with an <i>ex-vivo</i> blood gas analyzer	
In-vivo continuous blood gas monitor with disposable probe; oxygen, pH value and	(3)
carbon dioxide monitored in dog blood; sensor is biocompatible, non-toxic, and	
sterilizable	
Fiber optic system for sensing blood oxygen (along with pH and CO <sub>2</sub> ) in radial artery;	(4)
excellent agreement with true values; thermocouple integrated to compensate for effects	
of temperature; sensor is nonhemolytic, nonthrombogenic	
Review on challenges of and methods in transcutaneous sensing of oxygen	(5)
Critical assessment of continuous systems (optical included) for <i>ex-vivo</i> and <i>in-vivo</i>	(6)
monitoring of blood gases	
Study on the performance of a triple sensor (oxygen, pH, CO <sub>2</sub> ); uses three 140-µm optical	(7)
fibers with respective sensor chemistries at the tip; good precision, stability, consistency	
and accuracy reported	
<i>In-vivo</i> visualization of oxygen-transport in microvascular network.	(8)
Continuous intra-arterial oxygen monitoring during cardiopulmonary resuscitation	(9)
Review: Performance of optical sensors for in-vitro blood gas analysis	(10)
Microdialysis system to monitor oxygen in blood; later extended to sensing $pO_2$ , pH and	(11)
$pCO_2$ ; uses the probe Ru(bipy) in a silicone matrix; another system employs micro-flow	
streams of reagents; excellent resolution ( $\pm$ 1.5 torr); response times 2 - 5 min	(1.5)
Optical fiber sensors for blood oxygen, carbon dioxide and pH, based on porous glass tips;	(12)
uses pyrenebutyric acid as the OSP	(1.0)
Review: On-line arterial blood gas analysis (oxygen included) with optical sensors	(13)
Intravascular oxygen distribution in subcutaneous 9L tumors and radiation sensitivity	(14)
Set of luminescence decay time based chemical sensors for clinical applications	(15)
Measurement of muscle microvascular oxygen pressures: Compartmentalization of	(16)
phosphorescent probe	
Exercise training prevents the inflammatory response to hypoxia in cremaster venules	(17)
Review: Critical care analyzer with fluorescent optical chemosensors for blood analytes	(18)
Dual-wavelength phosphorimetry for determination of cortical and subcortical	(19)
microvascular oxygenation in rat kidney and in hypoxic areas in the renal cortex	(20)
Review: Noninvasive imaging of oxygen in blood vessels by methods including imaging	(20)
Monitoring of renal venous oxygen and kidney oxygen consumption in rats by a near-	(21)
infrared phosphorescence lifetime technique	(22)
Imaging of oxygen during microcirculation using microscopy at high magnification	(22)
Imaging of microvascular $pO_2$ ; uses 2-photon microscopy with phosphorescence	(23)
quenching; down to 120 µm below the surface, data correlate well with data for normoxic	
and hyperoxic conditions.	(24)
Fiber optic fluorescence oxygen sensor for <i>in-vitro</i> and <i>in-vivo</i> systems	(24)
Optical monitoring of oxygen tension in cortical microvessels with confocal microscopy	(25)
I wo-photon high resolution measurement of partial pressure of oxygen in cerebral	(26)
	(27)
Miniaturized oxygen sensing in blood; uses optical fibers and a ruthenium based sensor	(27)
Chemistry, metime-based (phase muorometry with modulated LED light source)	(28)
Phase Huorometric determination of oxygen in blood: Validation of optical method and	(20)
Comparison with polarographic Clark-type electrodes	(29)
riber optic sensor to detect rapid changes in oxygen pressure in simulated cyclical	(29)
atelectasis (compromised lung lunction)	

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## S2. Sensing Oxygen in Breath Gas: Respiratory Diagnosis

Analysis of inspired and expired gases plays an important role in respiratory physiology, in lung function diagnosis, and in supervision of the critically ill. Severe trauma, shock, or sepsis also can result in abnormal metabolic activity. Compromised respiratory function results in enhanced levels of oxygen in exhaled air. Also, the maximum rate of oxygen intake while exercising is a measure of the overall cardiovascular fitness. Specific features of such sensors include fast response times (ideally <100 ms in order to keep pace with fast breath cycles), a resolution of 0.1 vol. % of oxygen, insensitivity to humidity, and a low temperature coefficient. The Figure below shows a smart arrangement for evanescent wave excitation of a thin sensor film on an optical fiber inside a gas flow cell in the form of a microchannel.

**Figure S2**. Breath gas sensor constructed by inserting an optical sensor fiber into a transparent capillary. The microchannel formed between the optical fiber and the capillary inner wall acts as a flow cell for the gas flowing through. The evanescent wave field produced on the surface of the fiber core excites the OSP (an iridium complex) to produce emission fluorescence that is quenched by oxygen. From ref. (9) with permission.



 Table S2. Applications of optical oxygen sensors to in breath gas analysis and respiratory diagnosis

Application keywords	Refs.
Evanescent wave sensor for monitoring oxygen in breath gas; uses planar waveguide with	(1)
thin silicone sensor film and the polycyclic aromatic hydrocarbon benzo(ghi)perylene as	
the OSP; excitation light and fluorescence emission spectrally separated by means of total	
internal reflection; response time 30 ms	
Fast-responding (< 0.18 s) optical sensor for oxygen in breath gas; uses Ru(dpp) in silicone rubber	(2)
Gas monitor (prototype module) for use in the mainstream breath-gas flow directly at a	(3)
patient's mouth; uses PtOEPK in plasticized PVC; response time 66 ms	
Breath oxygen analysis; ormosil sensor film doped with Ru(dpp); employs phase	(4)
fluorometry using a blue LED light source and photodiode detection; single point	
calibration	
Unclad optical fiber sensor for breath gas analysis; coated with an iridium probe doped into	(5)
a fluorinated ormosil; evanescent wave excitation; response time 1 s; low cross-sensitivity	
to humidity and temperature	
Rapid oxygen sensor for the respiratory cycle in laboratory animals; uses a porphyrin as the	(6)
OSP; response times 20 ms reported; no cross-sensitivity to water vapor if the OSP is	
adsorbed on hydrophobic particles	
Breath-by breath measurement of oxygen using a compact optical sensor	(7)
Design of a test system for fast time response fiber optic oxygen sensors; used to calibrate	(8)
and determine the time response, linearity and temperature sensitivity of optical oxygen	
sensor for breath gas analysis	
Integrated micro-volume fiber optic sensor for oxygen determination in exhaled breath;	(9)
uses an iridium(III) probe immobilized in fluorinated xerogels; sensitivity: 13; response	
time 1 s; LOD: 0.009% of oxygen in gas	
Combined sensing platform for advanced diagnostics in exhaled mouse breath	(10)

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## **S3.** Sensing and Imaging of Oxygen in Skin (Tumor) Research, Diagnosis, and Therapy

Skin is a special organ that also is involved in the transport of oxygen to tissue. Cancerous skin is under-oxygenated, and wounds can have various levels of oxygen partial pressure. Oxygen supply also is a critical parameter for the vitality of human skin itself. There is a substantial oxygen gradient in the upper-most layers of skin due to diffusion of oxygen through skin to tissue. In wounded skin, this barrier does not exist. See Fig. S3. Sensors can be placed directly on skin (with an upper layer that is impermeable to oxygen), or underneath the skin (transcutaneous sensing). In the latter case, the absorbance and scatter of light caused by skin is a serious problem, and longwave (>600 nm) excitable and emitting OSPs are preferred. Tissue oxygenation in skin tumors is heterogeneous compared to that in normal tissue owing to structural and functional abnormalities of its microvasculature. This represents a major therapeutical obstacle. Sensing oxygen also is a viable tool to monitor the progress of photodynamic therapy (PDT) of skin tumors.



**Figure S3**. Changes in the oxygen partial pressure in wounded skin during the healing process and reflected in pseudo colors. (a) One day after split-thickness skin graft harvesting, the wound is rather inhomogeneous, and vast areas (orange to red ) lack a significant epidermal oxygen barrier. (b) After 6 days, large areas are partially re-epithelialized (lower  $pO_2$  values; darker colors). (c) 14 days postoperatively, most of the donor site wound is re-epithelialized (dark blue or purple areas), and  $pO_2$  is back to between 0 and about 10 mmHg. Reprinted from ref. (7).

#### Table S3. Applications of optical oxygen sensors in skin (tumor) research

Application keywords	Ref.
Planar sensors and phase-modulation used to image oxygen flux in skin; proof for close	(1)
relationship between local microcirculation and oxygen flux into skin; tested under clinical	
conditions; changes of $pO_2$ visualized after skin irritation with histamine	
Transcutaneous optical oxygen sensor; 635 nm-excitable and longwave emitting (710 nm)	(2)
osmium probe (highly toxic!) in silicone rubber layer placed under chicken skin (causes a 50-	
fold loss in intensity); uses phase fluorimetry	
Diagnosis of non-melanoma skin cancer at an early stage using oxygen sensors	(3)
Imaging of oxygen on skin with a spatial resolution of at least 25 $\mu$ m; local pO <sub>2</sub> of solid	(4)
tumors is lower than that of normal tissue; also used to monitor the progress of PDT	
Light-dependent oxygen consumption in bacteriochlorophyll-treated melanoma during PDT	(5)
of type II; oxygen microsensor inserted up to 8 mm in tissue	
Simultaneous photographing of oxygen and pH in wounds; uses dual sensor films and the	(6)
RGB technique	
Two-dimensional luminescence imaging of physiological wound oxygenation; uses sensor	(7)
film and time-resolved fluorescence detection	
Transcutaneous imaging of oxygen during tourniquet-induced forearm ischemia; uses planar	(8)
sensors and lifetime imaging	
Role of singlet oxygen and oxygen concentration in photodynamic disinfection (inactivation	(9)

of Staph. aureus on human skin)	
Effects of light fractionation and fluoresce rates on PDT; uses planar oxygen sensors on skin;	(10)
oxygen imaged by fluorescence lifetime	
Imaging the hypoxic tumor microenvironment in preclinical models	(11)
Imaging of oxygen on mouse skin revealed that hypoxia impairs the NO-dependent	(12)
Leishmanicidal activity of macrophages and prevails in the skin lesions of infected mice	

