Oxygen in Action

Planar optical sensors capable of measuring chemical analytes can now be used to map oxygen distributions in animal and plant tissue, and pave the way for breakthroughs in cultivating cell constructs.

Gregor Liebsch at PreSens Precision Sensing GmbH

The market for digital imaging devices is constantly growing and new products with increased resolution and scaled down size are continually being developed. The use of such devices is also of great interest in the field of chemical optical sensing, where the response of fluorescent sensors is recorded in order to measure chemical analytes. Fluorescent optical sensor foils, combined with imaging technology, allow easy visualisation of oxygen distributions and their spatial or temporal changes. This new technology opens up a whole range of applications in all fields of scientific and industrial research.

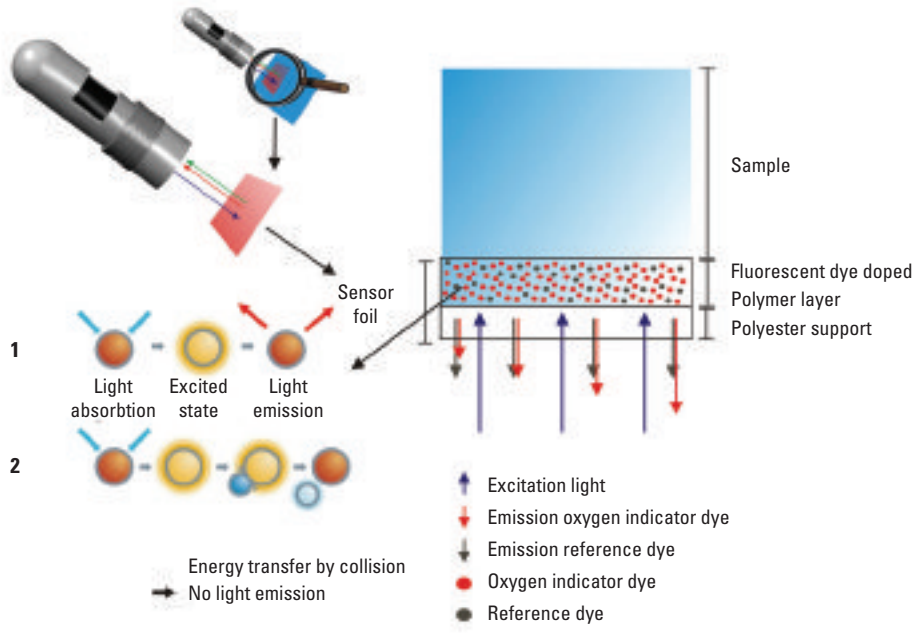
So far, microsensors have been widely used in the life sciences to measure oxygen concentrations. These sensors are often miniaturised Clark-type electrodes, with oxygen diffusing through a membrane and producing a measureable electric current by its reduction at the cathode. As this method consumes oxygen in the measurement process, these microsensors are now often replaced by chemical optical sensors. With these, the oxygen content is detected through dynamic quenching of a luminophore, and precise measurements with high spatial resolution are possible. But as most biological samples – for example animal or plant tissue – are not homogeneous, measurement with these sensors within localised regions or transects can only provide an outline, and lots of information is lost.

A planar optical sensor allowing analyte mapping in 2D can give a detailed picture of oxygen distributions within a sample. The sensor translates the oxygen content into a light signal which can then be recorded pixel by pixel with a digital imaging device, so a single image contains the information of a whole array of sensor points. While sophisticated but bulky camera systems have been used in the life sciences so far, the rapid process in imaging technology now enables the use of cost-effective and compact handheld microscopes for recording the sensor signal.

A New Oxygen Sensing Approach

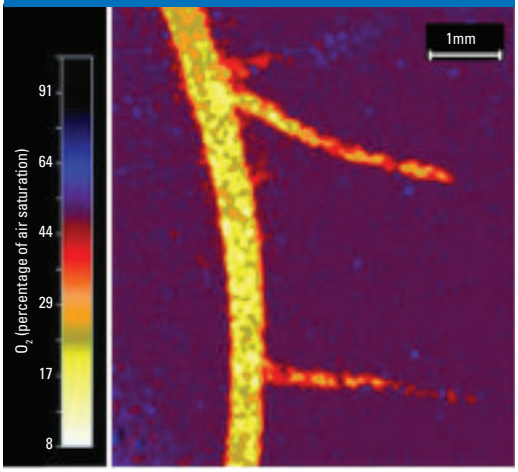
Optical sensor foils contain an oxygen sensitive dye and a reference dye which are immobilised in an oxygen permeable polymer matrix layer. The indicator dye emits red fluorescence which is dynamically quenched by oxygen; energy of the excited dye is transferred to the oxygen molecules and consequently the intensity of the sensitive dye is reduced with increasing oxygen content of the sample (see Figure 1). The reference dye on the other hand is not affected by oxygen and gives a constant green light signal. When the sensitive dye and reference dye are excited with an identical light source

