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AN INNOVATIVE LASER-BASED SENSING PLATFORM FOR REAL-TIME OPTICAL MONITORING OF OXYGEN

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Abstract – Known as "gas of life", molecular oxygen is a crucial metabolic factor. Especially in the field of cell biology, the content of ambient oxygen determines proliferation and differentiation. Therefore, it is a mandatory step to monitor and to control the oxygen concentrations in bioreactors for three-dimensional tissue engineering. From experience, the oxygen content of the culture medium does not reflect the circumstances within the cell tissue. Hence, it is important to develop advanced measurement techniques, which indicate the oxygen concentration within the cell carrier. To avoid interferences of the flow conditions and cell growth non-invasive or at least minimal-invasive detection techniques are strongly preferable.
In this paper we will describe the principle of the oxygen determination, results from measurements in biological samples.

Keywords: optical oxygen sensor, phase modulation, distributed sensing

1. INTRODUCTION

In contrast to the conventional Clark electrode, optical oxygen probes do not need any connection wires and show a nearly unlimited miniaturization potential. Optical oxygen measurements base on phosphorescent dyes entrapped in a polymer matrix. Both, the phosphorescence intensity and the decay time decrease in presence of ambient molecular oxygen. Since intensity-based measurements are explicitly susceptible to background signals, time-resolved techniques are applied, such as phase modulation spectroscopy for real-time monitoring. Unfortunately, the basic configuration of this technique, which is used by commercially available optical oxygen meters, is interfered by fluorescence arising from the sample. Especially, during the processing of poor signals of micro- and nanoprobes, background signals distort the result seriously. Therefore, a dual-frequency modulation technique has been developed, which allows the elimination of background fluorescence and is hardly prone to scattering, absorption or ambient light. This method has proven to be most valuable for measurements using micro- and nanoprobes within insect's salivary glands and chlorophyll-containing plant tissue [1, 2].

The new technique has now been implemented into a versatile system consisting of hardware platform, software, detector and light source. It is compatible to existing fluorescence microscopes and bioreactors. The setup is usable for non-invasive measurements with micro- and nanoprobes as well as for the use with fibre optical sensors, which are available in various sizes with tip diameters down to 5 µm. Special probes are obtainable for oxygen detection at trace levels.
For the determination of oxygen, phosphorescent metal complexes of tetrapentafluorophenylphorphyrin (TPFPP) and diphenylphenanthroline (DPP) embedded in specific polymer mixtures were used as sensor material. For applications, in which a broad range of oxygen concentration shall be monitored, the Pt-TPFPP and Ru-DPP probes are most suitable. For measurements, where only traces of oxygen have to be determined, the Pd-TPFPP probe is recommended.

2. RESULTS

2.1. Experimental

The measurement of the decay time is performed by using phase modulations spectroscopy. Here, the intensity of the excitation light is modulating with the frequency *f*. In the case of a monoexponential manner, the decay time τ is calculated by equation (1).

$$\tan(\phi) = 2\pi f \cdot \tau \qquad (1)$$

The usage of two modulation frequencies allows measurements in samples with intrinsic fluorescence or in high scattering media. The technique is described in detail in [1]. Within the last years the electronically as well as the optical set-up was miniaturised for commercialization of the sensing scheme.
Fig. 1 shows the electronic part of the miniaturized equipment [3], in which the lock-in amplifier and the frequency generator are implemented (left) and the optical device (right) containing a violet laser, optical filters, a dichroic mirror and the photodetector.
Depending on the system parameters and the specific requirements, two types of sensor designs can be applied. Similar to a Clark-Electrode, optical sensor fibres with tip diameters down to a few µm can be

built, yielding a fibre optical sensing (FOCS) scheme (Fig. 2, left).

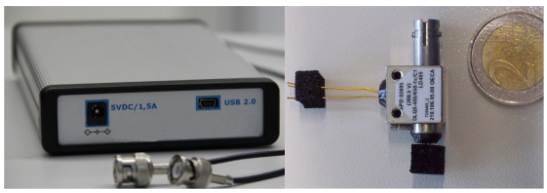

Fig. 1: Left: electronic device, right: optical device for two-frequency modulation technique.

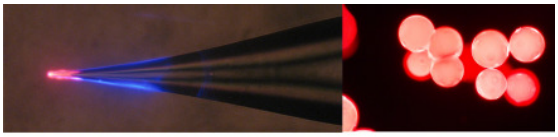

Fig. 2: Left: coated fibre, tip diameter 10 µm; right: 50 µm polystyrene beads coloured by Pt-TPFPP.