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#### S4. Sensing Oxygen in Medicine and Lab (Test) Animals (except skin)

Sensing dissolved oxygen has a large potential in the diagnosis of complications resulting from diabetes, peripheral vascular diseases, cerebrovascular and cardiovascular events, and eye pathology (such as diabetic retinopathy and macular degeneration), in the detection of tumors and to monitor the progress of photodynamic therapy. The groups of Wilson and Vinogradov have designed smart nanosized sensor particles (Oxyphor) that are based on the use of small (1 nm or smaller) dendrimers. This approach benefits from the benign environment of the OSP (usually a metalloporphyrin) that warrants biological compatibility, water solubility, and protection from interactions with blood protein, while access of oxygen to the probe in the inner domains of the dendrimer is slowed down but not suppressed. The size of the construct (less than 15 kDa) permits its clearance from the body via kidney dialysis. Depending on whether hydrophobic or hydrophilic dendrimers are used, folding of the dendritic matrix in water can vary and affect the permeability and solubility of oxygen diffusion barriers. In addition, peripheral chemical functions can be introduced at the periphery of the dendrimer to serve as anchor points for linking modifiers such as poly(ethylene glycol) which minimize interactions with proteins and other macromolecules. The Oxyphor dendrimers typically are injected into an organ, tissue, or a vascular system, and their luminescence intensity or lifetime is measured or imaged.

Other widely used techniques include the use of planar sensor films placed on (or in) the organ

of interest, or fiber optic sensing where fiber tips are placed at the site of interest, for example in an organ, in interstitial fluid, or a vessel. Longwave (>600 nm) excitable probes (and 2-photon excitation) are preferred because longwave light penetrates much deeper into tissue than shorter-wave light.

Optical sensing of intracellular oxygen can be traced back to the early 1970s and is often based on the use of nanoparticles whose volume often is less than 1 ppm of the total volume of a cell. Sensing intracellular oxygen is complex as its levels vary from site to site and is low, for example, in the proximity of functional mitochondria for obvious reasons. Oxygen affects cell signaling, growth, enzyme expression, stem cell differentiation, and apoptosis. Oxygen is a key substrate in aerobic biological systems in being the terminal electron acceptor of the electron transport chain. Adult humans consume >200 g of oxygen per day. On the other side, excess oxygen can lead to an overproduction of reactive oxygen species. In addition to the Oxyphor systems, the MitoExpress® and MitoImage® methods (commercial products from LuxCell; Cork; Ireland), and related products are used for sensing oxygen in mitochondria and for live cell imaging in general.

**Table S4.** Applications of optical oxygen sensors in (mammalian) tissue (including tumorous tissue), in organs, and in laboratory (test) animals

Application keywords	Ref.
Oxygen distribution in perfused isolated rat liver; perfusion through the portal vein with media	(1)
containing the probe PdTCPP; marked heterogeneous patterns of tissue reoxygenation, there	
are regional inequalities in oxygen delivery	
Intraocular oxygen tension measured with a fiber-optic sensor in normal and diabetic dogs;	(2)
uses perylene dibutyrate as a probe	
Localization of tumors and evaluation of their state of oxygenation by phosphorescence	(3)
imaging	
Oxygen distribution imaged in the retinal and choroidal vessels of the cat	(4)
Overview on non-invasive imaging of oxygen in tissue and in- vivo; mainly using near-	(5)
infrared phosphors	
Distribution of oxygen on the surface and in deep areas of the kidney imaged using	(6)
phosphorescent nanoprobes	
Oxygen distributions within R3230Ac tumors growing in dorsal flap window chambers in rats.	(7)
Oxygen distribution in a subcutaneous tumor growing in rats; uses the Oxyphor G2 probe to	(8)
noninvasively image $pO_2$	
Microscopic interstitial imaging of oxygen.	(9)
Measurement of tumor oxygenation using new frequency domain phosphorometers	(10)
Review on sensing oxygen in tissue using (mainly) Oxyphor sensor particles and	(11)
phosphorescence quenching.	
Regulation of tissue oxygen levels in the mammalian eye lens; distribution of oxygen imaged;	(13)
steep gradient of pO <sub>2</sub> found within tissue, with pO <sub>2</sub> of $<2$ torr in the core; mitochondrial	
respiration accounts for approximately 90% of the oxygen consumed by the lens; low oxygen	
leads to opaquification which is a major cause of blindness	
Temporal dynamics during adrenergic receptor stimulation in the isolated perfused rat heart	(13)
Oxygen distribution in murine tumors (malignant melanoma, renal cell carcinoma, and Lewis	(14)
lung carcinoma) using the Oxyphor G2 nanoparticles; both decay time and intensity measured;	
includes real-time oxygen monitoring	
Capillary probe for dissolved oxygen in the interstitial fluid of pigs; uses a microdialysis	(15)
catheter to extract the interstitial fluid from adipose tissue	
Validation of an optical sensor-based high-throughput bioreactor system for mammalian cell	(16)
culture	
Oxygen pressures in the interstitial space and their relationship to those in the blood plasma in	(17)
resting skeletal muscle	
Imaging oxygen pressure in the rodent retina by using Oxyphor nanosensors particles;	(18)

lifetime-based (FLIM)	
Generation of reactive oxygen species in endothelial hypoxia; reoxygenation stimulates kinase	(19)
signaling and kinase-dependent neutrophil recruitment in endothelial cells; uses planar oxygen	
sensor	
Imaging of oxygen in cortical vessels and tissue; uses 2-photon excitation and	(20)
phosphorescence quenching microscopy; small excitation area and at low flash rate.	
Monitoring oxygen and pH in mammalian cell culture using optical sensors: also studied with	(21)
CHO cells and mouse myelona cells	
Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue	(22)
Fluorescence optical fiber sensor for monitoring oxygen in rabbit with acute lung injury;	(23)
Ru(dpp) used as a probe along with a biocompatible methacrylate copolymer matrix	
Chemoradiation interactions under reduced oxygen conditions; cellular characterization of an	(24)
in-vitro model; includes studies in cell viability and profileration	
Dually emissive materials for imaging tumor hypoxia	(25)
Thin sensor film used for in-vitro monitoring of oxygen in cell cultures	(26)
Phosphorescent iridium probes used to sense hypoxia in tumors of living animals	(27)
Porcine kidneys procured; renal artery and vein cannulated and connected to perfusion system;	(28)
kidneys perfused at 8 °C, oxygen pressures measured with fiber optic sensor	
Simultaneous 2-photon imaging of oxygen and blood flow in deep cerebral vessels	(29)
Human P450 protein vertically integrated on an oxygen sensing film; analysis of the metabolic	(30)
activity of P450	
Evaluation of hyperbaric oxygen therapy for free flaps using planar optical oxygen sensors;	(31)
para-scapular flap transplanted to the lower limb; transcutaneous oxygen partial pressure	
imaged vial luminescence lifetime imaging.	
Modulation of tumor cell metabolism and proliferation in multicellular tumor spheroids by	(32)
drug (Pioglitazone) monitored via sensing oxygen	
Myocardial oxygen pressure assessed using the nanoprobe Oxyphor G3; myocardial infarction	(33)
induced in male rats, Oxyphor then injected, and oxygen distribution measured <i>in-vivo</i> by	
applying a fiber light guide to the beating heart	
Oxygen in the vasculature of the microcirculation and the interstitial spaces of resting muscle	(34)
tissue; uses Oxyphor dendrites; mean oxygen pressures are somewhat higher in the blood	
stream than in the interstitial space	
Phosphorescent nanoparticles for quantitative measurement of oxygen profiles in vitro and in	(35)
vivo	
Review on fluorescence imaging using chemical sensors (oxygen included)	(36)
Precise spatial and temporal control of oxygen within in-vivo brain slices via microfluidic gas	(37)
channels	
Cardiopulmonary function studied using dendritic nanoprobe sensors in the peripheral tissue;	(38)
gas-permeable tubing attached to optical fibers; in case of hemorrhage, blood flow to "non	
essential" peripheral tissues is restricted in favor of blood flow to essential internal organs	
Continuous oxygen monitoring of mammalian cell growth on space shuttle mission STS-93	(39)
with a novel radioluminescent oxygen sensor	
Review: Small molecule phosphorescent probes for oxygen imaging in 3D tissue models	(40)
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## **S5. Intracellular Sensing and Imaging of Oxygen**

Once nanosized sensor have become available, it was obvious to apply these to sense oxygen inside cells. Early work by Lübbers, Knopp, Wilson and Kopelman is cited below. Such sensors need to be very small, ideally have a diameter of <100 nm, better <10 nm. Critical issues include cell (membrane) permeability, toxicity, specific placement (cytosol, mitochondria, membrane), choice of probes and spectroscopies that are not interfered by the strong background luminescence of most (single) cells, in particular of algae.