Figure 1: Schematic diagram of the sensor foil, and principle of dynamic fluorescence quenching. The indicator dye is immobilised in a polymer matrix which is fixed to a transparent polyester support. The excited dye emits red fluorescence and transfers energy via collision to oxygen molecules resulting in fluorescence quenching with increasing oxygen content of the sample



Images: Presens Precision Sensing GmbH

Figure 2: Imaging of oxygen consumption of *Brassica napus* roots – image of oxygen distribution after six hours of dark incubation



they emit red or green light respectively. These emissions meet exactly the red and green channel sensitivity of a colour RGB chip. With a non-toxic, non-invasive sensor foil the typical biological range from 0 to 100 per cent air saturation (corresponding to 6.04mL of oxygen per litre at 25°C and 1013hPa) can be detected. The LED-based excitation light source, optical filters and a lens, together with a colour camera, are incorporated in a handheld microscope used for sensor read out.

This new technology can be used in a wide range of applications, from biological and medical research to industrial process monitoring. The two-dimensional visualisation of oxygen distributions can give new insights where only partial information was available so far.

Imaging in Experimental Biology

The planar optical sensors can also be used for oxygen measurements in plants, which are both producers and consumers of oxygen. The complex anatomy of tissue often hinders oxygen diffusion and results in diffusion gradients and local oxygen deficiencies. To visualise oxygen dynamics between tissue and the surrounding media, 2D imaging with fluorescent optical sensors can be used. An experiment using two plant models illustrates this using the respiring root system of the crop plant *Brassica napus* (see Figure 2) and the photosynthetically active leaf of the water plant *Cabomba caroliniana*. All experiments were carried out inside an incubating chamber to limit oxygen diffusion from the outside. The use of oxygen sensor foils allowed the alignment between the sample structure and the measured oxygen concentrations.

To visualise the oxygen consumption of intact roots, *B napus* was grown on 0.9 per cent Difco-agar for 14 days. Root segments of the seedlings were covered with sensor foils. Oxygen distribution in the sample was recorded over six hours at a sampling rate of 15 minutes. Measuring a decline in oxygen concentration the respiration rate of the central root zone – in a defined region of interest – was calculated to be 0.015 per cent air saturation min^{-1} on average, which corresponds to approximately 12.5µmol oxygen h^{-1}. The planar sensor allowed mapping of oxygen consumption for distinct root regions in sub-millimetre scale.

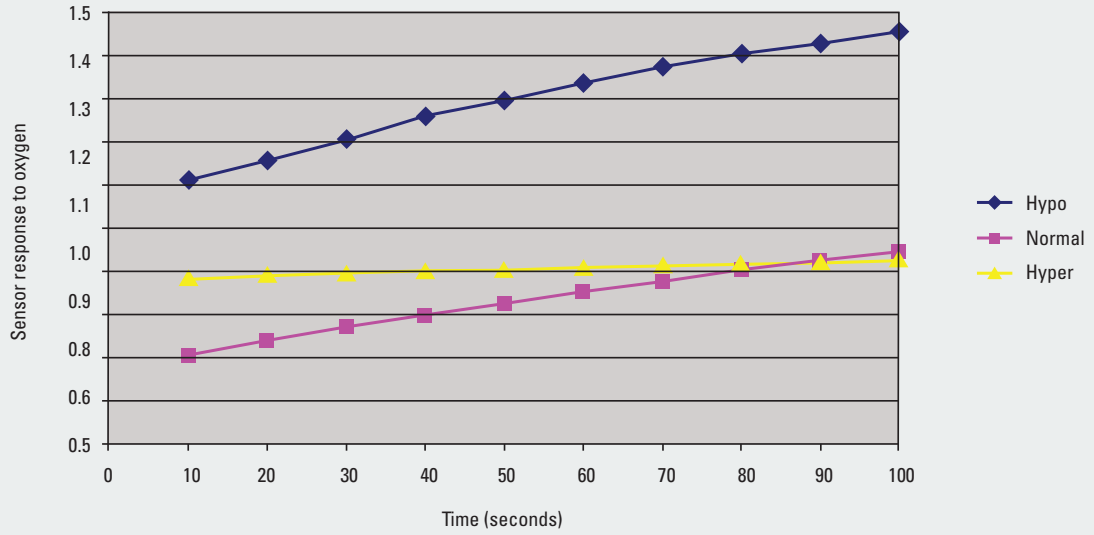
To visualise both consumption and production of oxygen, light/dark experiments with Cabomba leaf were conducted. Under non-lit conditions, a remarkable decrease in oxygen concentration was detected, which was localised with the characteristic leaf shape of the plant. Oxygen declined to 13 per cent air saturation after 60 minutes in the dark. Illumination of the leaf led to a quick increase in oxygen concentration, presumably indicating oxygen production by photosynthesis. To prove this assumption the test was repeated with the photosynthesis inhibitor DCMU (3-(3,4-Dichloropheyl)-1,1-dimethylurea). The addition of 20µmol DCMU completely abolished the light-dependent oxygen production. Measured differences between the oxygen exchange rates in darkness and light were remarkable and demonstrate the high photosynthetic capacity of *C caroliniana*. The planar sensor can be used to monitor respiration rates in plant roots, and responses of leaves to dark/light switches. Both spatial and temporal oxygen monitoring could be performed.

