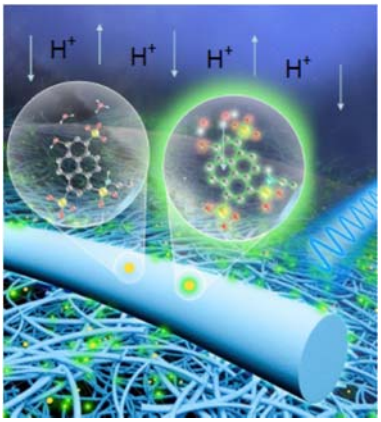
List of Papers Related to Optical Imaging

vs. of 24-Jan-2021; edited by O. S. Wolfbeis

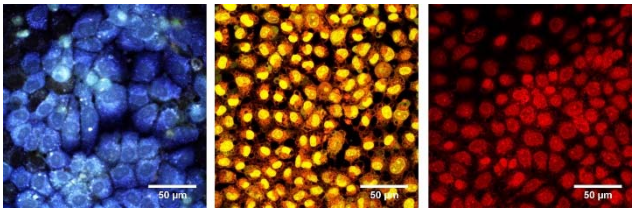
609. *Review:* **Optical Sensing and Imaging of pH Values: Spectroscopies, Materials and Applications.** A. Steinegger, O. S. Wolfbeis, S. M. Borisov; *Chem. Reviews* (**2020**) *120*(22) 12357 - 12489. DOI: 10.1021/acs.chemrev.0c00451. IF: 54.3.

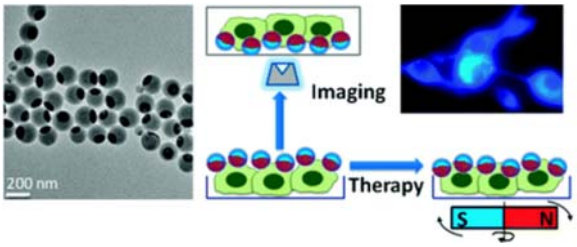


Abstract: This is the first comprehensive review on methods and materials for use in optical sensing of pH values, and on applications of such sensors. The Review starts with an introduction that contains subsections on the definition of the pH value, a brief look back on optical methods for sensing of pH, on the effects of ionic strength on pH values and pKa values, on the selectivity, sensitivity, precision, dynamic ranges and temperature dependence of such sensors. Commonly used optical sensing schemes are covered in a next main chapter, with subsections on methods based on absorptiometry, reflectometry, luminescence, refractive index, surface plasmon resonance, photonic crystals, turbidity, mechanical displacement, interferometry and solvatochromism. This is followed by sections on absorptiometric and luminescent molecular probes for use pH in sensors. Further large sections cover polymeric hosts and supports, and methods for immobilization of indicator dyes. Further and more specific sections summarize the state of the art in materials with dual functionality (indicator and host), nanomaterials, sensors based on upconversion and 2-photon absorption, multiparameter sensors, imaging, and sensors for extreme pH values. A chapter on the many sensing formats has subsections on planar, fiber optic, evanescent wave, refractive index, surface plasmon resonance and holography based sensor designs, and on distributed sensing. Another section summarizes selected applications in areas such as medicine, biology, oceanography, bioprocess monitoring, corrosion studies, on the use of pH sensors as transducers in biosensors and chemical sensors, and their integration into flow-injection analyzers, microfluidic devices and lab-on-a-chip systems. An extra section is devoted to current challenges, with subsections on challenges of general nature and those of specific nature. A concluding chapter gives an outlook on potential future trends and perspectives.

590. *Review:* **An Overview of Nanoparticles Commonly Used in Fluorescent Bioimaging.** O. S. Wolfbeis, *Chem. Soc. Rev.* (**2015**), *44*, 4743-4768. DOI: 10.1039/c4cs00392f. Open access. IF: 33.4.

Abstract: The article gives an overview on the various kinds of nanoparticles (NPs) that are widely used for purposes of fluorescent imaging, mainly of cells and tissue. Following an introduction and a discussion of merits of fluorescent NPs compared to molecular fluorophores, labels and probes, the article assesses the kinds and specific features of nanomaterials often used in bioimaging. These include fluorescently doped silicas and sol-gels, hydrophilic polymers (hydrogels), hydrophobic organic polymers, semiconducting polymer dots, quantum dots, carbon dots, other carbonaceous nanomaterials, upconversion NPs, noble metal NPs (mainly gold and silver), various others nanomaterials, and dendrimers. Another section covers coatings and methods for surface modification of NPs. Next, examples are given on the use of nanoparticles in (a) plain fluorescence imaging of cells, (b) targeted imaging, (c) imaging of chemical species, and (d) imaging temperature. A final section covers aspects of multimodal imaging (for example fluorescence/nmr), imaging combined with drug and gene delivery, or imaging combined with therapy or diagnosis. A *Supporting Information* gives specific examples for materials and methods used in imaging, sensing, multimodal imaging and theranostics such as imaging combined with drug delivery or photodynamic therapy. The article contains 270 references in the main part, and 157 references in the *Supporting Information*. The graph on the *left* shows cell images obtained by using carbon dots, and how the wavelength of excitation affects the color of luminescence. The graph on the *right* shows blue fluorescent nanobeads made from poly(styrene-b-allyl alcohol) and labeled with pyrene. They possess a magnetic core and can be applied to fluorescent imaging of imaging cancer cells (top) and, simultaneously, to magnetically induced heat lysisof cell membranes.



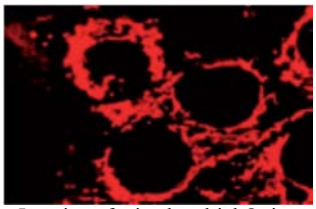


587. **Luminescent Sensing and Imaging of Oxygen: Fierce Competition to the Clark Electrode.** O. S. Wolfbeis, *Bioessays* (**2015**) *37(8)*, 921-928. DOI: 10.1002/bies.201500002. IF: 4.7. Open access.

Abstract: Luminescence-based sensing schemes for oxygen have experienced a fast growth and are in the process of replacing the Clark electrode in many fields. Unlike electrodes, sensing is not limited to point measurements via fiber optic microsensors, but includes additional features such as planar sensing, imaging and intracellular assays using nanosized sensor particles. This essay discusses, in layman's terms, (a) the commons solid-state sensor approaches based on the use of indicator dyes and host polymers; (b) fiber optic and planar sensing schemes as well as nanoparticle-based intracellular sensing; and (c) common spectroscopies. Optical sensors also are capable of multiple simultaneous sensing (such as O_2 and temperature). Sensors for O_2 are produced nowadays in large quantities. Fields of application include plant and animal physiology, clinical chemistry, marine sciences, the chemical industry and process biotechnology.

Optoelectronic system containing an RSB port (*left*) and a fiber connector (*right*) for fiber optic decay time-based sensing of O_2.

Schematic of the state of the art in imaging O_2 . *Left*: PC with sioftaware and for display.*Center*: Handheld portable instrument for lifetime imaging. *Red film:* Sensor membrane incorporating a quenchable luminescenct probe for O_2 to be placed on the object of interest (a bioreactor, or skin, or an aircraft, etc.; symbolized by a cube)

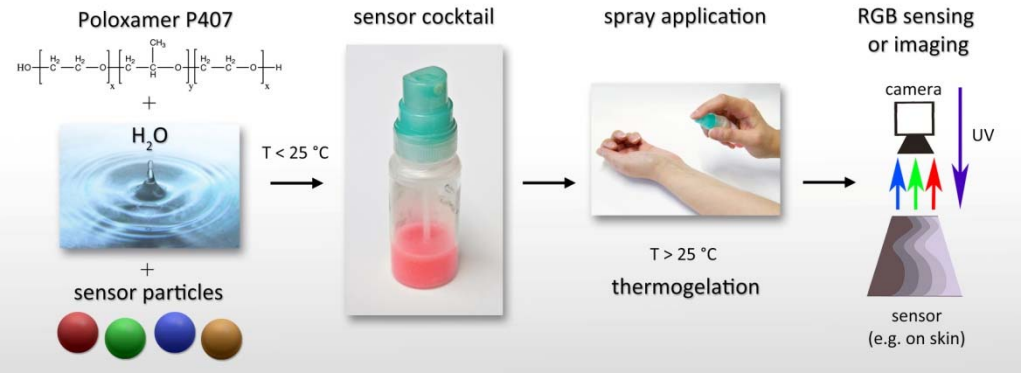


Imaging of mitochondrial O_2 in cells using sensor NPs. Pictures are comparable to those obtained with a mitotracker but luminescence intensity depends on local pO_2

586. **A Water-sprayable, Thermogelating and Biocompatible Polymer Host for Use in Fluorescent Chemical Sensing and Imaging of Oxygen, pH Values and Temperature.** X. Wang, R. J. Meier, C. Schmittlein, S. Schreml, M. Schäferling, O. S. Wolfbeis; *Sensors & Actuat., B: Chem.* (**2015**), *221*, 37-44. DOI: 10.1016/j.snb.2015.05.082. IF: 4.1.