**Table S5.** Application of optical oxygen sensors to intracellular sensing and imaging of oxygen in single cells

Application keywords	Ref.
Intracellular sensing of oxygen by quenching of fluorescence of pyrenebutyric acid	(1)
Oxygen dependence of mitochondrial oxidative phosphorylation measured with sensor nanoparticles	(2)
Interaction of oxygen-sensitive luminescent ruthenium probes with animal and plant cells in	(3)
vitro; mechanism of phototoxicity and conditions for non-invasive oxygen measurements	
Imaging fluorescence lifetime modulation of a ruthenium-based dye in living cells: the potential for oxygen sensing	(4)
Real-time measurements of dissolved oxygen inside live cells by organically modified silicate fluorescent nanosensors	(5)
Optical nanoparticle sensors for quantitative intracellular imaging.	(6)
Uptake of oxygen by HEPG2 liver cells encapsulated in alginate matrices; cells grown on cover glass slides and then encapsulated in an alginate matrix;	(7)
Intracellular oxygen sensing via fluorescence lifetime imaging microscopy (FLIM) using cell lysates; in-vitro calibration; and to account for the high variability between different cell lines; oxygen levels evaluated in living human normal squamous and adenocarcinoma esophageal epithelial cells	(8)
Review on nanoparticle ("PEBBLE") sensors for use in live cells and in-vivo	(9)
(a) Pt(II)-coproporphyrin probe covalently linked to positively charged TAT-derived peptides; these are well loaded into live mammalian cells without transfection reagents; (b) various other probes also reported and used to image oxygen gradients	(10)
Real-time ratiometric monitoring of oxygen inside living cells using sol-gel based spherical optical nanosensors: applications to rat C6 glioma	(11)
Oxygen monitored during metabolic responses of mouse embryonic fibroblast cells; uses nanosensors (35 nm); also used for screening experiments with a time-resolved plate reader; single cell analysis performed on a microscope; probe: PtTCPP immobilized on cell-penetrating positively charged peptides; also shown to work with nanoparticle sensors and with various detection modes	(12)
Cell-penetrating conjugates of oxygen-sensitive coproporphyrins with arginine peptides; application to sense intracellular oxygen	(13)
Nanosensors for oxygen in the cytosol; also measures pH; nanosensors densely covered with PEG to make them membrane-impermeable; small size (12 nm); internalized via electroporation; oxygen and pH in cytosol imaged with good spatial resolution	(14)
Sensing of cellular oxygen; sensor based on Ir(III) octaethylporphyrin complex on a peptide carrier	(15)
Review on probes and sensors for sensing oxygen in extracellular and intracellular space; emphasis on performance, convenience and applicability to measure consumption rates, sample oxygenation, and imaging of oxygen in tissue, vasculature and individual cells	(16)
Monitoring of oxygen in subcutaneous adipose tissue using microdialysis	(17)
Monitoring pO <sub>2</sub> and pCO <sub>2</sub> by microdialysis of the critically ill	(18)

# FRET-based ratiometric sensing of oxygen in active brain; sensor based on a Pt(II)(19)ketoporphyrin probe and nanocystal quantum dots in a polymer matrix; sub-second response*References*

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### **S6. Oxygen Sensors in Tissue Engineering and Stem Cell Research**

Tissue engineering is critically dependent on adequate oxygen supply. Sensors for oxygen can be applied to image oxygen so to warrant proper growth of engineered tissue, for example of chondrocytes. Sensors are typically placed at the bottom of a Petri dish in which cultures of cells are grown. The sensor also can detect irregularities during growth due to inadequate oxygen supply. Tissue defects in the engineered cartilage often correlate with oxygen concentrations which can reach almost zero level in less accessible sites and if not adequate supplied (**Figure S4**).



**Figure S4**. Oxygen distribution in engineered tissue, and a scan from the outer surface to the center showing a steep gradient. Reprinted from ref. (1) with permission.

Table S6. Applications of optical oxygen sensors in tissue engineering and stem cell research

Application keywords	Refs.
Determination of oxygen gradients in engineered chondrocyte tissue; culture grown in Petri	(1)
dish; fluorescent oxygen sensor layer placed on bottom	
Analysis of intracellular oxygen and metabolic responses of mammalian cells; uses time- resolved fluorometry	(2)
Hypoxia in static and dynamic 3D culture systems for tissue engineering of bone; oxygen	(3)
monitored via integrated sensor layers	
Review on sensor technologies for the quantitative evaluation of engineered tissue (oxygen	(4)
sensors included)	
Evaluation of oxygen and shear stress distributions in 3D perfusion culture systems during	(5)
tissue engineering (chondrocytes)	
Oxygen levels scaffolds for bone engineering constructs using human jaw periosteal cells;	(6)
includes proliferation assay and osteogenic differentiation	
Review on hypoxia and stem cell-based engineering of mesenchymal tissues; includes a	(7)
comparison of optical methods for sensing oxygen	
Effects of hypoxic culture conditions on umbilical cord-derived human mesenchymal stem	(8)
cells; uses shake flasks with integrated oxygen sensor spots	
Physiological characterization of primary human hepatocytes in a 3D hollow-fiber	(9)
bioreactor; oxygen sensor placed in tubing before and after the bioreactor cell	
Review on cardiotoxicity testing using pluripotent stem cell-derived human cardiomyocytes;	(10)
includes methods based on the use of optical sensors for oxygen	
Oxygen diffusion through collagen scaffolds at defined densities; implications for cell	(11)
survival in tissue models; uses an optical fiber sensor	
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## S7. Oxygen Sensors in Animal Biology

This section covers selected applications of oxygen sensor to study the physiology of (healthy) animal species. It does not include model studies with laboratory test animals which are treated in section S4. Sensors are mostly of the fiber optic microsensor type, while imaging is less common so far. Given the number of animal species, of organs and of physiological and environmental condition, the number of conceivable studies is virtually endless. **Table S7** gives typical examples.

Table S7. Selected Examples of Optical Oxygen Sensors Used in Animal Biology

Application keywords	Refs.
Effects of exercise and hypoxia upon oxygen tensions in the red muscle of rainbow trout	(1)
Crater landscape: Two-dimensional oxygen gradients in the circulatory system of the	(2)
microcrustacean Daphnia magna	
Intragel oxygen promotes hypoxia tolerance of fish; metabolism and regulation	(3)
Adult neural stem cells express glucose transporters and regulate GLUT3 expression;	(4)
studied in rat brain	
Oxygen consumption of frog (Rana pipiens) muscle under stress	(5)
Continuous sensing of oxygen tension in the air-breathing organ of Pacific tarpon (Megalops	(6)
cyprinoides) in relation to aquatic hypoxia and exercise	
Detection of oxidative stress in a Drosophila model; hypoxia in mitochondria and in	(7)
embryos	
Effect of aerial oxygen partial pressure on bimodal gas exchange and air-breathing behavior	(8)
in fish (Trichogaster leeri)	
Essential fatty acids influence metabolic rate and tolerance of hypoxia in Dover sole (Solea	(9)
solea) larvae and juveniles	
Network activity in submerged hippocampal slices depends on oxygen supply; sharp wave-	(10)
ripple oscillations observed in animal	
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## **S8.** Oxygen Sensors in Plant Biology

Oxygen is one product of photosynthesis. Its determination in various kinds of photosynthetic plants obviously can give deep insight into the biology of plants. The Kautsky effect was discussed above. Fiber optic oxygen sensors are mainly used in plant biology to determine oxygen at specific sites. Few studies have been reported so far on the use of nanosensors in plant cells. Leaves of all photosynthetic plants display strong intrinsic (red) luminescence due to the presence of chlorophyll which may interfere in fluorometric sensing. The spectroscopic techniques given in Section 9 of the main part are particularly useful here in order to mask autofluorescence.

Again, the number of studies that may be performed with either planar, or fiber optic, or nanoparticle-based sensors, some in combination with imaging, and with the millions of plants and their subunits is virtually endless.

**Table S8.** Applications of optical oxygen sensors in plant biology

Application keywords	Refs.
Oxygen production during photosynthesis; uses the Kautsky method (a phosphorescent dye	(1)
such as trypaflavine absorbed on silica gel); can detect as little as $5x10^{-8}$ mL of O <sub>2</sub> per min	
In-situ measurement of oxygen in the sapwood of woody plants	(2)
Sensing intracellular oxygen concentration in plants; uses multi-frequency phase-modulation	(3)
and sensor microbeads	
Low oxygen sensing and balancing in plant seeds; study on its role for nitric oxide and ATP availability; storage metabolism also studied	(4)
Oxygen concentration shown to regulate microsomal oleate and linolate desaturase in	(5)
developing sunflower seeds	
Overview on methods and significance of microsensor-based oxygen mapping in plant seeds	(6)
and crop	
Self-referencing fiber optic microsensor for sensing spatially resolved metabolic rhizosphere	(7)
oxygen flux	
Review on the use of optical oxygen micro- and nanosensors in diverse tissues (mainly	(8)
plant); includes a discussion of the pitfalls	
Oxygen dynamics in rhizomes of <i>Phragmites australis</i> , and presence of methanotrophs in	(9)
root biofilms in a constructed wetland for wastewater treatment	
Study on photosynthetic circadian rhythmicity patterns of Symbiodium, a coral	(10)
endosymbiotic algae; uses a commercial 4-channel optical fiber oxygen meter	
Respiration of Mediterranean cold-water corals studied with fiber optic sensor; found not to	(11)
be affected by ocean acidification	
Planar optodes for visualizing oxygen distribution during rhizosphere processes	(12)
Imaging of oxygen distribution, respiration and photosynthesis at a microscopic level of	(13)
resolution using a sensor foil	
Methods to determine oxygen in plants (a review)	(14)

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## **S9.** Oxygen Sensors in Drug Screening, Testing Toxicity and Mitochondrial Activity, and in Related Areas

Screening has become an indispensible tool in various field of (bio)sciences. This section is related to screening for drugs, to toxicity screening, and related areas. Screening methods for cell viability are mainly covered in the section on applications in biotechnology. Drugs and toxic compounds exert a strong effect on respiration and mitochondrial activity, and this can be detected via sensors for oxygen.

Oxygen is consumed in mitochondria to generate the energy needed to maintain cellular metabolism. Sensing of oxygen concentration in mitochondria (such as in the heart) can provide abundant information for studying the normal metabolism of cells, the cause of diseases, health status of cells, and is beneficial in sorting tumor cells from normal cells. The oxygen dependence of respiration is modulated by the cellular metabolic state. At near maximal levels of respiration or on recovery from hypoxic episodes, oxygen diffusion becomes an important determinant of oxygen dependence of drug-induced toxicity. Early identification of new chemical entities that perturb mitochondrial function is of significant importance to avoid attrition in later stages of drug development.

