Oxygen Detection

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Photographing Oxygen Distribution**

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Oxygen, or more correctly dioxygen, is one of the most fundamental chemical species on earth. As a result, sensing and imaging of oxygen is essential in numerous areas including medicine[1] and physiology,[2] biology,[3] biotechnology,[4] clinical diagnosis,[1] cancer research,[5] the chemical industry,[3,6] environmental sciences,[7] and in less obvious areas such as coal mines,[8] food packaging,[9] and marine research.[10] Oxygen is also the species that is sensed in so-called pressure-sensitive paints[11] (which are indispensible tools in automotive and aerodynamic research), and in biosensors based on enzyme-associated reactions during which oxygen is consumed, for example, in biosensors for glucose.[12]

Sensors for oxygen can be based on measurement of either (gas) pressure, electrical current (such as with the Clark electrode), or signals of appropriate optical (usually luminescent) indicators. They are now fabricated in large numbers and widely applied. Optical sensors for oxygen have become a subject of particular research in recent years[13] in view of their distinct advantages over other kinds of sensors, and because they enable chemical species to be detected remotely or invasively if combined with optical fiber technology[14] or in nanometer dimensions.[15] Recently, oxygen has been detected by dual-color systems rather than with single emitting probes.[16] Such methods enable semiquantitative colorimetric determination of oxygen through visual readout. However, sophisticated instrumentation is required for quantitative sensing and imaging, and this represents a substantial drawback.

A recent trend in sensor technology involves the use of devices such as computer screens[17] or mobile phone cameras[18] to monitor chemical targets. This can substantially reduce the costs and time that is needed for development of diagnostic instrumentation. Moreover, such devices are affordable and familiar to potential users. Herein we show that a combination of two-color digital photographic cameras (another "familiar" device) and two-color sensor technology can lead to a very simple method for sensing and imaging of oxygen that may replace more complex systems.[19]

Practically all digital photographic cameras are based on the use of chips containing so-called red/green/blue (RGB) channels (see Figure S1 in the Supporting Information) that are sensitive to the red, green, and blue parts of the visible spectrum. The final color picture seen by the user is composed of the three virtually independent RGB data sets. The distribution of the brightness of the colors is recorded in the RGB image channels and reflected in the form of histograms. We perceived that this may pave the way to quantitative readout in dual-color based optical chemical sensing, which is particularly useful in case of oxygen, for which fluorimetry is the method of choice.

To achieve this goal, a sensor layer for oxygen was designed that employs two dyes that spectrally match the red and the green channels, respectively, of an RGB chip. The known[20] platinum(II) *meso*-tetrakis(pentafluorophenyl)porphyrinato complex (PtTFPP) has red luminescence and was chosen as the probe for oxygen. It can be excited efficiently at 409 nm (which is close to the peak of the strong Soret band) to give a fairly narrow red emission band with a peak at 650 nm. Thus, it perfectly matches the red channel of the RGB chips as shown in Figure S1 (see the Supporting Information). The search for a reference dye that is also excitable at 409 nm but displays green emission led to the fluorophore *N*-(5-carboxypentyl)-4-piperidino-1,8-naphthalimide, which has a fairly narrow fluorescence band peaking at 510 nm (in the polymeric solvent used herein). The green emission is detected by the green channel only. A commercially available and highly biocompatible polyurethane hydrogel was chosen as the polymer matrix ("solvent") to host the two dyes. This polymer is a good solvent for the two dyes and possesses excellent oxygen permeability. Polymer and dyes were dissolved in an ethanol/water mixture and this "paint"[21] was spread onto a glass support to give a 6 μm thick sensor film after solvent evaporation.

The fluorescence of the red dye is dynamically quenched by oxygen. This quenching does not spectrally shift the red emission, but the brightness of the red pixels varies strongly with p_{O_2}. The emission of the naphthalimide fluorophore, in contrast, is not measurably quenched by oxygen so that the brightness of the pixels does not change. Rather, the green dye acts as an optical contrast and also gives a reference (fluorescence) signal in the RGB channel system.