Abstract: We report on the use of a sprayable and thermogelating biomaterial (Poloxamer™; a.k.a. Pluronic™) in optical imaging of pH values, local oxygen and temperature (*T*). The material is highly biocompatible and easy to handle. We also show that the material is well permeable to oxygen (thus making it a good choice for use in oxygen sensors), and is stable in liquid solution and at elevated T. We demonstrate its applicability in optical sensors for oxygen, pH values and T. This was accomplished by incorporating appropriate luminescent probes in various kinds of microparticles (which act as hosts for the probes and prevent dye leaching and aggregation), and then dispersing the microparticles in the thermogelating polymer. The resulting sensor gels were deposited on the surface of interest via spraying at T's of <20 °C. At these T's, the gels adhere well to the target, even on uneven surfaces such as skin, wounds, and bacterial cultures.

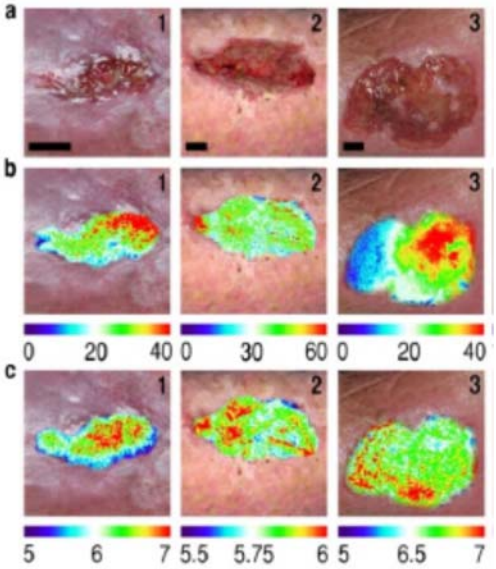
If T is risen to above 25 °C, the gels form a thin and soft but solid sensing layer which, however, can be removed from surface of interest by cooling and wiping it off, or by washing with water. Sprayable thermogelating sensors present obvious advantages over other sensors by not causing damage to the surface of interest. In our perception, the sensing materials also have wide further applicability in sensors for other species including clinically relevant gases, enzyme substrates (such as glucose or lactate) and ions



579. **Luminescent Dual Sensors Reveal Extracellular pH-radients and Hypoxia on Chronic Wounds that Disrupt Epidermal epair.** S. Schreml, R. J. Meier, M. Kirschbaum, O. S. Wolfbeis, M. Landthaler, P. Babilas; *Theranostics* (**2014**), *4*, 721-735. DOI: 10.7150/thno.9052. IF: 7.8. *Open Access.*

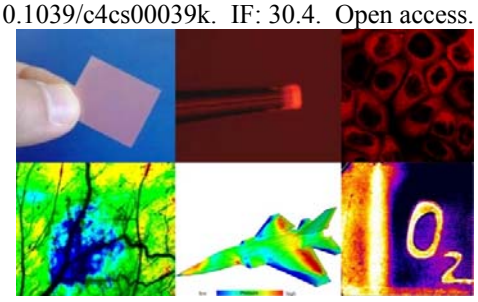
Abstract: We are presenting a method to simultaneously image extracellular wound pH and oxygenation in-vivo. It is based on hydrogel-based biocompatible luminescent dual sensor foils doped with pH-responsive and oxygen-responsive microparticles, respectively. The sensor foils were placed on wounds, and fluorescence images were acquired by two kinds of lifetime imaging (td-DLR and LLI). The pH-gradients were identified as governors of cell proliferation. Simultaneous imaging of oxygen also revealed marked hypoxia, albeit with no correlating gradient in oxygen partial pressure. pH-gradients in chronic wounds of humans are predominantly generated via centrifugally increasing pH-regulatory expression of Na^+/H^+-exchanger-1. The study has implications in terms of cell science where spatial variations of pH play key roles, e.g. in tumor growth. The article includes 3 supplementary movies.

The figure shows (a) three kinds of wound; 8B9 the variation of oxygen tension (in mmHg), and (c) the variation of locl pH values. Obviously, this is y very powerful methd for detecting even small varations in local pH values and oxygen partial pressures.



578. *Review:* **Optical Methods for Sensing and Imaging Oxygen: Materials, Spectroscopies and Applications.** X. Wang, O. S. Wolfbeis; *Chem. Soc. Rev.* (**2014**), *43*, 3666-3761. DOI: 10.1039/c4cs00039k. IF: 30.4. Open access.

Abstract: We review the current state of optical methods for sensing oxygen. These have become a powerful alternative to electrochemical detection and are in the process of replacing the Clark electrode in many fields. The article (with 693 refs.) is divided into main sections on direct spectroscopic sensing of oxygen, on absorptiometric and luminescent probes, on polymeric matrices and supports, on additives and related materials, on spectroscopic schemes for read-out and imaging, and on sensing formats (such as waveguide sensing, sensor arrays, multiple sensors and nanosensors). We finally discuss future trends and applications and summarize the properties of the most often used indicator probes and polymers. A *Supporting Information* (with 385 refs.) gives a selection of applications of such sensors in medicine, biology, marine and geosciences, in intracellular sensing, aerodynamics, industry and biotechnology, among others.

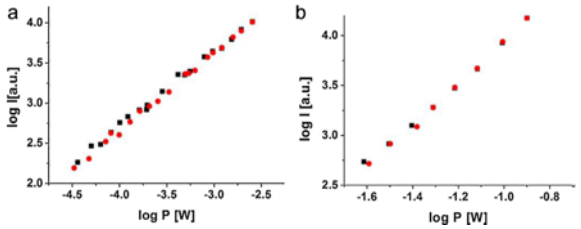


574. **Direct Formation of Mesoporous Upconverting Nanoparticles for Bioimaging of Living Cells.** T. Liu, L. Sun, Y. Qiu, J. Liu, F. Li, L. Shi, O. S. Wolfbeis; *Microchim. Acta* (**2014**), *181*, 775-782. DOI: 10.1007/s00604-013-1073-9. IF: 3.7.

Abstract: We describe a single-step method for the synthesis of mesoporous upconverting nanoprobes (MUCNs) of the type $NaYF_4$:Yb,Er@mSiO_2, with the mesoporous and assisted by CTAB which serve as both phase transfer assistant agents and pore-generating templates. With effective emission upon 980-nm light excitation and low cytotoxicity according to the thiazolyltetrazolium assay, the MUCNs can be applied to image human nasopharyngeal epidermal carcinoma cells in-vitro via laser scanning upconversion luminescence microscopy. silica directly encapsulating the hydrophobic upconversion nanoparticles

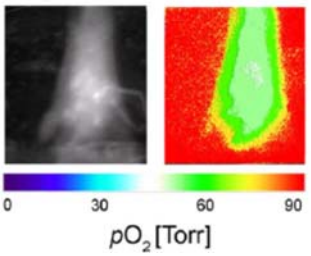
569. **Imaging of Cellular Oxygen via Two-Photon Excitation of Fluorescent Sensor Nanoparticles.** X. Wang, D. E. Achatz, C. Hupf, Sperber, J. Wegener, S. Bange, J. M. Lupton, O. S. Wolfbeis; *Sensors & Actuat., B (Chem.)* (**2013**), *188*, 257-262. DOI: 10.1016/j.snb.2013.06.087. IF: 3.8.

Abstract: Polystyrene nanoparticles (PSNPs) with an average size of 85 nm and loaded with an oxygen-quenchable luminescent ruthenium complex were used to image oxygen inside cells following 2-photon excitation (2-PE). The ruthenium probe possesses a large 2-photon absorption cross-section, and 2-PE is achieved by irradiation in the near infrared with fs-pulsed laser systems.
The luminescence of the dye-loaded PSNPs is strongly quenched by oxygen, and Stern–Volmer plots are linear for both conventional 1-PE and for 2-PE. The particles are readily taken up by mammalian cells (MCF-7), presumably via membrane mediated pathways. The 2-PE is considered to be advantageous over conventional imaging techniques because it works in the near-infrared where background absorption and luminescence of biomatter is much weaker than at excitation wavelengths of <600 nm. The Fig. shows double-logarithmic plots of laser power versus emission intensity of the oxygen probe Ru(dpp). Plot (a): excitation via single-photon absorption; the slope is 0.95. Plot (b): excitation via 2-PE (slope 2.04).