**Table S9.** Applications of optical oxygen sensors in drug screening, toxicity testing, cell respiration and related areas

Tespiration, and Telated areas	
Application keywords	Ref.
Cytotoxicity testing in primary and secondary cell lines; uses microtiter plates to determine	(1)
respiration of adherent cells (caco 2); hepatocytes	
Monitoring cellular respiration using a ratiometric method; sensing oxygen in live cells using	(2)
sol-gel-based spherical optical nanosensors	
Enzyme activity screening; uses microtiter plates with integrated sensors for oxygen and pH;	(3)
toxicological assay based on the respiratory activity of P. putida.	
Microtiter plates with oxygen sensor spots used to measure oxygen consumption by isolated	(4)
mitochondria; yields specific information about possible mechanisms of toxicity; method	
may accelerate development of drugs and improve drug attrition	
Analysis of mitochondrial function using phosphorescent oxygen-sensitive probes	(5)
Screening drugs against pathogenic yeasts; uses microtiter plates with oxygen sensor layers	(6)
In-vitro toxicity prediction for safety assessment during preclinical drug development using	(7)
hepatocytes G2 cells	
High throughput toxicity screening on adherent cells using respiratory measurements; studies	(8)
on rat hepatocytes and human hepatic cell line; dose-response curves established	
Monitoring of respiratory activity of isolated mitochondria; these are functional for 3 h but	(9)
stop respiring at a critical limit of 20% air saturation; inhibition and enhancement of	
respiratory activity by drugs also observed.	
High-throughput screening methods in toxicity testing (book); contains sections on the use of	(10)
optical sensors for oxygen	
Review on applications in enzymatic assays; analysis of respiration of mammalian and	(11)
microbial cells, small organisms and plants; food and microbial safety; live tissue,	
bioreactors and fluidic chips	
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## S10. Oxygen Sensors in Biotechnology and Microbiology

Oxygen is routinely monitored in bioreactors, during biofuel production, in the brewing and beverage industry (both in anaerobic and aerobic processing), and in quality control in general. Oxygen detection at both high and low concentrations is critical, and it is mandatory that sensors can be sterilized (autoclaved). One particular feature of optical sensors, viz. the possibility of contactless sensing, appears to be highly attractive here. **Figure S5** shows a typical arrangement. Table S10 gives selected applications of oxygen sensors in biotechnology, fermentation and microbiology. Numerous other imaging applications can be found under www.presens.de/products/category/category/imaging.html





**Table S10.** Selected Applications of Oxygen Sensors in Biotechnology, Fermentation and

 Microbiology

Application keywords	Refs.		
Sterilizable optical fiber oxygen sensor with high long-term stability; used for long-term			
monitoring in bioreactors			
Optical triple sensor for measuring pH value, oxygen and carbon dioxide in a bioreactor	(2)		
Triple sensor for simultaneous sensing of pH, oxygen and carbon dioxide in bioreactors	(3)		
Sensing dissolved oxygen in cell cultures and bioreactors; lifetime-based sensing scheme;	(4)		
sensing spots fixed on the inner wall of a bioreactor, and signals read from outside via fiber			
optics			
Determination of cell viability via the consumption of dissolved oxygen during			
metabolism; uses a fiber optic oxygen sensor			
Oxygen sensors for bioreactors; study on standing autoclavation	(6)		
Review on optical sensor systems for bioprocess monitoring; covers optical methods based			
on UV, IR, Raman, fluorescence, terahertz spectroscopy, and reflectometric interference			
spectroscopy; also covers optical sensors (oxygen included) and SPR sensors			
Oxygen-sensitive patches used for non-invasive measurement of dissolved oxygen in shake	(8)		
flasks; uses frequency domain fluorometry.			
Optical sensor spots for dissolved oxygen inside microtiter plates; determination of oxygen	(9)		
uptake rate; bioprocess optimization (cultivation of Corynebacterium glutamicum)			

Up to 180-day continuous monitoring of dissolved oxygen in cell culture medium for	(10			
perfused bioreactors: uses Ru(dpp) in silicone as a quenchable probe: applied to baby				
hamster kidney cell culture				
Sansing avugan during high throughput bioprocessing in membrane coroted	(11)			
minimiser activities and the second s	(11)			
	(12)			
Rate of oxygen uptake and culture viability measurement of animal cell culture using	(12)			
microplates with integrated oxygen sensors	(10)			
Milliliter-scale bioreactor for high-throughput bioprocess design using <i>E. coli</i> and fed-	(13)			
batch methods; oxygen transfer coefficients also determined.				
On-line monitoring of <i>E. coli</i> culture parameters in shaken 24-well microtiter plates and a	(14)			
dish reader; pH also measured				
Studies on oxygen transfer between sample, air, and plate material; uses microtiter plates	(15)			
with integrated sensors; paraffin wax coating used to exclude oxygenation of shaken				
sample by air				
Competition between oxygen and nitrate respiration in continuous culture of <i>Pseudomonas</i>	(16)			
aeruginosa performing aerobic denitrification	, ,			
Monitoring the growth of $F_{coli}$ and $P_{scudomonas}$ mutida: toxicological assay based on	(17)			
the respiratory activity of <i>P</i> , putida	(17)			
Ui de respiratory activity of <i>F</i> . <i>puttuu</i> .				
High cell density cultivation of recombinant yeasts and bacteria under non-pressurized and				
pressurized conditions in stirred tank bioreactors; uses planar oxygen sensors	(18)			
Imaging of oxygen in microbial biofilms; lifetime based; can be used in flow chambers and	(19)			
other growth devices				
Oxygen transfer rate determined in shake flasks via fiber optic microsensor; respiration	(20)			
activity also monitored; applied to <i>E. coli</i>				
Scale-down and parallel operation of the riboflavin production process with <i>Bacillus</i>	(21)			
subtilis; uses microtiter plates with integrated optical sensors layers				
Polystyrene nanoparticles stained with Ru(dpp) was used to monitoring oxygen				
consumption in the proximity of Sacch, cerevisiae cells: surface of nanosensors coated with				
polyethylenimine which enables them be assembled on individual cells in a controlled				
manner at physiological pH				
Comparison of ontical oxygen sensor with electrochemical ("Clark") probes during				
mammalian cell culture				
Determination of ovygen gradients in biofilms: steen concentrations observed within the	(25)			
Determination of oxygen gradients in biofilms; steep concentrations observed within the				
$M_{\text{substantian}} = \frac{1}{2} \sum_{i=1}^{n} 1$	(26)			
Monitoring the growth of <i>P. punda</i> in 24-well microplates using a dual planar optical	(20)			
sensor for dissolved oxygen and pH values; highly parallelized and miniaturized				
bioprocessing; also applied to cell-based high-throughput screening				
Review on techniques for oxygen transfer measurement in bioreactors				
Oxygen-mediated regulation of biofilm development in Staph. Epidermidis				
A microwell array device capable of measuring single-cell oxygen consumption rates.				
10-mL stirred bioreactors for cultivation of mycelium-forming microorganisms; oxygen	(30)			
introduced via surface aeration; oxygen transfer coefficients determined;				
Microfluidic reactor for continuous cultivation of Sacch. Cerevisiae	(31)			
Review on optical oxygen sensors for applications in microfluidic cell culture systems	(32)			
including stem cells				
High-throughput screening of <i>Hansenula nolymorpha</i> clones: batch mode compared with				
the controlled-release fed-batch mode on a small scale				
Ontical daviage for norallal onling maggirement of dissolved awagen and nU in shales fleat				
Optical device for parallel online measurement of dissolved oxygen and pH in snake flask				
Dissolved oxygen in shake flasks, also using a rotating flexitube optical sensor.				
Growth and recombinant protein expression with <i>E. coli</i> in different batch cultivation	(36)			

media; optical sensor layer placed on bottom	
On-line monitoring of oxygen in a small-scale disposable bioreactor (called "Tubespin")	(37)
Optical oxygen sensor in rocking T-flasks a tool for upstream bioprocessing	(38)
Study on the effect of sterilization methods on sensors; efficacy and compatibility assessed	(39)
of the following: gamma-ray; electron beam; steam; ethylene oxide; hydrogen peroxide	
gas; low-temperature hydrogen peroxide plasma sterilization	
Oxygen supply in disposable shake-flasks: prediction of oxygen transfer rate, oxygen	(40)
saturation and maximum cell concentration during aerobic growth	
Sensing oxygen in microbioreactors using a NIR luminescent copolymer of a platinum	(41)
porphyrin and a reference dye; suitable for high-throughput sensing	
Sensor film containing Ir (III) complex as a high-permeability sensor for oxygen in	(42)
fermentation bioprocesses	
System of miniaturized stirred bioreactors for parallel continuous cultivation of yeast with	(43)
online measurement of dissolved oxygen and off-gas	

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## S11. Oxygen Sensors in Marine and Freshwater Research

Dissolved oxygen is one of the most important dynamic parameters to characterize marine systems, and the knowledge of oxygen concentration gradients is paramount for understanding the function and regulation of most marine microbial communities. The concentration of oxygen decreases in deep water, and therefore the species, population and behavior of marine organisms are quite different. The fish industry also is using oxygen sensors in order to optimize the growth of fish. Oxygen also plays a key role in benthic microbial ecology, and flux rates of oxygen have been used to quantify primary production, nutrient cycling of shallow-water environments, total community respiration, and mineralization rates. Additionally, oxygen cycling within various photosynthetic communities has a major impact on the biogeochemical cycling and the ecology of microorganisms and fauna living within such communities. **Figure S6** shows pseudo-color images of the oxygen and pH distribution at an sediment-water interface using a dual sensor and acquired via luminescence lifetime imaging. Planar optical sensor also can be used to image oxygen in flowing marine waters after environmental disasters such as the Deepwater Horizon catastrophe. **Table S11** gives a selection of articles on the use of optical sensors for oxygen in marine and freshwater research.