Figure 1 (top) shows that the response of the sensor layer to oxygen partial pressure can be both visually observed and be imaged with a camera. Figure 1 (bottom) shows the green and red channel histograms corresponding to these colorimetric pictures. The peaks of the histograms of the green channel are displayed in green, and those of the red channel in red. The green peaks obviously are not shifted when the

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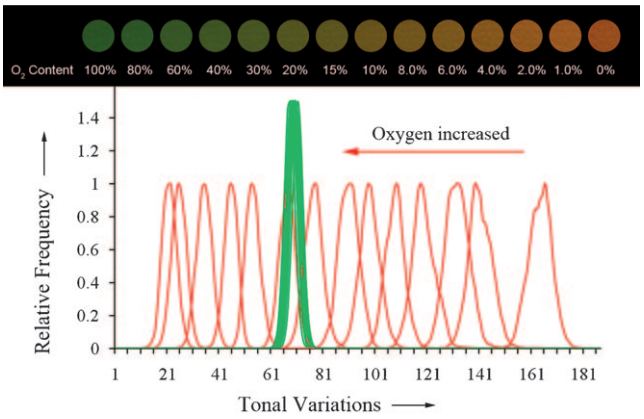

Figure 1. Top: Apparent colors of the sensor layer at different concentrations of oxygen. Bottom: Corresponding image histograms (200×200 pixels) of the red and green channels. The relative frequency represents the relative number of pixels for each tonal value.

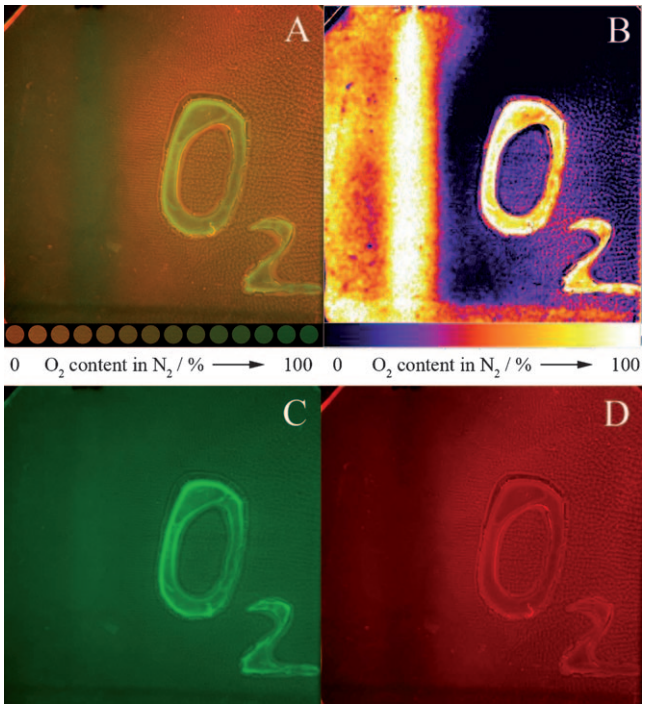

Figure 2. Photographing oxygen distribution: A) Regular photo (14-bit color depth per channel) obtained for colorimetric readout. B) Pseudo color image of the G/R ratio calculated with data of the green channel, shown in (C), and the red channel, shown in (D).

oxygen concentration changes, whereas the red peaks move to lower values with increasing concentration of oxygen.

As shown in Figure S2 a in the Supporting Information, an exponential quenching curve was obtained by dividing the data of red channel by those of the green channel (the so-called R/G ratio). The reciprocal value of the R/G ratio (the G/R ratio) is linearly related ($y = 0.028x + 0.4452$, $R^2 = 0.9960$) to oxygen concentration (Figure S2 b). Hence, it can be used for precise quantitative determination of oxygen at single spots but of course also enables oxygen distribution to be imaged. The visible color change of the sensor layer, in turn, results from changes in the ratio of the intensities of the red and green luminescence (Figure 1, top, and Figure S3). Most notably, this ratio is independent of the intensity of the purple LED used for photoexcitation.