The luminescence probe is immobilized in a suitable polymer matrix at the fibre tip and the fibre itself is used to direct the excitation light to the tip and the luminescence signal from the tip to the detector. Alternatively, the dye can be embedded in polystyrene beads, which have diameters from few µm to tenth of nm (Fig. 2, right). Excitation and signal detection can be accomplished afar. In the simplest case a standard fluorescence microscope can be used. The beads can be embedded for example in cell cultures during growth and the concentration of oxygen can be monitored in vivo continuously with high spacial resolution (*vide infra*).

2.2. Principle of Oxygen Quenching

In Fig. 3 the absorption and emission spectra of the oxygen sensitive Pd-TPFPP probe are presented. The emission is located at $\lambda_{em} > 550$ nm with an oxygen sensitive peak at $\lambda_{em} = 675$ nm. This wavelength is beneficial for environmental and life science applications since the intrinsic absorption in the long wavelength region of the electromagnetic spectrum is usually decreasing in real-world samples. Moreover, the large separation between absorption and emission minimizes interferences arising from residual excitation light in the detection channel. In addition, the outstanding long luminescence decay time of this class of luminescence probes allows the use low priced equipment in time-resolved detection schemes.
The intensity of the emission at 675 nm is dependent on the oxygen concentration and decreases in the presence of oxygen due to an effective collisional quenching process (see Fig. 3). Due to the dynamic character of the collisional quenching also the luminescence decay time is decreased.

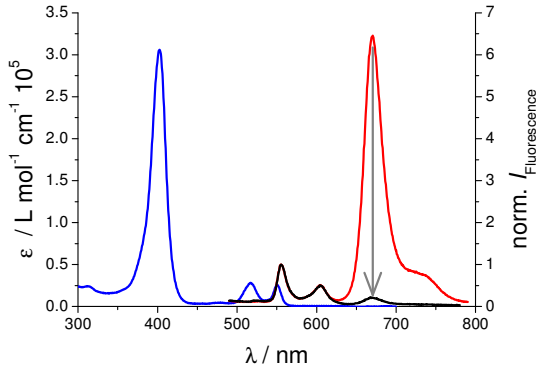

Fig. 3: Absorption (blue) and emission spectra (λ_{ex}= 405 nm) of Pd-TPFPP probe dissolved in EtOH bubbled with 10 *vol. %* oxygen (black) and nitrogen (red).

In contrast to intensity-based measurements the luminescene decay time is independent on the concentration of the emitting species and consequently less prone to matrix-induced changes of the sensor (e.g., photobleaching, sorption of matrix components to the polymer, bleeding of the dye).

2.3. Calibration of sensors

Fig. 4 shows a calibration curve of the Pd-TPFPP fibre optical sensor in aqueous solution for the trace level oxygen concentration range (preferred working range: 0-5 *vol. %*). In Fig. 5 the results of Pd-TPFPP probe embedded in 50 µm polystyrene beads are presented. To convert concentrations in the gas phase (*vol. %*) into dissolved oxygen (µmol·L^{-1}), the solubility table shown in [4] was used (at 20 °C and 1013 mbar, 20.95 *vol. %* O_2 correspond to 284.1 µmol·L^{-1}).

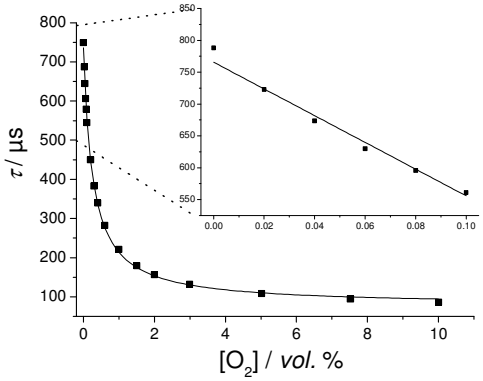

Fig. 4: Calibration curve of fibre-optic Pd-TPFPP sensor in aqueous solution at different oxygen concentrations.

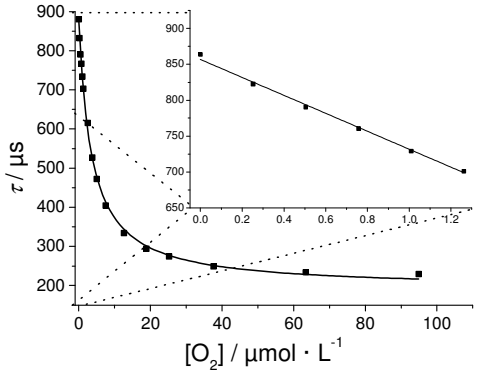

Fig. 5: Calibration curve of Pd-TPFPP probe embedded in 50 µm-polystyrene beads in gas phase at different oxygen concentrations.