**Figure S6**. Oxygen and pH distribution at sediment-water interface based on luminescence lifetime imaging. (A): Pseudo-color maps of the pH and  $pO_2$  distributions in natural marine sediment. (B): profiles extracted from the maps as well as profiles measured with micro-optical sensors ("optodes") and micro-electrodes. Reprinted from ref. (26)

**Table S11.** Applications of optical oxygen sensors in marine and freshwater research

Application keywords	Refs.
Traces of oxygen in seawater; uses phosphorescence quenching of dyes such as acriflavine	(1)
or acridine orange adsorbed on various silica gels	
Oxygen in seawater using a glass-immobilized (non-specified) PAH in a moored fluorescent	(2)
sensor; complete sensor system described; oxygen depth profile measured in the ocean near	
Victoria, B. C. (Canada)	
Fiber optic oxygen microsensors, a new tool in aquatic biology	(3)

Microenvironment and photosynthesis of Zooxanthellea in scleractinian corals studied with	(4)			
Planar optical oxygen sensor used to image oxygen distribution in seawater above				
sediments; spatial resolution around 26 μmFiber optic oxygen sensor for use in oceanography; lifetime measured with two time				
windows in the decay profile; effects of salinity and hydrostatic pressure also investigated				
Oxygen imaged in a benthic photosynthetic mat; consumption and production in the microbial mat shown to strongly vary between day and night.	(7)			
Oxygen dynamics in marine sediments (review)	(8)			
Fiber optic sensors for monitoring dissolved oxygen in seawater	(9)			
Sensing oxygen and temperature in sediments using a fiber optic microsensor array or high-				
resolution oxygen imaging to determine 2-dimensional distribution; also in heterogeneous				
living systems	(10)			
Planar oxygen sensor films for measurements at benthic interfaces	(11)			
Oxygen microsensors used in sea ice; oxygen dynamics during sea ice formation; detection of brine channels	(12)			
Use of oxygen optical fiber microsensors in aquatic animal ecology	(13)			
A new microcosm to investigate oxygen dynamics at the sea ice water interface; includes effects on photosyntheis:	(14)			
Diffusive boundary layers and photosynthesis of algal community of coral reefs	(15)			
Hypoxic life and respiration of intertidal acorn barnacles	(16)			
Oxygen dynamics in sediments with wave-driven nore water exchange: uses a planar oxygen	(17)			
sensor film;	()			
Effect of elevated temperature on aerobic respiration and oxygen uptake of coral recruits	(18)			
Oxic microzones andradial oxygen loss from roots of Zostera Marina	(19)			
Study on the spatio-temporal variation in oxygen distribution within a marine sediment Ru(dpp) used in an ormosil matrix (prepared from diphenyldiethoxysilane and phenyl				
Comparative evaluation of lifetime based enticel fiber sensors for evagen in equation				
comparative evaluation of metime-based optical fiber sensors for oxygen in aquatic	(21)			
Systems, manny deep sea	(21) (22)			
incorporation of benthic algae and hacteria in near-shore sandy sediment				
Small-scale spatial and temporal variability in coastal benthic oxygen dynamics: effects of				
fauna activity; solute exchange and carbon mineralization in subtidal sandy sediments: effect	(23)			
Or advective pole-water exchange	(24)			
Quantification of oxygen respiration of bacterial communities and plankton in freshwater ecosystems; uses oxygen sensor spots;				
Temperature and salinity effects on oxygen consumption of shrimp; uses fiber optic microsensors				
Simultaneous measurement of the distribution of oxygen and pH in natural marine sediments	(26)			
Porous plastic sensor also was reported for the determination of dissolved oxygen in	(27)			
seawater				
Small-scale oxygen measurement in riverine sediments using fiber optic sensors:	(28)			
measurement performed over a 12-month period;				
Oxygen consumption of reef-building corals (Acropora intermedia)				
Imaging of oxygen dynamics within the endolithic algal community of the massive coral norites lobata	(30)			
Oxygen microsensor for in-situ measurement of gross photosynthesis using a light-shade	(31)			
Sillit include	(32)			
of dissolved organic matter in freshwater	(32)			

Functional and structural imaging (or oxygen) of phototrophic microbial communities and	(33)	
symbioses		
Biomass-specific respiration rates of benthic meiofauna; demonstration of a novel oxygen	(34)	
micro-respiratory system		
Small-scale oxygen measurement in riverine sediments		
Planar optical sensor film used to image concurrent oxygen; applied to a benthic	(36)	
phototrophic community		
Tracking study on the plume transport and biodegradation of hydrocarbons at Deepwater	(37)	
Horizon; dissolved oxygen concentrations suggest that microbial respiration rates within the		
plume were not appreciably more than 1 micromolar oxygen per day		
Microbial respiration of oil from the Deepwater Horizon spill in offshore surface waters of	(38)	
the Gulf of Mexico		
High resolution color ratiometric planar optical sensor-based imaging; appllied to sensing of		
oxygen and pH <sup>•</sup> utilizes the inbuilt color filter of digital cameras to record the red green and		
blue (RGB) colors of luminophore emission	(39)	
Imaging of surface oxygen dynamics in corals using magnetic microsensor particles	(40)	
Fiber ontic oxygen sensor to measure the diffusion across sediment-water interfaces: has fast	(41)	
response ( $\sim 160$ ms): oxygen fluctuations used to calculate flux	()	
Method for in gity calibration of Aendered enticel evygen sensors on surface meetings	(42)	
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#### S12. Oxygen Sensors in Environmental and Geosciences

Dissolved oxygen concentration is a key parameter in terms of assessing the quality of surface waters and of recycled wastewater. The so-called biochemical oxygen demand (BOD) is another important parameter that relates to the oxygen consumed in 5 days by aerobic microorganisms (BOD<sub>5</sub>) to the quality of (waste) water and its loading with biodegradable matter. Such sensors consists of layers of (a) an oxygen-sensitive fluorescent material, and (b) immobilized microbes such as *Trichosporon cutaneum* in matrices such as poly(vinyl alcohol). The quantity of oxygen consumed by the microbes is indicated by the oxygen sensor material. This scheme largely reduces the time for measuring BOD (typically response times are 10 min only), and the results correlate well with the conventional BOD<sub>5</sub> method that takes 5 days.

 Table S12. Applications of optical oxygen sensors in environmental and geosciences

Application keywords	Refs.		
Determination of biochemical oxygen demand using a sensor; takes 15 min only; uses	(1)		
immobilized yeast (Trichosporon cutaneum)			
Fiber optic sensor for seawater BOD; takes 15 min only; uses immobilized bacteria;	(2)		
response time 15 min, covers the $1 - 10$ mg/L BOD range; hardly influenced chloride (up to			
1 g/L); not affected by most heavy metal ions			
Distribution of microbes in sediments of a lowland river: relationships with environmental	(3)		
gradients and bacteria; spatial distribution imaged; applied to Protozoa bacterial respiration			
BOD sensor; uses a sensing film immobilized on the bottom of sample vials and activated	(4)		
sludge and Bacillus subtilis immobilized in a sol-gel modified with poly(vinyl alcohol) and			
poly(vinylpyridine); uses Ru(dpp) as the luminescent probe			
Composite thin film optical sensor for dissolved oxygen in contaminated aqueous	(5)		
environment			
Fiber optic BOD sensor; uses of three kinds of seawater microorganisms immobilized in a	(6)		
poly(vinyl alcohol)/ormosil hybrid matrix			
Assessment of in situ biodegradation of monochlorobenzene in contaminated groundwater			
and sediments			
Effects of iron ions on chemical sulfide oxidation in wastewater from sewer networks	(8)		
Diffusion and microbiologial activity in pyrite oxidation in abandoned lignite mines	(9)		
Kinetics of chlorobenzene biodegradation under reduced oxygen levels; uses the activity of	(10)		
chlorocatechol-1,2-dioxygnase			
Fiber optic oxygen sensors used in glacial ecosystems; freeze-thaw cycles are compromising	(11)		

function; modifications required to explore Antarctic sub-glacial lakes	
Fiber-optic oxygen sensors for harsh underground environments; thermostable sensor (a	(12)
silicone film containing a luminescent Ru(II) complex); thermostable; works well at 80 °C	
Oxygen diffusivity in waste heaps of a lead-zinc mine (pyrite); acid mine drainage also	(13)
studied	
High-resolution monitoring of oxygen; uses fiber optic sensor; reveals dissolved oxygen	(14)
dynamics in an Antarctic cryoconite hole	
Sensor array for long-term in-situ oxygen measurements in soil and sediment	(15)
Oxygen-sensitive gel placed on the bottom of a micro titer plate used for physiological	(16)
profiling of soil microbial communities	
Review on recent advances in optical biosensors for environmental monitoring and early	(17)
warning; includes the use of oxygen sensors acting as transducers	
Review on methods for assessing biochemical oxygen demand (BOD)	(18)

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## S13. Oxygen Sensors in Food Technology and Packaging

Oxygen is associated with food spoilage because its presence is essential for the growth of moulds and other aerobic microorganisms. Most food therefore is packed in modified (oxygen-free) atmosphere. The so-called modified atmosphere packaging (MAP) under reduced or zero levels of oxygen can keep food longer fresh. Sensors typically are placed inside packed food or bottles to detect their tightness. Colorimetric oxygen sensors with their distinct color changes, typically from colorless to blue or red (see **Figure S7**) are well accepted because they can be inspected with bare eyes. Such irreversible "sensors" (better: regenerable probes) are commercially available.

<sup>(1)</sup> C. Preininger, I. Klimant, O. S. Wolfbeis, Anal. Chem., 1994, 66, 1841

**Figure S7**. Chromogenic oxygen sensitive paints for "smart" food packaging and monitoring freshness. Reprinted from ref. (8) with permission.