Perfusion Monitoring in Medical Research

Free tissue transfer in neck and head reconstruction has a very high success rate. Still, in up to 10 per cent of all cases serious complications occur, caused by circulatory failure in free flaps. Technical devices are used for flap monitoring to detect vascular problems at an earlier stage and allow the possibility of a more timely intervention before complications can occur. Despite the many monitoring methods for flap transplantation available, no ideal procedure has been established yet. Now perfusion monitoring by 2D imaging of the oxygen flux into skin has been tested on its suitability for discovering

“Free tissue transfer in neck and head reconstruction has a very high success rate. Still, in up to 10 per cent of all cases serious complications occur, caused by circulatory failure in free flaps”

Figure 3: Perfusion monitoring of free skin flaps – sensor response to oxygen plotted over time, showing easy, fast and reliable assessment of the current perfusion status

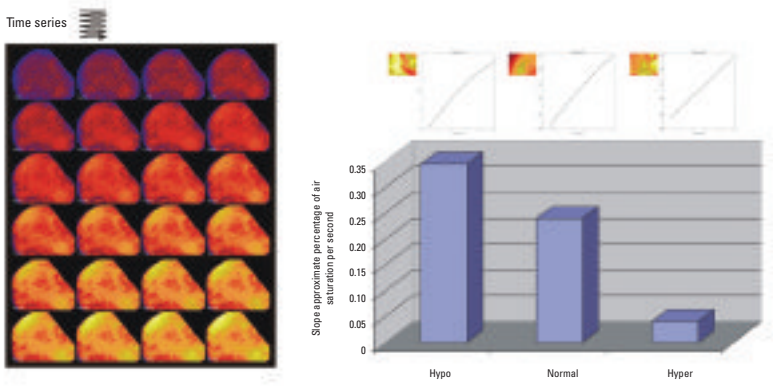


vessel thrombosis in free skin flaps. As the sensors are not medically approved products and so far only provided as a research tool, patients in this small clinical study had to sign informed consent. All these patients required reconstructions with free microvascular grafts after tumour surgery or because of non-healing wounds. Ten grafts were measured before explantation, after successful anastomosis and one day after surgery. The sensor foil was put with its sensing side directly onto the skin surface and every measurement was documented in pictures to optically judge flap perfusion (see Figure 3).

Tissue oxygenation depends on the oxygen supply provided by blood vessels and the oxygen consumption of the tissue cells. Oxygen partial pressure (pO_2) is an ideal parameter for monitoring tissue oxygenation as it is proportional to the concentration of physically dissolved oxygen. In these experiments the fluorescent sensor foil was loaded with oxygen from ambient air and released its reservoir of oxygen depending on the demand by the tissue. The reservoir was not reloaded during measurements, because the transparent polyester support of the sensor is impermeable. This way, oxygen release, which is driven by the oxygen flux, could be recorded measuring the sensor response over time. So measurements were only performed during equilibration time of the partial pressures of the sensor and the tissue, in a time series of 10 images every 10 seconds (100 seconds recording time). The sensor response to oxygen was plotted against the respective measurement. The perfusion dependent parameter, which is driven by the oxygen tension of the tissue (which again is driven by the perfusion rate and the oxygen consumption rate of the tissue), could be calculated from the slope of the graphs. Before obtaining the graphs, certain regions of interest (ROI) had to be determined. The time-depending change in oxygen level inside this defined region of interest over the observation period served as a basis for the graph. Low perfusion rates led to low oxygen tensions and therefore to high slopes, while high perfusion rates led to high oxygen tensions in the tissue and to low slopes. With this method, poorly perfused grafts could be clearly identified by means of the strongly altered perfusion-dependent slopes and three flaps were successfully revised by surgery (see Figure 4).

Though results in this trial depended on the experience of the examiner and the pressure applied with the examination tool, good results could be achieved.