The data were fitted with a modified Stern-Volmer equation [5, 6]:

$$\frac{\tau}{\tau_0} = \frac{x}{1 + K_{SV}[O_2]} + (1 - x) \qquad (2)$$

In equation (2) τ represents the luminescence decay time of Pd-TPFPP in the presence and τ_0 in the absence of oxygen, K_{SV} is the Stern-Volmer constant. The term (1 - x) accounts for a fraction of dye molecules embedded in the polymer matrix which can not interact with oxygen. The working range for oxygen detection based on decay time measurements is for the Pd-TPFPP sensor between 0-5 *vol. %* oxygen. For higher oxygen concentrations different probes such as Pt-TPFFP or Ru-DPP are recommended. In Fig. 6 the calibration of a fibre-based Pt-TPFPP fibre optical sensor for the oxygen concentration range of 0-63 *vol. %* is presented. A similar measurement range is accessible with Ru-DPP-based sensors.

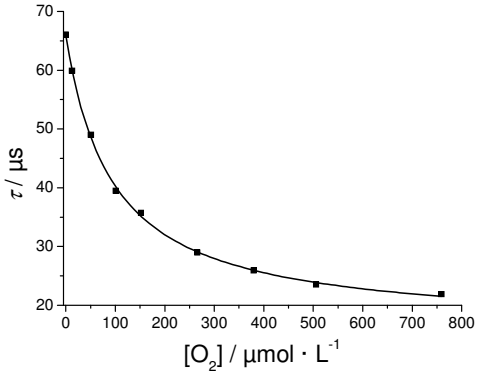

Fig. 6: Calibration curve of Pt-TPFPP sensor in aqueous solution at different oxygen concentrations.

2.4. Fibre optical measurements of oxygen in biofilms

Biofilms are known as populations of microorganisms that are immobilized at surfaces and are defined as matrix-enclosed bacterial populations [7-9]. Biofilms are of great importance in environmental science but also in technical applications such as waste water treatment since they can act as a sink (or source) for many pollutants. An important class of inorganic pollutants are heavy metals, which can be released to the environment from mining (e.g., uranium, which is released to the environment upon flooding of former uranium mining facilities).

In Fig. 7 a depth profile of the oxygen concentration within a biofilm in the absence and presence of uranium is shown. The biofilm has been characterised in-depth and it was suggested that the extracellular polymeric substances (EPS) of the biofilm may fix different uranium species [10,11]. A fibre-optical sensor (tip diameter of about 10 µm) was injected into the biofilm using a micromanipulator. Every 50 µm the sensor was stopped for 10 s and the oxygen concentration was measured.

In the absence of uranium the concentration of oxygen decreases from 260 µmol·L^{-1} at the surface to 200 µmol·L^{-1} at a depth of 0.5 mm. After inducing stress to the organisms by the addition of uranium, a rapid decline of the oxygen concentration down to zero at a depth of 0.5 mm was found.

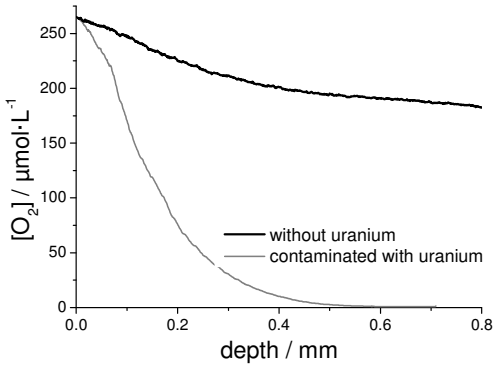

Fig. 7: Fibre optical determination of oxygen with a Pt-TPFPP fibre optic sensor in a biofilm.

2.5. Oxygen measurements in cell cultures using a microbead-based sensor scheme

The cultivation of chondrocytes for anaplasty applications is a major medical research topic [12]. For successful and projected growth of the cell cultures the oxygen content plays an important role [13,14].