Non-invasive but reversibly responding oxygen sensing systems for sealed packages and bottles also have been commercialized. These systems make use of quenchable (metal-organic) probes and can measure oxygen non-invasively, and both in the headspace and in solution. Oxygen sensors also have been used to detect the tightness of plastic bottles, mainly for soft drinks. **Table S13** gives representative examples.

Table S13. Selected applications of optical oxygen sensors in food technology

Application keywords	Ref.		
Sensors for oxygen, pH and ammonia for quality control of packaged organic substances and			
packaging			
Oxygen in modified atmosphere packaged ham; uses phase fluorometry;			
Reversible oxygen sensor; uses Ru(dpp) in an ormosil matrix; and frequency domain lifetime	(3)		
read-out; LOD: 0.05% oxygen (v/v); intended for use in MAP			
Review on (irreversible) oxygen indicator for use in food packaging	(4)		
Study of migration of active components of phosphorescent oxygen sensors for food	(5)		
packaging applications; six sensor materials tested;			
Oxygen levels in industrial modified atmosphere packaged cheddar cheese	(6)		
Quality and safety assessment of packaged green produce; uses the GreenLight <sup>™</sup> system for	(7)		
rapid enumeration of total viable counts in food homogenates, and the Optech <sup>TM</sup> system for			
non-destructive sensing of residual oxygen in package headspace			
Water-proof, irreversible, reusable, UV-activated, oxygen-sensitive plastic film consisting of	(8)		
Methylene Blue and a sacrificial electron donor (DL-threitol) coated onto an inorganic			
support with semiconductor functionality			
Non-invasive oxygen sensor applied to determination rapeseed oil shelf-life	(9)		
Study on the effect of modified atmosphere packaged bread; uses oxygen sensors and active			
ethanol emitters			
Characterization (oxygen barrier properties) of polylactate-limonene polymer blends for food			
packaging applications			
Novel water-resistant UV-activated oxygen indicator for intelligent food packaging; made	(12)		
from thionine, glycerol, $TiO_2$ , and zein; leaching strongly reduced			
Oxygen sensors for product component selection in the development of packages for ready-			
to-eat salads			
Method for measurement of antioxidant activity; antioxidants separated by HPLC, substrate	(14)		
TMAMQ is reduced and reoxidized by laccase; consumed oxygen is directly proportional to			
the concentration of antioxidants and measured by the oxygen sensor			
Review on methods for monitoring the quality of perishable food; also covers techniques	(15)		
based on sensors			
Optical sensors for determination of dissolved oxygen in wine; use optical sensor with Ru(II)			
probe			
Measurement of oxygen ingress into PET Bottles using fiber optic sensors	(17)		

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  - Publication\_2006\_Stangelmayer\_Oxygen\_Ingress\_measurement\_into\_PET.pdf (visited in March. 2014)

### S14. Oxygen Sensors for Use in Gas Pressure-Sensitive ("Barometric") Paints

Inspired by early work of Peterson & Fitzgerald (1) who demonstrated the feasibility of "seeing" oxygen partial pressure via quenching of luminescence, the method was soon extended not only to determine oxygen levels at ambient pressure but to sense (image) air pressure in general. The respective sensor materials often are referred to as a pressure-sensitive paints (PSPs). **Figure S8** gives a typical example of a pseudo-color image of the distribution of air pressure. The major initial application was in wind tunnels since this method is extremely powerful in that pictures of air pressure can be acquired within milliseconds. This is quite an advantage when engineering aircrafts, missiles, high-speed cars, or propeller or turbine blades.

PSP-based methods are clearly superior to those previously applied and have the additional attractive feature that no holes, taps and cables are needed (like in case of other sensors) which cause undesired turbulences. Current challenges include the generation of distance-independent signals, response time of <20 ms, mechanically stable paints with good adhesion (so to survive supersonic wind speeds up to 25 Mach in case of spacecrafts), paints that are photostable for up to 12 h, ease of calibration, and the use of affordable and environmentally friendly materials (both probes and polymers), among others. Various manufacturers are offering PSPs. Table S14 give selected examples. Many more can be found by searching for pressure-sensitive paints. Temperature-sensitive paints also have been reported. The state of the art in fast PSPs has been reviewed (23) and covers development of porous binders, the selection of luminophore molecules suitable for unsteady testing, dynamic calibrations of PSP, data-acquisition methods, and noteworthy applications for flow and acoustic diagnostics. Issues of calibration also are addressed. The dynamic response of fast PSP typically show a flat frequency response to at least 6 kHz, with some paint formulations exceeding a response of 1 MHz. Various applications of fast PSP are discussed that highlight the capabilities of the technique, and concluding remarks highlight the need for the future development of fast PSP.

**Figure S8**. Visualization of the air pressure on 3-D surfaces using a pressure sensitive paints. Pressure is given in pseudo-colors. Reprinted from ref. (10)



**Table S14.** Selected applications of optical oxygen-sensitive materials as gas pressure-sensitive paints (PSPs)

Application keywords(Keys.)Oxygen quenching used for the first time to visualize (image) the flow of gas(1)Polysiloxane-grafted copolymer pressure-sensitive adhesive composition and sheet material(2)Review on criteria, materials and methods in temperature- and pressure-sensitive paint(3)technology by the year 1995(4)Competent and comprehensive overview quenching of luminescence by oxygen , and its application to sensing air pressure in wind tunnel research(4)PtOEP and polydimethylsiloxane in toluene; sprayed onto a model of an aircraft; barometric pressure recorded in a wind tunnel via luminescence intensity; first PSP(5)Porphyrin-based PSP; response times as low as 5 ms; used at wind speeds of up to Mach 8.(6)Study on the ideality of pressure-sensitive paint using PtTFPP in a fluoroacrylic polymer (7)(7)temperature-independent PSP; uses a bichromophoric luminophore consisting of Ru(bipy) and pyrene, dispersed in a co-polymer of poly(ethylene glycol)ethyl ether methacrylate and tri(propylene glycol) diacrylate.(9)(a) Optical oxygen sensor coating based on the fluorescence quenching of a new pyrene derivative; (b) Study of the mechanism of degradation of pyrene-based pressure sensitive paints(10)PSP based on the probe PtTFPL and the temperature sensitive probe MgTFPP in FIB polymer (see Section 7.1.2)(11)PSP using the probe pyrene and a europium complex as a reference dye and for temperature compensation; require UV excitation(12)Water-based and sprayable paint for imaging pressure and temperature; paint can be easily removed from the surface by washing with water(14)
Oxygen queriening used for the instrume to visualize (image) the forw of gas       (3)         Polysiloxane-grafted copolymer pressure-sensitive adhesive composition and sheet material       (2)         Review on criteria, materials and methods in temperature- and pressure-sensitive paint       (3)         technology by the year 1995       (4)         Competent and comprehensive overview quenching of luminescence by oxygen, and its application to sensing air pressure in wind tunnel research       (4)         PtOEP and polydimethylsiloxane in toluene; sprayed onto a model of an aircraft; barometric pressure recorded in a wind tunnel via luminescence intensity; first PSP       (5)         Porphyrin-based PSP; response times as low as 5 ms; used at wind speeds of up to Mach 8.       (6)         Study on the ideality of pressure-sensitive paint using PtTFPP in a fluoroacrylic polymer       (7)         temperature-independent PSP; uses a bichromophoric luminophore consisting of Ru(bipy)       (8)         and pyrene, dispersed in a co-polymer of poly(ethylene glycol)ethyl ether methacrylate and tri(propylene glycol) diacrylate.       (9)         (a) Optical oxygen sensor coating based on the fluorescence quenching of a new pyrene derivative; (b) Study of the mechanism of degradation of pyrene-based pressure sensitive paints       (10)         PSP based on the probe PtTFPL and the temperature sensitive probe MgTFPP in FIB polymer (see Section 7.1.2)       (11)         PSP/TSP dual paint using two iridium(III) complexes       (12)         Wat
(3)         Review on criteria, materials and methods in temperature- and pressure-sensitive paint       (3)         technology by the year 1995       (3)         Competent and comprehensive overview quenching of luminescence by oxygen , and its       (4)         application to sensing air pressure in wind tunnel research       (5)         PtOEP and polydimethylsiloxane in toluene; sprayed onto a model of an aircraft; barometric       (5)         pressure recorded in a wind tunnel via luminescence intensity; first PSP       (6)         Study on the ideality of pressure-sensitive paint using PtTFPP in a fluoroacrylic polymer       (7)         temperature-independent PSP; uses a bichromophoric luminophore consisting of Ru(bipy)       (8)         and pyrene, dispersed in a co-polymer of poly(ethylene glycol)ethyl ether methacrylate and tri(propylene glycol) diacrylate.       (9)         (a) Optical oxygen sensor coating based on the fluorescence quenching of a new pyrene derivative; (b) Study of the mechanism of degradation of pyrene-based pressure sensitive paints       (10)         PSP based on the probe PtTFPL and the temperature sensitive probe MgTFPP in FIB       (10)         polymer (see Section 7.1.2)       (11)         PSP/TSP dual paint using two iridium(III) complexes       (12)         Water-based and sprayable paint for imaging pressure and temperature; paint can be easily removed from the surface by washing with water       (14)
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Competent and comprehensive overview quenching of luminescence by oxygen , and its application to sensing air pressure in wind tunnel research(4)PtOEP and polydimethylsiloxane in toluene; sprayed onto a model of an aircraft; barometric pressure recorded in a wind tunnel via luminescence intensity; first PSP(5)Porphyrin-based PSP; response times as low as 5 ms; used at wind speeds of up to Mach 8.(6)Study on the ideality of pressure-sensitive paint using PtTFPP in a fluoroacrylic polymer(7)temperature-independent PSP; uses a bichromophoric luminophore consisting of Ru(bipy) and pyrene, dispersed in a co-polymer of poly(ethylene glycol)ethyl ether methacrylate and tri(propylene glycol) diacrylate.(8)(a) Optical oxygen sensor coating based on the fluorescence quenching of a new pyrene derivative; (b) Study of the mechanism of degradation of pyrene-based pressure sensitive paints(9)PSP based on the probe PtTFPL and the temperature sensitive probe MgTFPP in FIB polymer (see Section 7.1.2)(10)PSPTSP dual paint using two iridium(III) complexes(12)Water-based and sprayable paint for imaging pressure and temperature; paint can be easily removed from the surface by washing with water(14)
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Referenced PSP/TSP with color camera read-out   (14)
PSP based on PtTFPP or Ru(dpp) adsorbed on silica nanoparticles; slurry used as a (15)
sprayable paint; pressure sensitivity is -0.94%/kPa for PtTFPP and -0.64%/kPa for
Ru(dpp); response time <0.1 ms but substantial temperature sensitivity (ca. 1.5%/°C)
2-color pressure- and temperature-sensitive paint; uses a Pt(II) porphyrin in I-
(trimethylsilyl)phenyl-2-phenylacetylene; blue-green fluorescence of polymer is
temperature-sensitive but not affected by oxygen; red emission of the Pt complex is both
pressure- and temperature-sensitive; 10 ms response time
pressure-sensitive paint (PSP) measurement system based on an electroluminescence (EL) as (17)
a surface infumination, consists of an inorganic EL foil used for infumination, uses a platinum
Destastable 2 luminonhore procesure consistive point based on mesonerous silice (18)
Photostable 2-luminophore pressure-sensitive paint based on mesoporous silica (16)
Mation conturing programs consistive point method: uses a two color DSD control surface with (19)
a high-speed color camera, acquires blue and red images simultaneously; blue emission from
a man-speed color camera, acquires once and red mages simulaneously, once emission nominated a granting dot pressure-sensitive red luminescence from Bu(nhen)
PSP measurements of transient shock phenomena: uses thin-layer chromatography plate (20)
dipped in a luminophore solution: sample imaged at 100 kHz: applied at Mach 2 wind speed