Figure 4: A time series of recorded images allows self-evident delineating of the regional oxygen flux over a total skin region of several square centimetres



This measurement method was found to allow non-invasive and two-dimensional assessment of tissue oxygenation, without causing the patient too much discomfort. Of course, the method needs to be tested and evaluated with a higher number of measured flaps but it might be an objective measurement tool for monitoring free flaps, aiding the evaluating surgeon in flap examination and giving clear indication on the extent of flap perfusion. The possibility of monitoring an entire flap over time with oxygen imaging conveys an important advantage.

Oxygen Imaging in Tissue Engineering

The method of two-dimensional oxygen sensing via fluorescent sensor foils also gives new possibilities to research in the field of tissue engineering.

Incomplete bone fracture healing or damage in cartilage tissue, especially in patients with systemic diseases, is a challenge in medicine. Methods used so far, including the implantation of own, donator or artificial material bears risks such as infection, rejection of the implant, or fatigue of material. A promising alternative to these methods are cell-based regenerative therapies, which are intended to heal tissue defects using multipotent precursor cells, such as mesenchymal stem cells (MSCs). These cells – extracted in a small surgical procedure – are expanded *in vitro* and transplanted into the damaged region on a carrier material. For bone damage solid carrier materials are normally used which degrade slowly and require surgical procedures involving soft tissue damage. This is why hydrogels are now tested, as a new and innovative method for healing tissue defects. Hydrogels mostly consist of aqueous media which are combined with a polymer. They can be applied to the damaged tissue minimal invasion by injection.

A limiting factor in the use of hydrogels as cell carriers is the restricted oxygen and nutrient supply to the cells after application of the gel until blood vessels grow into the area. Moreover, cells need adhesion surface to proliferate, which is especially problematic when using populated gels for bone regeneration. Very interesting in this context are new smart hydrogels which show temperature-dependent properties and solidify at body temperature. This way the gels can be seeded with cells at room temperature and injected in the damaged tissue, where they solidify and keep the cells in place. To overcome oxygen gradients and insufficient oxygen supply to the cells, oxygen releasing additives for the hydrogels have been tested. Experiments with certain of these additives showed promising results in enhancing the oxygen supply. Chemical optical microsensors had been used in these experiments to determine oxygen gradients within the cell-seeded hydrogels.

Forthcoming projects will focus on intelligent hydrogels with temperature-dependent properties for tissue regeneration, which are able to supply oxygen and give adhesive enough surfaces to the incorporated cells. For monitoring oxygen gradients within the gels 2D imaging over a cross section of the sample will be performed. In this way not only the actual oxygen supply over a cross section of the sample but also its continuity in oxygen release and homogeneity when adding an oxygen carrier can be evaluated. The fluorescent sensor foils are expected to give far better insight into oxygen distributions during experiments.

Planar Oxygen Sensing in the Future

Fluorescence imaging is a new approach for oxygen sensing and visualisation of oxygen distributions. The data and experience obtained in handling and operating such an imaging device illustrates the major advantages compared to methods used so far. As well as the future application of this technology in tissue engineering, other projects are on the way where 2D visualisation of oxygen distributions can give insight into processes and deliver data that could not otherwise be obtained to this extent. The simple application and robustness of sensor foils makes it possible to apply this imaging technique in a range of applications. Ongoing research will make it possible to measure other analytes with fluorescence imaging in the near future.

References

1. Tschiersch H, Liebsch G, Stangelmayer A, Brisjuk L and Rolletschek H, Planar oxygen sensors for non invasive imaging in experimental biology, *Microsensors* pp281-294, 2011
2. Gosau M, Meier JK, Müller S, Moralis A, Liebssch G and Prantl L, Simple, fast and reliable monitoring of microvascular flaps, *Clinical Hemorheology and Microcirculation*, accepted, 2011
3. Kellner K, Liebsch G, Klimant I, Wolfbeis OS, Blunk T, Schulz MB and Göpferich A, Determination of oxygen gradients in engineered tissue using a fluorescent sensor, *Biotechnology and Bioengineering* 80(1): pp73-83, 2002
4. Leicht U, Volkmer E, Wiese H and Schieker M, Polyoxamer-based hydrogels made with different chain extenders as oxygen releasing cell carriers for tissue engineering, 14th Annual Meeting on Surgical Research, *Langenbeck´s Archives of Surgery* 395(6), 2010

About the author

Gregor Liebsch is Head of R&D Imaging Solutions at PreSens Precision Sensing GmbH. In 2000 he received his doctorate at the Institute of Analytical Chemistry, Chemo- and Biosensor at the University of Regensburg. Following his time as postdoctoral researcher at the University of Regensburg he worked in the field of imaging technology for medical application in cancer diagnostics at Biocam GmbH. He joined PreSens in 2010 where, based on his experience on optical sensors and imaging technologies, he focuses his work on the development of 2D imaging measurement systems for oxygen, pH and CO_2. **Email: gregor.liebsch@presens.de**