Fig. 8 shows polystyrene microbeads containing Ru-DPP as oxygen probe embedded in a culture of chondrocytes (of *Sus scrofa domestica*) during the growth process. The microbeads were suspended within the hydrogel cylinder, which was used as cell carrier. Due to their small size (50 µm diameter), the beads are not incorporated by the cells and consequently cause no harm to the cells. A homogeneous distribution of the microbeads in the cell culture allows a three-dimensional monitoring of the oxygen concentration within the cell culture. Based on such 3D-mapping of oxygen, an improved monitoring and an optimization of the cultivation conditions can be carried out.

At different times during the growth process of the cell culture, luminescence measurements using a con-

focal microscope setup were performed, and the oxygen concentration at different locations (lateral and depth) were determined at 37 °C. From the results received at the position of each microbead, the 3D distribution of oxygen in the cell culture was constructed. After the measurement series, the cells were stained in order to check the vitality using a commercial live/dead kit (Invitrogen L-3224, result see Fig. 8.).

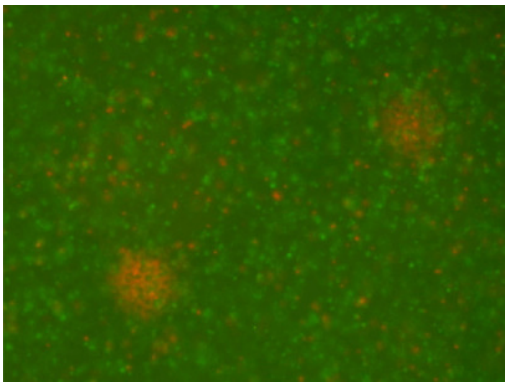

Fig. 8: 50 µm-beads embedded in a culture of chondral tissue cells. The cells were stained in order to check the vitality. The tiny red spots represent death cells, the light green spots living cells. The big red dot at the left bottom and the right top are the oxygen sensor beads.

Fig. 9 shows the oxygen distribution at the beginning of the experiment. The cell carrier (gel cylinder, 10 mm diameter, 3 mm height) was put into a 35 mm-dish and covered with culture medium. The sample was placed on the scanning stage of a Zeiss Axio Observer invert microscope. The microscope was equipped with the Colibri Photonics measurements system [3], consisting of the optoelectronic hardware, external light source and detection optic with a custom confocal unit.

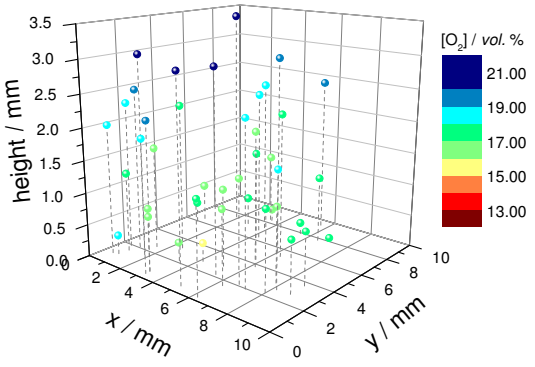

Fig. 9: 3D-plot of the oxygen distribution in a cell culture of chondrocytes at the starting point of the experiment. Every sphere represents one microbead located within the sample and the observed corresponding oxygen concentration at the particular location.

A slight oxygen gradient from the surface to bottom of the cell culture is observed. A change in the oxygen concentration ($\Delta[O_2]$) of about 4 *vol. %* was found. After four days (Fig. 10) the oxygen concentration at the bottom of the cell culture clearly decreased with an overall $\Delta[O_2]$ of 8 *vol. %* compared to the top of the cell culture. A minimum was found at the bottom in the center of the cell carrier. The observed effect can be attributed to the high respiratory activity of the cells during the growth process and the diffusion limitations thru the cell culture medium and thru the gel of cell carrier, which both represent diffusion barriers.

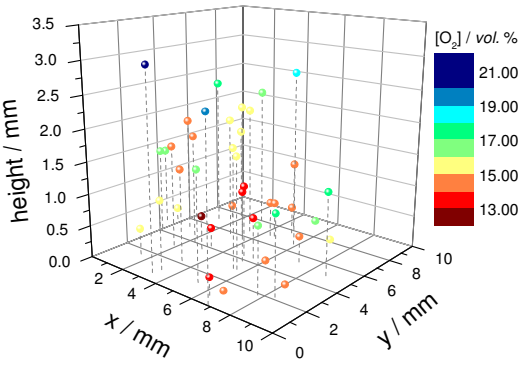

Fig. 10: 3D-plot of the oxygen concentration in a cell culture of chondrocytes after 4 days of growth process. Every sphere represents one microbead located within the sample and the observed corresponding oxygen concentration at the particular location.