In-flight application of a PSP to a pylon surface of an aircraft; three methods employed: (a)	(21)	
intensity method with LED array, (b) intensity method with electroluminescent foil, and (c)		
lifetime imaging; methods (a) and (b) yield good-quality images		
Dual luminescent arrays sensor fabricated by inkjet-printing of pressure- and temperature-		
sensitive paints		
Review on PSPs for flow and acoustic diagnostics	(23)	

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#### S15. Oxygen Sensors as Transducers in Biosensors

Oxygen is a substrate or co-substrate of numerous enzymes out of the group of oxidases and oxygenases. In fact, any biochemical reactions involving the formation or consumption of oxygen (e.g. those catalyzed by peroxidases or catalases as well) can be monitored over time via optical sensors for oxygen. Oxidase and related enzymes catalyze the oxidation if their substrates, and at the same time cause the consumption of oxygen which can be measured via sensors for oxygen. A sensor materials therefore often consists of an OSP for oxygen, an oxidase, and a (polymer) materials that incorporates both. However, most present-day sensors are more complex in that probe and enzyme are contained in different phases and/or in micro- or nano-particles. Reviews (see refs. (1 and 2 in the Table below) on optical biosensors also cover sensors for substrates of oxidases and oxygenases. Sensing glucose is considered as the "holy grail" in sensor technology and by far the

most important kind of biosensor given the fact that 4% of lycol len suffer from diabetes.

Such sensors require a continuous supply of oxygen (which must not become rate-limiting), and a constant background of oxygen. This is often not the case. Varying background levels of oxygen in the sample can be compensated for by a 2-sensor technique, with one sensor containing the enzyme, and the other not. Respective mathematical models have been derived. Obviously, such sensors do not work properly (or at all) if oxygen becomes rate limiting or is completely absent.

Oxygen sensors also can be used to lycol len the activity of catalase, peroxidase, and polyphenol oxidase. Oxygen sensors also have been applied in transducers for the determination of biochemical oxygen demand (see section 11 before) and in a (commercialized) test for the detection of *Mycobacillus tuberculosis* in blood. In such a system, the growth of *M. bacillus* (if present) is associated with a decreases in the particle pressure of oxygen inside a closed vial, and this is detected by an oxygen sensor placed at the bottom of the vial. This is shown in **Figure S9**.

**Figure S9**. Test tube (type BD 960) for visual detection of *M. tuberculosis*. The bottom layer is composed of a silicone layer containing a ruthenium probe for oxygen. If *M. tuberculosis* is present, its growth will cause consumption of most of the oxygen in the vial, and this results in reduced quenching (i.e. brighter luminescence) as can be seen in some samples. Other systems with instrumental readout (such as the BD MGIT series) also known. Reprinted from http://catalog.bd.com/ with permission.



Oxygen sensors may also serve as transducers in the assay of hydrogen peroxide (HP), a species produced by oxidases. If HP is decomposed by a catalyst (catalase, or silver nanoparticles) to give water and molecular oxygen, a local increase in  $pO_2$  will be observed which may be monitored via an oxygen sensor. The method is not very sensitive, however.

<b>Table S15.</b> Applications of optical	oxygen sensitive materials	as transducers in biosensors based on
the use of oxidases, oxygenases,	peroxidases and catalases.	. Gox: glucose oxidase. LOD: limit of
detection; OSP: oxygen-sensitiv	e probe.	-

Application keywords	Refs.
Review on optical biosensors, also for enzyme substrates	(1)
Review on optical methods (enzymatic and others) for glucose	(2)
Review on enzyme-based biosensors including those based on optical sensors acting as	(3)
transducers	
Ascorbate (vitamin C) (a) via ascorbate oxidase (from cucurbita species) or (b) by catalytic	(4)
oxidation using copper(II) ion as a catalyst	
Aspartame via a bienzyme system immobilized on eggshell membrane on top of an oxygen-	(5)
sensitive membrane; LOD: 32 µM	
Bilirubin via bilirubin oxidase; fiber optic system; for bilirubin in serum: LOD: 0.1 µM;	(6)
OSP: Ru(dpp)	
BOD (biochemical oxygen demand); fiber optic; consists of layers of (a) an oxygen-sensitive	(7)
fluorescent material, (b) <i>Trichosporon cutaneum</i> immobilized in poly(vinyl alcohol), and (c)	
a permeable polycarbonate membrane to retain the yeast cells; 20 min response time	
BOD sensor using other bacteria; 15 min, works best at 30 °C and pH 7.0; working range 1 –	(8)
10 mg/L; response hardly influenced by chloride (up to 1 g/L) and by most heavy metal ions	
BOD sensor using three kinds of seawater microorganisms immobilized in a poly(vinyl	(9)
alcohol)/ormosil hybrid matrix	
Catalase activity determined using an oxygen transducer; applied to activity assays in raw	(10)
coffee beans	
Cholesterol via cholesterol oxidase; enzyme in a hydrogel network; oxygen sensitive	(11)

microparticles; works best in micellar solutions: LODs 0.05 to 0.2 mM; OSPs: decacyclene or Ru(dpp)	
Choline in phospholinids of serum using a choline oxidase and an oxygen sensor membrane	(12)
Ethanol in aqueous solution via alcohol oxidase: LOD 10 mM: Ru(bipy) used as the OSP	(12)
Ethanol in breath air using alcohol oxidase: Ru(II) complex used as a probe for oxygen	(13)
Ethanol in organic solvents: uses immobilized spongiform species in a hydrogel co-	(14)
entrapped with alcohol oxidase and horseradish peroxidase with octadecyl silica	
glutamate via immobilized oxidase: flow injection mode and using fiber ontics with	(15)
decacyclene in silicone rubber as the oxygen sensor	(-)
Glucose via Gox: LOD: 0.05 mM: OSP: decacyclene	(16)
Glucose and lactate in animal cell cultures: uses fiber ontic oxygen sensor as a transducer:	(17)
applied in a flow injection mode	
Glucose lactate and alcohol using the respective oxidases and the oxygen probe	(18)
nyrenebutyric acid	()
Glucose via glucose ovidase: various ovygen probes used various materials employed: some	(19)
recent work very similar to earlier work	(1))
Glucose sensor based on an oxygen sensor (ruthenium probe and immobilized Gov: coated	(20)
with carbon black as an ontical isolation: multi-layer design	(20)
Glucose vie Cov: LOD: 0.05 mM lifetime based OSP: DtOED:	(21)
Glucose via Gox: LOD: 0.1 mM: OSP: Pu(dnn)	(21)
Chucose via Gox, LOD. 0.1 mill, OSF. Ku(upp)	(22)
Glucose via Gox using a sol-gel based oxygen transducers, includes a method for	(23)
Chippensating for variable oxygen background. LOD. 0.1 milli, OSP. Ru(upp)	(24)
Chucose via Gox, LOD. 80 µW, OSP. Al(III)/Teriori complex	(24)
Chucose and oxygen via a dual fiber optic sensor	(25)
(lycol)protein subunits and loaded with Gox; OSP: Ru(dpp)	(20)
Glucose biosensor employing an eggshell membrane as an enzyme support and an oxygen	(27)
sensor as the transducer	
Glucose biosensor with enzyme entrapped in a sol-gel and in contact with an oxygen-	(28)
sensitive sensor membrane;	
Glucose biosensor for the rapid determination of glucose in human serum	(29)
Glucose via an optical colorimetric sensor strip for direct (bare-eye) readout	(30)
Glucose and temperature and temperature simultaneously using probes of different decay	(31)
time; application to monitor glucose while temperature varies	
Glucose in microtiter plates with integrated optical sensors for oxygen	(32)
Glucose monitoring in subcutaneous tissue using a miniaturized fiber optic dual sensor	(33)
Glucose via a fiber optic flow-through sensor; enables on-line monitoring	(34)
Glucose, other systems (all using Gox as the enzyme and oxygen transduction)	(35)
Hydrogen peroxide via its decomposition by catalyse or silver particles to form oxygen and	(36)
water; oxygen is detected.	
Immunoassay where the secondary antibody is labeled with Gox; demonstrated for the IgG-	(37)
anti-IgG system in a sandwich format; glucose added as an enzyme substrate; consumption	
of oxygen measured with oxygen probe	
Lactate (a) via lactate oxidase; OSP: pyrenebutyric acid; (b) via lactate oxidase; OSP:	(38)
decacyclene; LOD: 0.02 mM; (c) via lactate monooxygenase; LOD: 0.3 mM	
Lipids using lipoxidase (from soybean),	(4)
Methanol via alcohol oxidase and peroxidase; LOD: 80 µM-60 mM ; OSP: Ru(dpp)	(39)
Oxygen sensor used for the for the detection of <i>Mycobacillus tuberculosis</i> in blood:	(40)
measures consumption of oxygen in closed vial within $2 - 4$ days; commercialized by	
Becton-Dickinson and in widespread use; other bacterial tests also discussed	
Organic-phase phenol using enzymatic oxidation with chemical reduction	(41)