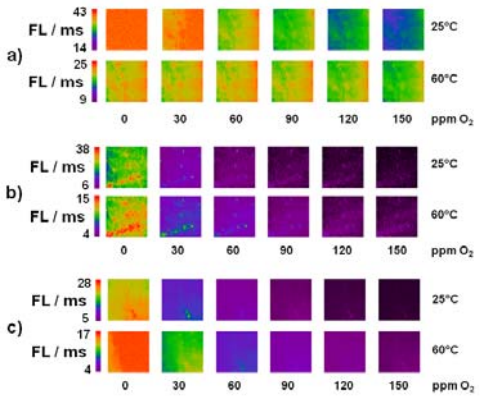
568. **Ratiometric Luminescence 2D *In-vivo* Imaging and Monitoring of Mouse Skin Oxygenation.** J. Hofmann, R. J. Meier, A. Mahnke, V. Schatz, F. Brackmann, R. Trollmann, C. Bogdan, G. Liebsch, X. Wang, O. S. Wolfbeis, J. Jantsch; *Meth. Appl. Fluoresc. (London)* (**2013**) *1*, 045002. DOI: 10.1088/2050-6120/1/4/045002. IF: 2.3.

Abstract: Tissue oxygenation plays a critical role in the pathogenesis of various diseases, but non-invasive, robust and user-friendly methods for its measurement in vivo still need to be established. Here, we are presenting an in vivo oxygen-detection system that uses ratiometric luminescence imaging (RLI) as a readout scheme to determine the skin oxygen tension of mouse hind footpads via side-by-side comparison with more established techniques including luminescence-lifetime imaging using planar sensor films and the polarographic electrode as the gold standard. We also demonstrate that this technology allows the detection of changes in mouse skin tissue oxygenation induced by subjecting mice to systemic hypoxia. The data demonstrate oxygen imaging based on RLI to be a most useful tool for reliably and easily analyzing and monitoring skin tissue oxygenation in vivo. This technology will advance our understanding of local regulation of skin tissue oxygenation in various disease conditions.



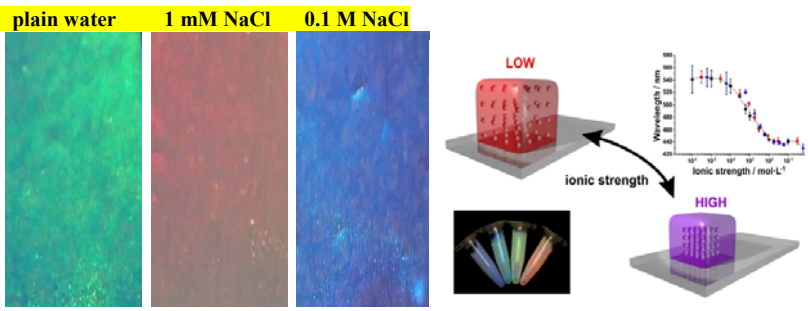
564. **Sensing and Imaging of Oxygen with Parts per Billion Limits of Detection and Based on the Quenching of the Delayed Fluorescence of $^{13}C_{70}$ Fullerene in Polymer Hosts.** S. Kochmann, C. Baleizão, M. N. Berberan-Santos, O. S. Wolfbeis; *Anal. Chem.* (**2013**), *85*, 1300-1304. DOI: 10.1021/ ac303486f. IF: 5.9.

Abstract: The method for sensing trace oxygen in the gas phase is based on the extreme efficiency of the quenching of the thermally activated delayed fluorescence of isotopically enriched (85%) carbon-13 fullerene C_{70} ($^{13}C_{70}$). The fullerene was dissolved in polymer matrices of varying oxygen permeability, viz. polystyrene (PS), ethyl cellulose (EC), and an organically modified silica gel ("ormosil"; OS). The sensor films (5 – 10 µm thick), on photoexcitation at 470 nm, display a strong delayed photoluminescence with peaks between 670 and 700 nm. Quenching by oxygen was studied at 25 ºC and 60 ºC, and at levels from zero to 150 ppmv of oxygen in nitrogen gas. The rapid lifetime determination (RLD) method was applied to determine oxygen-dependent decay imes and to perform fluorescence lifetime imaging of oxygen. The oxygen sensors reported here are the most sensitive ones described so far.



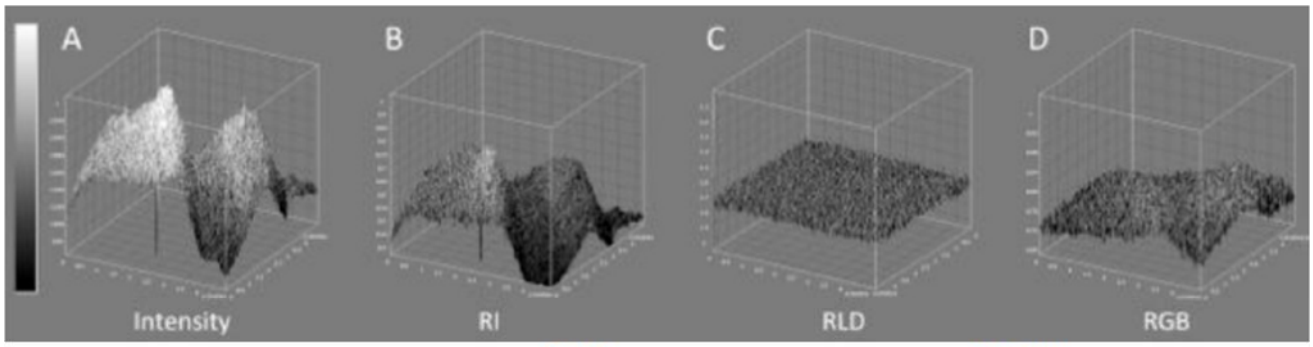
563. **Optical Sensing of Ionic Strength Using Photonic Crystals in a Hydrogel Matrix.** Ch. Fenzl, Th. Hirsch, O. S. Wolfbeis; *ACS Appl. Mat. Interfaces* (**2013**), *5*, 173-178. DOI: 10.1021/am302355g. IF: 6.7.

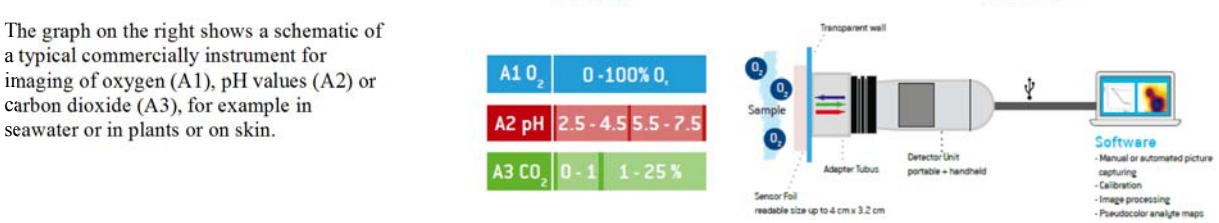
Abstract: Monodisperse, highly neg. charged, crosslinked polystyrene nanoparticles with a diam. between 80 to 120 nm have been incorporated into a polyacrylamide hydrogel where they display an iridescent color that conventionally is attributed to the so-called photonic crystal effect. The film is red if placed in plain water but turns to green in the presence of 1 mM soln. of an electrolyte, and to purple in 100 mM solns. of electrolytes. See the Figure. Quantitative reflection spectroscopy resulted in plots of reflected light wavelength vs. ionic strength (IS) that are almost linear in the logarithmic concentration range from $5 \cdot 10^{-5}$ to 10^{-2} mol•L^{-1}. Such films are capable of monitoring the IS of aqueous solutions in the pH range from 5 to 9. In addition to visual and instrumental readout, the sensor films can be analyzed with a digital camera at fixed angle. The digital images were separated into their red, green and blue (RGB) channels and analyzed. The red channel was found to be best suited for determination of IS and resulted in calibration plots that are comparable if not better than those obtained by reflectometry.



562. **Referenced Luminescent Sensing and Imaging with Digital Color Cameras: A Comparative Study.** L. H. Fischer, R. J. Meier, M. Schaeferling, O. S. Wolfbeis; *Sensors Actuat. B (Chemical)* (**2013**), *177*, 500-506. DOI: 10.1016/j.snb.2012.11.041. IF: 3.9.