3. CONCLUSION

The outstanding performace and capabilities of the dual-frequency phase modulation technique for oxygen determination was shown for two complex biological examples.
The used versatile and robust sensing platform can employed with fibre optical as well as microbead based sensors for measurements at trace level concentrations but also at higher concentrations of oxygen. Further, the method can be used in aqueous as well as in gas phase. It is foreseen to include other chemical parameters such as pH-measurements yielding a multiparameter distributed sensing platform. Due to the obvious advantages of the decay-time based sensing approach compared to intensity-based sensors an outstanding potential for on-line and in-situ measurements in many different application ranging from environmental monitoring to life science can be envisaged.

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REFERENCES

[1] E. Schmälzlin, B. Walz, I. Klimant, B. Schewe, H.-G. Löhmannsröben, "Monitoring hormone-induced oxygen consumption in the salivary glands of the blowfly, Calliphora vicina, by use of luminescent microbeads", *Sensors and Actuators B*, 119, 251–254, 2006.

[2] E. Schmälzlin, J. T. van Dongen, I. Klimant, B. Marmodée, M. Steup, J. Fisahn, P. Geigenberger, H.-G. Löhmannsröben, "An optical multi-frequency phase modulation method using micro-beads for measuring intracellular oxygen concentrations in plants", *Biophys. J.*, 89, 1339-1345, 2005.

[3] www.colibri-photonics.com

[4] B.B. Benson, and D. Krause "The concentration and isotopic fractionation of gases dissolved in freshwater in equilibrium with the atmosphere." 1. Oxygen. *Limnol. Oceanogr*., 25, 662-671, 1980.

[5] J. R. Lakowicz, "Principles of Fluorescence Spectroscopy", *Springer Science and Business Media*, 2006 (third edition), chapter: 8.8, 288-289.

[6] S. M. Borisov, T. Mayr, I. Klimant, "Poly(styrene-block-vinylpyrrolidone) Beads as a Versatile Material for Simple Fabrication of Optical Nanosensors", *Anal. Chem.*, 80, 573-582, 2008.

[7] A.B. Ganesh, T.K. Radhakrishnan, "Fiber-optic sensors for the estimation of oxygen gradients within biofilms on metals", *Optics and Lasers in Engineering*, 46, 321-327, 2008.

[8] H. Beyenal, R. K. Sani, B. M. Peyton, A. C. Dohnalkova, J. E. Amonette, Z. Lewandowski; "Uranium Immobilization by Sulfate-Reducing Biofilms", *Environ. Sci. Technol.*, 38, 2067-2074, 2004.

[9] J.W. Costerton, Z. Lewandowski, D.E. Caldwell, D.R. Korber, H.M. Lappin-Scott, "Microbial biofilms" *Annu. Rev. Microbiol.*, 49, 711-745, 1995.

[10] E. Krawczyk-Bärsch, K. Grossmann, T. Arnold, S. Hofmann, A. Wobus, "Influence of uranium (VI) on the metabolic activity of stable multispecies biofilms studies by oxygen microsensors and fluorescence microscopy", *Geochim. et Cosmochim. Acta*, 72, 5251-5265, 2008.

[11] E. Krawczyk-Bärsch, D. Steinbrueck, M. U. Kumke, "Comparative Study on Electrochemical and Laser-based Fiber-optic oxygen microsensors applied to uranium contaminated biofilms" In: *Wissenschaftliche Berichte - Karlsruhe Institut für Technologie*, (KIT-SR 7557), 103-110, 2010.

[12] S. Marlovits1, S. Aldrian, B. Tichy, C. Albrecht, S. Nürnberger, „Biomaterialien für die autologe Knorpelzelltransplantation", *Der Orthopäde*, 11, 1044 -1052, 2009.

[13] A. Krinner, M. Zscharnack, A. Bader, D. Drasdo, J. Galle, "Impact of oxygen environment on mesenchymal stem cell expansion and chondrogenic differentiation", *Cell Prolif.*, 42, 471-484, 2009.

[14] J. Malda, J. Rouwkema, D. E. Martens, E. P. le Comte, F. K. Kooy, J. Tramper, C. A. van Blitterswijk, J. Riesle, "Oxygen Gradients in Tissue-Engineered PEGT/PBT Cartilaginous Constructs: Measurement and Modeling", *Biotechnology and Bioengineering*, vol. 86, 9-18, 2004.