Figure 2 demonstrates the potential of photographing oxygen distribution. The image was obtained by photographing the sensor layer described above. The characters "O_2" were written onto the sensor layer using a solution of the block-copolymer poly(acrylamide-*co*-acrylonitrile), which is impermeable to oxygen. Figure S4 in the Supporting Information nicely demonstrates the impermeability of this polymer to oxygen in that virtually oxygen-independent spectra are obtained if the sensor matrix is made from this polymer. The sensor layer with the characters O_2 painted onto its surface was first placed in an atmosphere of nitrogen, then exposed to a flow of pure oxygen, and then photographed under illumination with the 409 nm LED. The characters "O_2" and the flow of oxygen are easily visible in the "normal" photographic picture (Figure 2 A). However, the processed picture (Figure 2 B) exhibits better contrast and reveals a more detailed image of oxygen distribution. Digital processing of the two channels (R and G) was performed by using free software (herein Image J). In future, this process may be integrated in the camera firmware.

It was noted that the color depth is affected by aperture, ISO setting, and shutter speed. Therefore, the effects of these parameters were investigated more systematically. The results are shown in the Supporting Information (Figures S5 to S7). However, their effects on color depth can be eliminated by comparing relative peak positions in the photographic method and by calculating (and normalizing) the R/G ratio. The results also reveal that the photographic method allows readout of rather dark images, which is difficult with conventional colorimetric methods.

RGB-based imaging was used recently by Stich et al.[22] in context with dual sensing. The brightness of the pixels in two channels was used as relative information. The method is not applicable to quantitative chemical analysis but rather yields relative data on light intensity. Pixel brightness is, however, affected by variations in the intensity of the light source, the length of the light path, by photodecomposition of probes, inhomogeneous dye distribution, inhomogeneous light field, and certain camera parameters. The method presented herein, in contrast, enables direct and quantitative fluorescence imaging of oxygen on the basis of ratiometric analysis of image histograms by dividing the values of the red and green channel. The adverse effects of variations such as in the light source, as outlined above, are almost completely eliminated by comparison with the reference channel. In addition, the use of image histograms allows the analysis of oxygen distribution by comparing the positions of the peaks of the histogram in red and green channels, respectively. This function is available in most digital cameras but has not been used so far for chemical sensing purposes. The large differences found in histograms provide ample space for more detailed data analysis.

Other attractive features include high colorimetric resolution, precision (calculated to be ±0.2 % at air oxygen level),

14-bit color depth per channel in the photographic readout, broad operational range, and applicability under ambient conditions. We note that the materials used are easily accessible and environmentally friendly. Rather incidentally, we found that the two dyes undergo identical, albeit very slow, photodecomposition so that the ratio of the intensities of the red and the green channels remains identical over time. Toxic quantum dots[23] are not needed, sensors can be fabricated in a single step, sensing films are highly uniform, and the use of an established hydrogel makes the material highly biocompatible. The analytical range may be fine-tuned by incorporating the indicator probes into polymers of appropriate permeability for oxygen. The dyes used herein are quite photostable (Figure S8) and do not measurably leach out of the sensor film (notwithstanding the option of covalently immobilizing the green fluorophore in a matrix through its carboxy group). In terms of features of the optical system, we note that a conventional digital camera can be used along with a commercial purple LED flashlight. While not used in this work, the blue channel of the RGB chip may be used for gaining additional spectral information, for example, in context with multiple sensing.[24] We therefore believe that this approach, because of its simplicity and versatility, is of interest whenever oxygen is to be sensed, and potentially can be extended to other species if appropriate probes are available.

Experimental Section

Preparation of oxygen sensor: A sensor "cocktail" was prepared by mixing solutions of *N*-(5-carboxypentyl)-4-piperidino-1,8-naphthalimide (90 μL; 2 mg mL^{-1}) in ethanol, PtTFPP (110 μL; 2 mg mL^{-1}) in ethanol, and a hydrogel (type D4; a highly biocompatible polyurethane; Cardiotech Intl. Inc.; 500 μL; 5.0 % w/w) in ethanol/water (90:10 v/v). The mixture was shaken for 4 h until a homogeneous solution was obtained. This solution ("cocktail") was spread onto a 125 μm thick support matrix from poly(ethylene terephthalate) (Mylar; Dr. D. Müller GmbH) using a knife coating device. The thickness of the sensor film after solvent evaporation at room temperature was 6 μm.

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