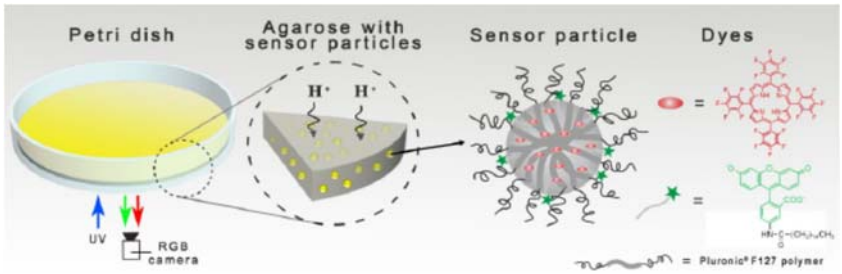
Abstract: We have performed a comparative study on different imaging techniques for optical chemical sensors with the aim to assess the utility of red–green–blue (RGB) color cameras for quantitative analysis. A luminescent film for sensing barometric pressure (via quenching by oxygen) was used as a model system and calibrated by four fluorescence imaging methods including intensity imaging, referenced intensity imaging, lifetime imaging, and RGB based imaging using a customary digital color camera. The results are compared with respect to standard deviations, lateral signal homogeneity, and resolution. The imaging methods were applied to the sensor film under identical experimental conditions in order to warrant comparable results.
The figure shows images of a sensor film at 100 mbar air pressure at 25◦ with inhomogeneous illumination. The results are shown for intensity (a), referenced intensity (RI) (b), RLD (c), and RGB imaging (d) as 3D surface plots to show the homogeneity of the sensor response.



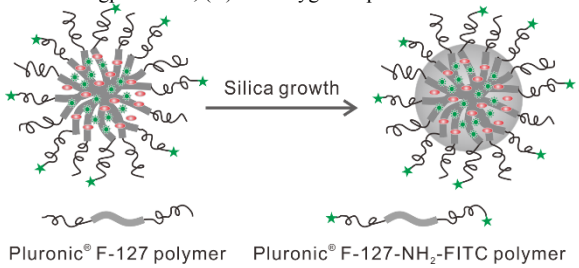


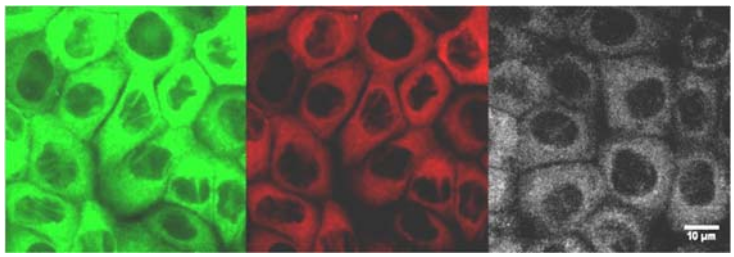
561. **Fluorescent pH-Sensitive Nanoparticles in an Agarose Matrix for Imaging of Bacterial Growth and Metabolism.** X. Wang, R. J. Meier, O. S. Wolfbeis; *Angew. Chem. Int. Ed.* (**2013**), *52*, 406-409. DOI: 10.1002/anie.201205715. IF: 13.4.

Abstract: We report on novel nanosensors for fluorescent imaging of physiological pH values. Features include (a) very small diameters (12 nm); (b) biocompatibility due to the use of a hydrogel kind of material [a commercial poly(ethylene glycol)-co-poly-ethyleneoxide)], non-covalent immobilization (based on strong hydrophobic interactions), and (c) lack of toxicity. Such nanosensors, if incorporated into an agar film, enable continuous monitoring of the pH value of bacterial cultures, and thus of their growth.

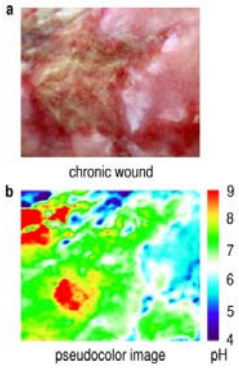


556. **Ultra-Small, Highly Stable and Sensitive Dual Nanosensors for Imaging Intracellular Oxygen and pH in Cytosol.** X. Wang, J. A. Stolwijk, T. Lang, M. Sperber, R. J. Meier, J. Wegener, O. S. Wolfbeis; *J. Am. Chem. Soc.* (**2012**), *134*, 17011-17014. DOI: 10.1021/ja308830e. IF: 9.9. Article featured in *JACS Spotlights (J. Am. Chem. Soc.* (**2012**)*, 134,* 18151−18152). *Abstract:* We report on the first dual nanosensors for imaging of pH values and oxygen partial pressure in cells. The sensors have a unique nanostructure in that a soft core structure is rigidized with a silane reagent, while poly(ethylene glycol) chains form an outer shell. Lipophilic oxygen-sensitive probes and reference dyes are encapsulated inside the hydrophobic core, while a pH-sensitive probe is covalently attached to the poly(ethylene glycol) end-group on the shell. The core/shell structure renders the nanosensors well dispersed and highly stable in various kinds of aqueous media. Their average size is 12 nm, and they respond to both pH velues and oxygen in the physiological range. They do not pass cell-membranes, but can be internalized into the cellular cytosol by electroporation, upon which they enable sensing and imaging of pH values and oxygen with high spatial resolution. The *Figure* shows confocal laser scanning microscopy images of the nanosensors internalized into normal rat kidney cells via electroporation. (A) The green luminescence of the pH-dependent signal of the nanosensors as seen with a 520-nm bandpass filter; (B) The red luminescence as seen with a 650-nm longpass filter; (C) The oxygen-dependent NIR luminescence (black/white) of the nanoparticles.





551. **Sprayable pH Sensor and its Use for Photographic Wound Imaging in-vivo.** S. Schreml, R. J. Meier, J. Cattani, D. Flittner, S. Gehmert, O. S. Wolfbeis, M. Landthaler, P. Babilas; *Exptl. Dermatol.* (**2012**), *21*, 942–970. DOI: 10.1111/exd.12042. IF 4.4.

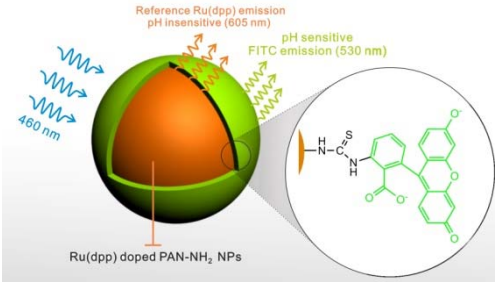


Abstract: Non-invasive luminescence imaging is of great interest for studying biological parameters (such as oxygen and pH) in cutaneous wound healing. Recently, we developed the first method for 2D luminescence imaging of pH in vivo on humans, and a method for one-stop-shop visualization of oxygen and pH using the RGB read-out of commercial cameras. Both methods make use of semitransparent sensor foils. Here, we describe a sprayable ratiometric luminescent pH sensor, which combines properties of both these methods and is suitable for in vivo use. Fluorescein isothiocyanate (FITC) was used as the pH indicator, and the ruthenium(II) complex Ru(dpp) as the reference dye. A digital (RGB) photo of the spray on the tissue is then taken, and the signals of the green fluorescent pH indicator are stored in the green channel, while that of the reference dye are stored in the red channel. Images are processed by ratioing the intensities of the two channels to result in pseudo-color pH maps of tissue surfaces, e.g. wounds.

550. **Referenced Dual Pressure and Temperature Sensitive Paint for Color Camera Read-Out.** L. H. Fischer, C. Karakus, R. J. Meier, O. S. Wolfbeis, E. Holder, M. Schaeferling; *Chemistry – Eur. J.* (**2012**) *18*, 15706-15713. DOI: 10.1002/chem.201201358. IF: 5.9.

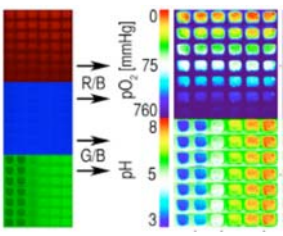
Abstract: We are presenting the first fluorescent material for referenced simultaneous RGB imaging of barometric pressure (oxygen partial pressure) and temperature (*T*). The dually sensitive coating contains two Pt(II) complexes acting as indicator for pressure (oxygen) and *T*, and a blue emitting reference dye, respectively. They are incorporated in polymer nanoparticles dispersed in a polyurethane hydrogel which is spread onto a solid support. The luminescence of the pressure probe (PtTFPP) matches the red channel of a RGB color camera, while that of the T probe matches the green channel. The blue-emitting reference dye (9,10-diphenyl-anthracene), in turn, matches the blue channel. In contrast to other dually sensitive materials, this new coating allows for simultaneous imaging of both indicator signals and the reference signal in one RGB color picture without having to separate the signals with additional optical filters. All dyes can be excited with a 405-nm LED and display good photostability under continuous illumination. Barometric pressure was determined with a resolution of 22 mbar, and *T* with a resolution of 4.3 °C. The formulae related to the probes and polymers (PAN, PVDCAN, PS, PVP) used are given below.