Phenol via tyrosinase; LOD: 0.08 mM; OSP: Ru(dpp)	(41)
Phenol via phenolase (from potato)	(4)
Sulfite via sulfite oxidase; can detect ppm levels; OSP: perylene	(42)
Uric acid; uses uricase and oxygen transduction	(43)
Xanthine via xanthine oxidase on an oxygen sensor film	(4)
Biogenic amines determined with oxygen sensitive magnetic particles; diamine oxidase	(44)
immobilized either on chitosan-coated magnetic microparticles or on commercial	
microbeads modified with a ferrofluid	
Histamine sensing platform based on a diamine oxidase and oxygen nanosensors particles	(45)
for in vivo tracking	
Glucose biosensor using glucose oxidase and a crystalline It(III) complex acting as a probe	(46)
to detected the quantity of oxygen consumed	

A review has been published<sup>47</sup> on recent developments in enzyme-based biosensors for biomedical analysis with respect to design characteristics, performances, and applications, with a focus on electrochemical and optical sensors. It mainly covers emerging technologies and innovative biosensing designs such as nanosensors, paper based-sensors, lab-on-a-chip methods, biochips, and microfluidic devices.

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## S16. Oxygen Sensors Integrated into Microfluidic Devices and Lab-on-a-Chip Systems

This is a comparatively new area. It was soon recognized that microfluidic systems and so-called labs-on-a-chip (LOACH) are limited by the size and costs of detectors. Numerous articles can be found where elegant microchips and LOACHs are described but with detectors that are in acceptably large, complex, or expensive. It was soon recognized that (luminescent) optical sensors for oxygen can be fairly easily integrated directly into such systems and that read-out is accomplished by using solid-state electronic components such as LED light sources and photodiode detectors. The Table below gives a couple of impressive examples of such systems. These are often used now for purposes of cell culturing, combinatorial biotechnology, and for screening. Related work can be found in the biotechnology applications section. **Figure S10** shows a typical layout for a microfluidic chip that also incorporates a sensor for oxygen.

**Figure S10**. Integrated microfluidic chip (referred to as a "chemostat and turbidostat") with control of flow rate, oxygen, and temperature for dynamic continuous culture monitoring. Reprinted from ref. (18) with permission.



Table S16. Microfluidic Devices and Lab-on-a-Chip Systems with Integrated Sensors for C	Dxygen
System and Function	Ref.
Optical sensor instrumentation using absorption- and fluorescence-based capillary	(1)
waveguide sensors	
Flow-through capillary (µm-sized) with optical sensors placed on the inner surface	(2)
Integrated optical oxygen sensor fabricated using rapid prototyping techniques	(3)
Membrane-aerated microbioreactor for high-throughput bioprocessing	(4)
The design and fabrication of 3-chamber microscale cell culture analog devices with	(5)
integrated dissolved oxygen sensors	
Integrated microfluidic platform for dynamic oxygen sensing and delivery in a flowing	(6)
	(7)
Multiplexed microbioreactor system for high-throughput bioprocessing	(/)
Optical imaging in microfluidic bioreactors; applied to continuous monitoring of oxygen	(8)
The application of frequency-domain Eluorescence Lifetime Imaging Microscony as a	(9)
quantitative analytical tool for microfluidic devices	(-)
Quantitative measurement and control of oxygen levels in microfluidic	(10)
poly(dimethylsiloxane) bioreactors during cell culture	
Patterning, integration and characterization of polymer optical oxygen sensors for	(11)
microfluidic devices	
Hard top soft bottom microfluidic devices for cell culture and chemical analysis.	(12)
In-situ measurement of cellular microenvironments in a microfluidic device	(13)
Culturing aerobic and anaerobic bacteria and mammalian cells with a microfluidic	(14)
differential oxygenator	
Precise control over the oxygen conditions within the Boyden chamber using a	(15)
microfabricated insert.	
Pressure-sensitive molecular film for investigation of micro gas flows	(16)
Review on optical oxygen sensors for applications in microfluidic cell culture systems	(17)
including stem cells	(10)
Microfluidic chemostat and turbidostat with flow rate, oxygen, and temperature control	(18)
for dynamic continuous culture (see the figure above)	(10)
Integrated microfluidics on waveguide-based photonic platforms fabricated from	(19)
Microfluidic oxygen imaging using integrated ontical sensor layers and a color camera	(20)
Microfluidic capillary ontical fiber with a ring-shaped waveguide: inner surface of	(21)
capillary coated with ratiometric oxygen-sensitive doped organosilica gel	
Measurement of dissolved oxygen diffusion coefficient $(2.32 \times 10^{-9} \text{ m2/s})$ in a	(22)
microchannel using 450-nm LED induced fluorescence method	
Spatially resolved monitoring of oxygen in 3D microfabricated epithelial Caco-2 cell	(23)
systems using optical oxygen sensing beads	
Review on optical oxygen sensors for micro- and nanofluidic devices	(24)
Microfluidic in-fiber oxygen sensor derivates from an optical fiber with a ring-shaped	(25)
waveguide	

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## S17. Oxygen Sensors in Industry, Material Research, Production and Surveillance

Oxygen (aside from water) probably is the most important "reagent" in industry. It is required in combustion, steel-making welding, in car motors and jet engines (rockets included), power plants, and of course the chemical industry. Oxygen also has to be monitored in the exhaust stream of combustion engines, in fuel-based power plants and in millions of simple boilers. The control of oxygen in fuel cells represents a particularly large future market. Thermostable sensors may be applied as a lambda probe in car exhaust gases. Aside from combustion, oxygen also plays an important role in manufacturing silicon oxides as used in integrated circuits. Oxygen detection is also important in from a safety standpoint, as oxygen leaks can cause fires and explosions and can be harmful in storage chambers. Sensors for oxygen were used to determine the permeability of polymers. Numerous other applications have been, or will be, found but this exceeds the length of the article.

 Table S17. Selection of Oxygen Sensors Used in Industry, Material Research, Production, and

 Surveillance

Surveinance	
Application keywords	Ref.
Spatially resolved sensing of oxygen in microfluidic devices; uses a sensor layer (PtOEPK in	(1)
PS) integrated into PDMS-based microchannels; range: 0 to 34 mg/L of oxygen in water	
Environmentally-friendly manganese-based catalyst for alkyd emulsion coatings; mechanism of	(2)
drying; ethyl linoleate as a model substance	
Onset of oxygen production in water electrolysis on raising the voltage from 0 to 2.6 Volt; uses	(3)
phosphorescent dye adsorbed on silica gel	
Determination of dissolved oxygen in perfluorocarbon emulsions and other solutions;	(4)
diffusivity also studied; oxygen consumption microchamber coupled to a glucose oxidase	
reaction for the quantitative determination of oxygen; flow chambers fitted with fluorescence	
lifetime oxygen sensors	

Sensing oxygen in aircraft fuel tanks; intensity-based approaches contrasted with frequency	(5)
domain (= lifetime) techniques; complete analytical system suggested	
Long-term corrosion monitoring of radioactive-waste repositories; uses a ruthenium complex	(6)
with a dodecyl sulfate counterion in a silicone layer; sensor degradation in air between 150 °C	
and 250 °C is negligible.	
Corrosion monitoring in boilers; fiber optic sensing system; can detect 0.9 Torr of oxygen in the	(7)
gas phase, and 0.01 mg/L of oxygen in water	
Permeability of contact lenses for oxygen determined with a fiber-optic luminescent sensor	(8)
system; measures the $pO_2$ inside a chamber sealed by the contact lens, where a thin luminescent	
sensor film is deposited	
Fiber optic oxygen sensor leak detection system for space applications	(9)
Phosphorescence quenching used to for determine the permeabilities of polymeric films to	(10)
oxygen	
Spatially resolved sensing of oxygen in microfluidic devices; sensor layer (PtOEPK in PS)	(11)
integrated into PDMS-based microchannels; analytical range: 0 to 34 mg/L of oxygen in water	
Determination of oxygen diffusion coefficients in glassy PMMA films and effect of a	(12)
crosslinking additive; effect of a castor oil-derived additive on the oxygen diffusivity of	
polystyrene	
Dual-chromophore paint with oxygen-insensitive green and oxygen-sensitive red fluorescence	(13)
used to screen catalyst compositions (such as Ni/Al/Fe oxides) for their efficiency in terms of	
water oxidation	
Fiber optic sensor for power plants; uses clusters of Mo6Cl12 whose luminescence is quenched	(14)
by oxygen; can be continuously operated up to 700 °C	
Determination of solubility of oxygen in air-saturated organic solvents; HPLC columns retain	(15)
oxygen; outlet uses a plastic optical fiber sensor for detection	
Sensor foil for monitoring oxygen in microbial fuel cells	(16)
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