547. **Fluorophore-Doped Polymer Nanomaterial for Referenced Imaging of pH and Temperature with Sub-Micrometer Resolution.** X. Wang, R. J. Meier, O. S. Wolfbeis; *Adv. Funct. Mat.* (**2012**), *22*, 4202-4207. DOI: 10.1002/adfm.201200813. IF: 8.5.



Abstract: We report on a new kind of pH and temperature (*T*)-sensitive material. It is composed of dye-doped polymer nanoparticles incorporated into a thin film of a polyurethane hydrogel. The new pH/*T*-sensitive nanoparticles were obtained by post-staining oxygen-impermeable amino-functionalized polyacrylonitrile nanoparticles with a long-lifetime reference dye. Staining is followed by covalently linking fluorescein isothiocyanate onto the surface of the nanoparticle. The sensor material has distinct features: (a) It enables imaging of pH via time domain dual-lifetime referencing (td-DLR); (b) effects of *T* on pH sensing may be compensated for; (c) *T* can simultaneously be visualized via rapid lifetime imaging; (d) It offers superior spatial resolution due to the use of nanosized sensor particles.

538. **Simultaneous Photographing of Oxygen and pH in-vivo Using Sensor Films.** R. J. Meier, S. Schreml, X. Wang, M. Landthaler, P. Babilas, O. S. Wolfbeis; *Angew. Chem. Int. Ed.* (**2011**), *50*, 10893-10896. DOI: 10.1002/anie.201104530 IF: 12.7.

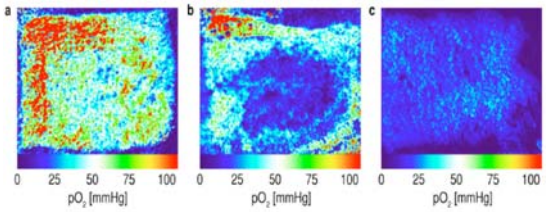


Abstract: We are presenting a method for real-time and simultaneous visualization of oxygen and pH in vivo using the RGB option of commercial digital cameras. Luminophores were used with emission peaks that correspond to the RGB channels of the camera. To create a 2D sensor, micro-particles were loaded (a) with an oxygen-sensitive platinum(II) complex whose data are stored in the red channel, (b) fluorescein isothiocyanate (the pH probe; data stored in the green channel), and (c) diphenyl-anthracene (as reference dye; data stored in the blue channel) and placed in a biocompatible hydrogel matrix. The sensor was characterized in vitro, and used to image oxygen and pH in human wounds. The novel imaging technique presented herein can be adapted to visualize various important chemical parameters, and may simplify imaging to a large extent.

535. **Two-Dimensional Luminescence Imaging of Physiological Wound Oxygenation.** S. Schreml, R. J. Meier, O. S. Wolfbeis, T. Maisch, R.-M. Szeimies, M. Landthaler, J. Regensburger, F. Santarelli, I. Klimant, P. Babilas; *Exp. Dermatol.* (**2011**), *20*, 550-554. DOI: 10.1111/j.1600-0625.2011.01263.x. IF: 4.2.

Abstract: We have studied the distribution of pO_2 during physiological wound healing. Split-thickness skin graft donor sites (n = 12) served as standardized wound models. Wound surface pO_2 was measured at 1, 6, and 14 days after split-skin harvesting using 2-dimensional luminescence lifetime imaging (2D-LLI) of Pd(II)-meso-tetraphenyl-benzoporphyrin in poly(styrene-co-acrylonitrile) particles on transparent foils. In another experiment, we removed the stratum corneum (SC) on the volar forearm (n = 10) by tape strippings to study the impact of the SC on the epidermal oxygen barrier. Split-skin donor site pO2 significantly decreased during the time course of physiological healing.

Figures: (a) One day after skin graft harvesting, where vast areas lack an epidermal oxygen barrier. (b) After 6 days, large areas within the donor site wounds are re-epithelialized; (c) 14 days p.o.; most of the wound is re-epithelialized.

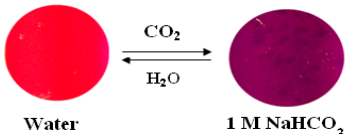


530. *Review.* **Fluorescent Sensing, Biosensing, and Screening Using Upconverting Nanoparticles.** D. E. Achatz, A. Reham, O. S. Wolfbeis; *Topics Curr. Chem.* (**2011**), *300*, 29-50. DOI: 10.1007/128_2010_98. IF: 4.3.

Abstract: Upconverting nanoparticles (UCNPs) display the unique property of converting near-infrared light (with wavelengths of typically 800 to 1000 nm) into visible luminescence. The main classes of materials are discussed. We also review the state of the art of using UCNPs (a) to label biomolecules such as antibodies and (synthetic) oligomers for use in affinity assay and flow assays; (b) to act as nanolamps whose emission intensity is modulated by chemical indicators, thus leading to a novel kind of chemical sensors; and (c), in FRET-based chemical sensors and biosensors.

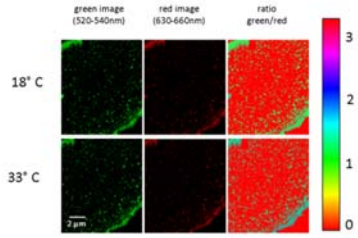
529. **Optical Sensing Scheme for Carbon Dioxide Using a Solvatochromic Probe.** R. Ali, T. Lang, S. M. Saleh, R. J. Meier, O. S. Wolfbeis; *Anal. Chem.* (**2011**), *82*, 2846-2851. DOI: 10.1021/ac200298j. IF: 5.8.

Abstract: The sensing scheme – unlike previous ones that are based on the use of pH indicator probes – is making use of solvatochromic probe Nile Red (NR). Dissolved in a matrix of ethyl cellulose, it can report the polarity of its microenvironment that is modulated by an additive (a hydrophobic amidine) which – in turn – is capable of reversibly binding carbon dioxide. The spectra of NR undergo a strong solvatochromic shift both in color (from brick-red to magenta) and in fluorescence (from orange to red) if the respective sensor layer is exposed to gaseous CO_2 (gCO_2) or dissolved CO_2 (dCO_2) Both visual and instrumental readout are possible. The detection limits are around 0.23% for gCO_2 and 1.53 hPa for dCO_2.The response time is in the order of10 min in the forward direction, and 3 min in the reverse direction for gCO_2,. The optical response also can be quantified using a digital camera by extracting the spectral information contained in the blue and green color channels (in reflectometry), or in the green and red channels (in fluorescence), resp.. Pseudo-color pictures also enable RGB imaging of the spatial distribution of pCO_2.



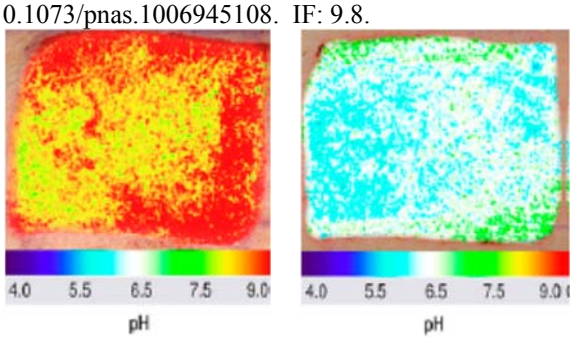
526. *Highlight Article.* **Upconversion Nanoparticles for Nanoscale Thermometry.** L. H. Fischer, G. S. Harms, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (**2011**) *50*, 4546-4548. DOI: 10.1002/anie.201006835. IF: 12.7.

Abstract: Lanthanide ion-doped nanoparticles display a strongly temperature-dependent luminescence that can be used to sense temperature in sub-µm dimensions, for example in cells or nanofluidics.



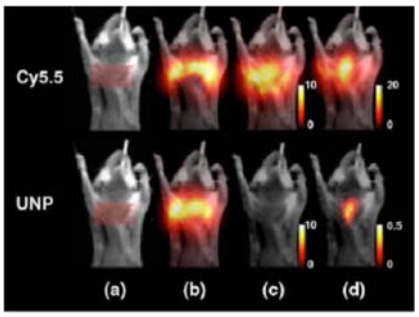
522. **2D Luminescence Imaging of Wound pH.** S. Schreml, R. J. Meier, O. S. Wolfbeis, M. Landthaler, R.-M. Szeimies, P. Babilas; *Proc. Natl. Acad. Sci. USA* (**2011**) *108*, 2432-2437. DOI: 10.1073/pnas.1006945108. IF: 9.8.

Abstract: We are presenting a luminescent sensor for 2D, high-resolution imaging of pH in vivo. The sensing scheme is based on luminescence imaging of fluorescein and a ruthenium phenanthroline complex. To create a biocompatible 2D sensor, these dyes were bound to or incorporated in microparticles (aminocellulose, poly-acrylonitrile) and particles were immobilized in polyurethane hydrogel on transparent foils. We demonstrate sensor precision and validity by conducting in vitro and in vivo experiments, and show the versatility in imaging pH during physiological and chronic cutaneous wound healing in humans. Implementation of this technique may open new vistas in wound healing, tumor biology and other biomedical fields. The graphs show images of the pH of an acute would (left) and 14 days p. o. (right).



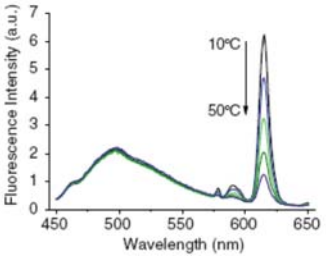
512. *Review.* **Upconverting Luminescent Nanoparticles for Use in Bioconjugation and Bioimaging.** H. S. Mader, P. Kele, S. M. Saleh, O. S. Wolfbeis; *Curr. Opin. Chem. Biol.* (**2010**), *14*, 582-596. DOI: 10.1016/j.cbpa.2010.08.014. IF: 8.3.

Abstract: Upconverting luminescent nanoparticles (UCNPs) display the unique property of emitting visible light following photoexcitation with near-infrared laser light. This results in features such as virtually zero auto-fluorescence of (biological) matter and easy separation of the emission peaks from stray light. Other features include rather narrow emission bands, very high chemical stability, the lack of bleaching, and the absence of blinking effects. This article reviews the work performed in the past few years with UCNPs in terms of surface modifications, bioconjugation, and optical (cellular) imaging.



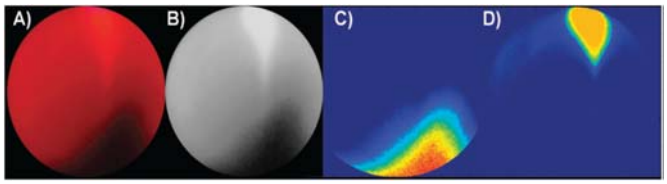
509. **Ratiometric Fluorescent Nanoparticles for Sensing Temperature.** H. Peng, S. Huang, O. S. Wolfbeis; *J. Nanoparticle Res.* (**2010**), *12*, 2729-2733. DOI: 10.1007/s11051-010-0046-8. IF: 2.5.

Abstract: Nanoparticles made from a poly(methyl methacrylate)-co-1,2-bis(trimethoxysilyl)decane composite and containing a red-luminescent europium(III) complex were prepared by the encapsulation-reprecipitation method. By introducing a green-emitting naphthalimide reference dye, the NPs display both a green and a red fluorescence under single-wavelength excitation. The ratio of fluorescence intensities is highly temperature dependent in the 25 - 45 °C range, with a sensitivity of –4.0 % per °C. Given their small size (20 – 30 nm) and biocompatibility (due to the presence of an outer layer of silica), such NPs are useful *T* sensors for cellular sensing and imaging.



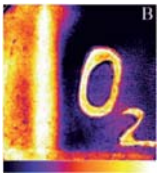
506. *Review:* **Multiple Fluorescent Chemical Sensing and Imaging.** M. I. J. Stich, L. H. Fischer, O. S. Wolfbeis; *Chem. Soc. Rev.* (**2010**), *39*, 3102-3114. DOI: 10.1039/b909635n. IF: 20.1.

Abstract: Optical sensors, unlike most others, enable multiple sensing of (bio) chemical species by making use of probes whose signals can be differentiated by spectral and/or temporal resolution. Multiple sensors are of substantial interest for continuous monitoring of chemical parameters in complex samples. Such sensors enable non-invasive, non-toxic and online detection. We are discussing here the state of the art in terms of spectroscopic principles, materials (mainly indicator probes and polymers), and are giving selected examples for dual and triple sensors (such as combined sensing of O_2/CO_2, pH/O_2, pH/temperature, O_2/T, O_2/T/pH, O_2/T/glucose and the like.



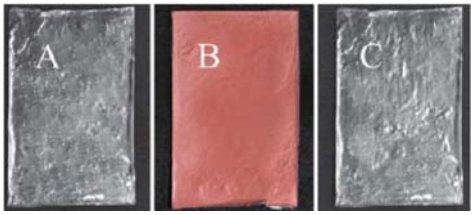
505. **Photographing Oxygen Distribution.** X. Wang, R. J. Meier, M. Link, O. S. Wolfbeis; *Angew. Chem. Int. Ed.* (**2010**), *49*, 4907-4909. DOI: 10.1002/anie.201001305. IF: 11.8.

Abstract: The spatial distribution of oxygen can be imaged with a conventional digital camera by making use of a specially designed sensor film containing a quenchable red-fluorescent probe for oxygen along with a green-emitting reference fluorophore. It exploits the RGB option of digital photography and this has resulted in a simple method for quantitative sensing and imaging of this important species.



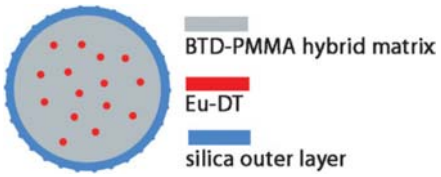
497. **Dual Sensing of *p*O_2 and Temperature Using a Water-Based and Sprayable Fluorescent Paint.** L. H. Fischer, S. M. Borisov, M. Schaeferling, I. Klimant, O. S. Wolfbeis; *Analyst* (**2010**) *135*, 1224-1229. DOI: 10.1039/b927255k. IF: 3.8.

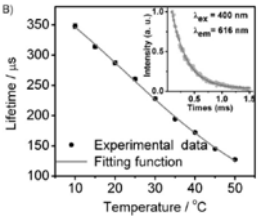
Abstract: Core-shell particles (NPs) composed of a polystyrene core and a poly(vinyl pyrrolidone) shell were dyed with a luminescent platinum(II) porphyrin probe for oxygen. In parallel, microparticles were dyed with a luminescent iridium(II) complex acting as a probe for temperature (*T*). The particles were deposited (by spraying) on a surface to enable continuous imaging of the distribution of oxygen (and thus of barometric pressure) and *T*. Unlike in most previous paints of this kind, a binder polymer is not needed and water can be used as a dispersant. This makes the paint environmentally friendly and reduces costs in terms of occupational health and disposal.
Both indicator probes can be excited at 405 nm using LEDs or diode lasers, whilst their emission maxima are spectrally separated by about 130 nm. Thus, two independent optical signals are obtained that allow for fluorescent imaging of barometric pressure (in fact oxygen partial pressure) and of *T*, but also to correct the oxygen signal for effects of *T*. The paint was calibrated at air pressures ranging from 50 mbar to 2000 mbar and at *T*'s between 1 °C and 50 °C.The images show the aluminum foil before and after coating and washing. (A) Before spraying it with the PS/PVP particles; (B) after spraying it with the red paint (dyed with PtTFPP), and (C) after removing the red paint with water.



496. **Luminescent Europium(III) Nanoparticles for Sensing and Imaging of Temperature in the Physiological Range.** H. Peng, M. I. J. Stich, J. Yu, L. Sun, L. H. Fischer, O. S. Wolfbeis; *Adv. Mat.* (**2010**), *22*, 716-719. DOI: 10.1002/adma.200901614. IF: 8.4.

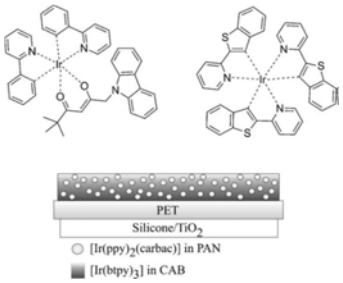
Abstract: Lanthanide-based nano-particles with a diameter of 20–30 nm are introduced for use in luminescent sensing and imaging of physiological temperatures. They are characterized by (i) visible-light photo-excitation, (ii) line-like emission (which facilitates multicolor (dual) sensing), (iii) inert-ness to external perturbers as a result of encapsulation of the europium probe into a biocompatible protective nanoshell, (iv) high photostability, (v) a dynamic range that covers *T*'s encountered in medicine, (cellular) biology, and biotechnology, and (vi) good resolution (typically +/- 0.3 °C).





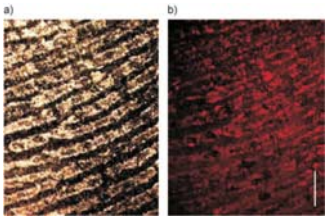
485. **Red and Green Emitting Iridium(III) Complexes for a Dual Barometric and Temperature Sensitive Paint.** L. H. Fischer, M. I. J. Stich, O. S. Wolfbeis, N. Tian, E. Holder, M. Schaeferling; *Chemistry – Eur. J.* (**2009**), *15*, 10857-10863. DOI: 10.1002/chem.200901511. IF: 5.5.

Abstract: A dually luminescent paint for measurement of barometric pressure (BP) and temperature (*T*) is presented. The green-emitting iridium(III) complex Ir-(ppy)(carbac) was applied as a novel probe for T along with the red-emitting complex [Ir-(btpy) which functions as a probe for BP (in fact for oxygen). The two iridium complexes were dissolved in two different polymer materials to achieve optimal responses. The effects of *T* on the response of the oxygen probe can be corrected for by simultaneous optical determination of *T*. The signals of the probes for T and BP can be separated by optical filters due to the ~75 nm difference in their emission maxima. The dual sensor is applicable to luminescence lifetime imaging of *T* and barometric pressure.



479. *Highlight Article*: **Nanoparticle-Enhanced Fluorescent Imaging of Latent Finger-prints Reveals Drug Abuse.** O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (**2009**), *48*, 2268-2269. DOI: 10.1002/anie.200805765. IF: 10.3.

Abstract: A Highlight Article on two recent papers by Russell et al. where the authors have combined three kinds of high technology: (1) magnetic nanoparticles, (2) fluorescence imaging, and (3) immunoassay, so to produce a method that has a high potential not only for the detection of various kinds of drugs, but also for the detection of other species such as chemicals formed in explosions.



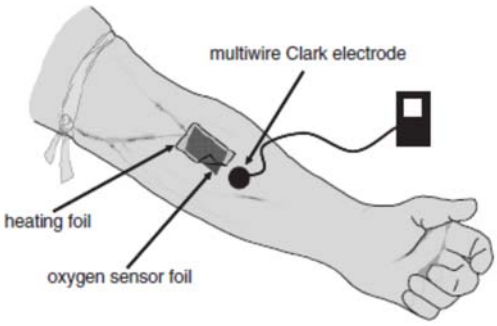
472. **Transcutaneous *p*O_2 Imaging During Tourniquet-Induced Forearm Ischemia Using Planar Optical Oxygen Sensors.** P. Babilas, P. Lamby, L. Prantl, S. Schreml, E. M. Jung, G. Liebsch, O. S. Wolfbeis, M. Landthaler, R. M. Szeimies, C. Abels, *Skin Res. Technol.* (**2008**), *14*, 304–311. DOI: 10.1111/j.1600-0846.2008.00295.x.

Background: Oxygen-dependent quenching of luminescence using transparent planar sensor foils was shown to overcome the limitations of the polarographic electrode technique in an animal model. This method was then transferred to a clinical setting to measure the transcutaneous pO2 (ptcO2).

Methods: In six healthy subjects, a cuff on the upper arm was occluded up to 20mmHg above systolic pressure and released after 8 min. PtcO2 was measured at the lower arm every 30 s before, during, and up to 20 min after cuff occlusion (at 40 °C skin temperature) using luminescence lifetime imaging (LLI) of platinum(II)-octaethyl-porphyrin immobilized in a polystyrene matrix. For validation, the polarographic Clark electrode technique was applied in close proximity, and measurements were conducted simultaneously.

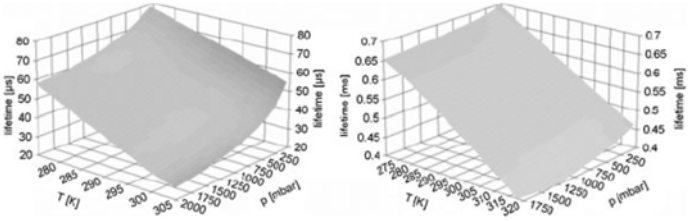
Results: $PtcO_2$ measurements before and at the end of ischemic and reperfusion phases did not differ significantly using the Clark electrode vs. LLI. At both the initial ischemic and the reperfusion phases, the Clark electrode measured a faster decrease or increase, respectively, in $ptcO_2$ because of the oxygen consumption occurring in the Clark method.

Conclusion: The method provides accurate and reproducible $ptcO_2$ values under changing microcirculatory conditions. The lack of oxygen consumption during measurement allows both a more realistic estimation of $ptcO_2$ than compared with the gold standard and permanent use in regions with critical oxygen supply.

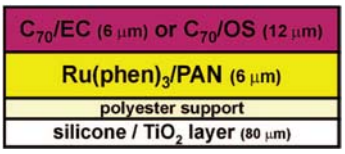


466. **Dual Luminescent Sensor Material for Simultaneous Imaging of Barometric Pressure and Temperature on Surfaces.** M. I. J. Stich, S. Nagl, O. S. Wolfbeis, U. Henne, M. Schaeferling; *Adv. Funct. Mat.* (**2008**) *18*, 1399-1406. DOI: 10.1002/adfm.200701199. IF: 7.0

Abstract: A composite material is presented for simultaneous luminescent sensing of air pressure and temperature (*T*) on surfaces. The sensor consists of a fluorinated platinum porphyrin complex as the oxygen-sensitive probe, and a highly *T*-sensitive europium complex acting as a probe for *T*. The signals are separated via the different luminescence lifetimes of the indicators by making use of a new 4-window technique that enables time-resolved determination of 2 decay times.

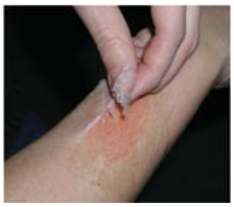


463. **Dual Fluorescence Sensor for Trace Oxygen and Temperature with Unmatched Range and Sensitivity,** C. Baleizão, S. Nagl, M. Schaeferling, M. N. Berberan-Santos, O. S. Wolfbeis; *Anal. Chem.* (**2008**), *80*, 6449-6457. DOI: 10.1021/ac801034p. IF: 5.6. *Abstract:* An optical dual sensor for oxygen and temperature (*T*) is presented which is highly oxygen sensitive and covers a broad *T* range. It contains two luminescent compounds incorporated into polymer films. Ruthenium tris(1,10-phenanthroline) has a highly *T* -dependent luminescence and is incorporated in poly(acrylonitrile) to avoid cross-sensitivity to oxygen. Fullerene C_{70} was used as the oxygen-sensitive probe owing to its intense thermally activated delayed fluorescence (TADF) at elevated *T*'s that is extremely oxygen sensitive. The cross-sensitivity of C_{70} to *T* is accounted for by means of the *T* sensor. C_{70} is incorporated into a highly oxygen permeable polymer, either ethyl cellulose (EC) or organosilica (OS). The two luminescent probes have different emission spectra and decay times and their emissions can be discriminated using both parameters. Spatially resolved sensing is achieved by means of fluorescence lifetime imaging. The dual sensor covers the temp. range from 0 and 120 ºC, and detection limits for oxygen are in the low ppbv range. These ranges outperform all dual oxygen and *T* sensors reported so far.



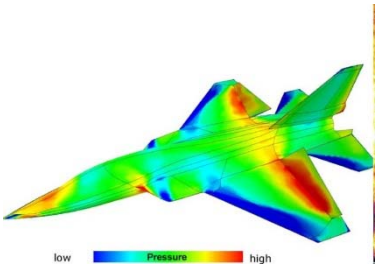
458. *Review:* **Sensor Paints.** O. S. Wolfbeis; *Adv. Mat.* (**2008**), *20*, 3759-3763. DOI: 10.1002/adma.200702276. IF: 8.2.

Abstract: Fluorescence microscopy is widely used for chemical imaging of intrinsically fluorescent chemical species, such as chlorophylls, or nonfluorescent species (e.g., DNA) that have been labeled appropriately or to which a relevant molecular probe has been added. However, there are numerous analytes that neither have an intrinsic luminescence nor can be rendered luminescent with the help of labels or probes. Such parameters include oxygen, pH, CO2, ammonia, and glucose. There are also numerous applications where the 2D distribution of a chemical or physical parameter is of interest. The use of a ''sensor paint'' is ideal in such situations. The state of this new technology is discussed herein with emphasis on current and future trends, for example, the use of pressure- and temperature-sensitive paints; known technological limitations are also discussed. These ''paints'' respond to a (bio)chemical parameter with a change in their optical properties. The object of interest is painted and the color or fluorescence of the paint is monitored by optical imaging. This technique enables monitoring of physical and chemical parameters over relatively large areas and in real time. In a typical application, the object of interest is painted and the fluorescence of the paint is monitored by methods of optical imaging. This technique represents a simple but exciting new technology to monitor (bio)chemical and physical parameters over relatively large areas and in real time.



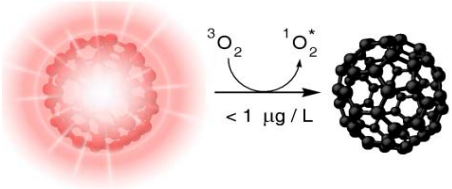
457. *Book Chapter:* **Fluorescence Sensing and Imaging Using Pressure-Sensitive Paints and Temperature-Sensitive Paints.** M. I. J. Stich, O. S. Wolfbeis; in: *Standardization and Quality Assurance in Fluorescence, vol. I* , U. Resch-Genger (ed.), Springer Berlin – Heidelberg (2008), pp. 429-461; ISBN 978-3-540-75206-6. DOI 10.1007/978-3-540-75207-3.

Abstract: Barometric-pressure-sensitive paints (PSPs) and temperature-sensitive paints (TSPs) are widely used in aerodynamic research and wind tunnel testing. Both systems are based on the incorporation of the respective indicators into a matrix polymer (often referred to as the "binder") to be cast on the area of interest. Spatially resolved distributions of oxygen partial pressure (pO2) and T can be instantly visualized by making use of respective paints and appropriate techniques of fluorescence imaging. This chapter summarizes state of the art in probes and polymers for use in PSPs and TSPs. Fluorescence spectroscopic methods for the interrogation of the paints are described along with the components and respective experimental setups. Finally, we discuss the advantages and drawbacks of various systems and methods, along with their utility in fields of applications such as measurement of barometric pressure on aircrafts.



447. **Optical Sensing and Imaging of Trace Oxygen with Record Response.** S. Nagl, C. Baleizão, S. M. Borisov, M. Schaeferling, M. N. Berberan-Santos, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* (**2007**), *46*, 2317-2319. DOI: 10.1002/anie.200603754. IF: 10.3.

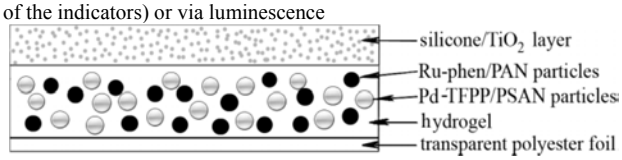
Abstract: Ultratrace quantities of oxygen can be determined over a temperature range of more than 100 °C by exploiting the extremely efficient quenching of the delayed fluorescence of fullerene C70 incorporated into organosilica or ethyl cellulose.



439. **Composite Luminescent Material for Dual Sensing of Oxygen and Temperature.** S. Borisov, A. Vasilevskaya, C. Krause, O. S. Wolfbeis; *Adv. Funct. Mater.* (**2006**), *16*, 1536-1542. DOI: 10.1002/adfm.200500778. IF: 6.8.

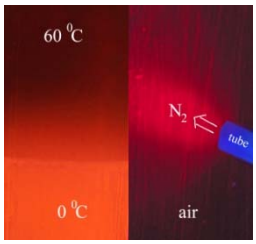
Abstract: A novel kind of composite material is presented that contains two indicators incorporated into a single polymer matrix, thus allowing simultaneous determination of oxygen partial pressure and temperature (*T*). The *T*-sensitive ruthenium complex was chosen for its highly *T*-dependent luminescence. A

fluorinated palladium(II) tetraphenylporphyrin served as the oxygen probe. The indicators were incorporated into either poly(styrene-co-acrylonitrile) microparticles (to sense oxygen) or into poly(acrylonitrile) (for sensing *T* since this polymer is virtually impermeable to oxygen). The luminescence of both dyes can be separated either spectrally (due to different absorption and emission spectra of the indicators) or via luminescence decay time. The material is suitable for *T*-compensated oxygen sensing, for example in high-resolution oxygen profiling, and for imaging *T* in the range between 0 and 60 °C. This enables *T* to be "seen" in this important ange.Simultaneous imaging of barometric pressure and *T* also has been achieved. It enables contactless imaging of the two parameters, for example in wind tunnels. Due to the use of a biocompatible hydrogel matrix, the material conceivably is suited for biomedical applications.



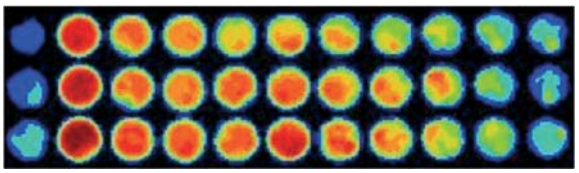
436. **Composite Material for Simultaneous and Contactless Sensing and Imaging of Oxygen and Carbon Dioxide.** S. M. Borisov, C. Krause, S. Arain, O. S. Wolfbeis; *Adv. Mat.* **(2006)**, *18*, 1511-1516. DOI: 10.1002/adma.200600120. IF: 7.9.

Abstract: A sensor is described that enables simultaneous imaging and monitoring of carbon dioxide and oxygen. It relies on the measurement of the phase shift of the luminescence decay time of a material that is composed of spectrally carefully selected indicators (with well-separated wavelengths of excitation and emission) that are contained in microbeads and polymers with excellent permeation selectivities along with optical and adhesive properties. The sensor material is used to monitor the growth of *Pseudomonas putida*. The *Picture* shows the orange-colored luminescence of the ruthenium probe contained in poly-acrylonitrile microparticles is highly temperature (*T*) dependent (luminescence drops with *T*). *Right*: After correction for *T* effects, the dark red luminescence of the Pd porphyrin contained in microbeads of an oxygen-permeable copolymer reflects areas of low oxygen (where nitrogen is blown on; this resulting in high luminescence) and areas of higher oxygen tension (where luminescence is weaker). The two signals (orange and red), though spectrally overlapping, can be resolved via luminescence lifetime measurements.



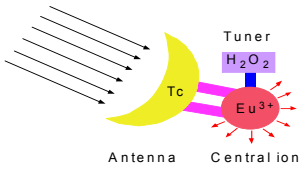
431. **Novel Method for Time-Resolved Fluorometric Determination and Imaging of the Activity of Peroxidase, and its Application to Enzyme-Linked Immunosorbent Assays**, Z. Lin, M. Wu, O. S. Wolfbeis, M. Schaeferling; *Chem. – Eur. J.* **(2006)**, *12*, 2730-2738. DOI: 10.1002/chem.200500884. IF: 5.0.

Abstract: A new format for enzyme-linked immunosorbent assays (ELISA) is described, using the europium(III) tetracycline complex [Eu(Tc)] as a fluorescent probe for hydrogen peroxide (HP). [Eu(Tc)] forms a strongly fluorescent complex with HP, and the peroxidase-catalyzed decoposition of the system [Eu(Tc)-HP] can be monitored via the decrease in fluorescence due to the formation of the weakly fluorescent [Eu(Tc)]. Due to this effect, the europium probe can be used to detect the presence of peroxidases linked to antibodies. Furthermore, the fluorescence decay time of [Eu(Tc)-HP] is in the range of 60 µs, which enables the application of time-resolved detection methods. These show superior properties compared to intensity-based techniques due to better elimination of background signals. The time-resolved ("gated") fluorescence assays cover a dynamic range from 0.1 – 8 ng/mL for the quantitation of bovine IgG by peroxidase-labeled anti-bovine IgG in asandwich-type ELISA. Multi-plexed samples can alternatively be visualized directly and evaluated quantitatively by means of fluorescent imaging with the help of an array of light-emitting diodes (LEDs) and a CCD camera. The figure shows a typical array of images as obtained by time-resolved imaging of microwell spots (sandwich assay format).



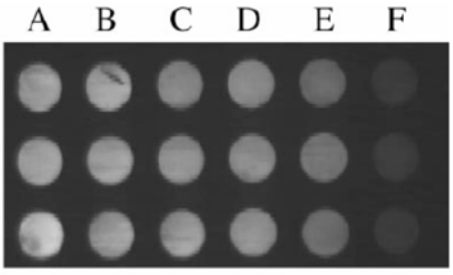
427. **Nonenzymatic Direct Assay of Hydrogen Peroxide at Neutral pH Using the Eu_3Tc Fluorescent Probe.** A. Duerkop, O. S. Wolfbeis; *J. Fluoresc.* **2005**, *15*, 755-761. DOI: 10.1007/s10895-005-2984-6. IF: 2.6.

Abstract: A detailed study is presented on the use of an easily accessible probe (the europium–tetracycline 3:1 complex; referred to as Eu_3Tc) for determination of hydrogen peroxide (HP). Eu_3Tc undergoes a 15-fold increase in luminescence intensity on exposure to an excess of HP. Data are given on the time dependence of the reaction, on the pH dependence of the absorption and emission spectra. HP can be quantified in aqueous solution of pH 6.9 over a 2 – 400 µM concentration range with a limit of detection of 960 nM. The assay is validated using standard additions, and mean recoveries are found to be between 97.0 and 101.8 %. Species that interfere in concentrations below 1 mM include phosphate, copper(II), fluoride and citrate. The method is critically assessed with respect to other optical methods for determination of HP.



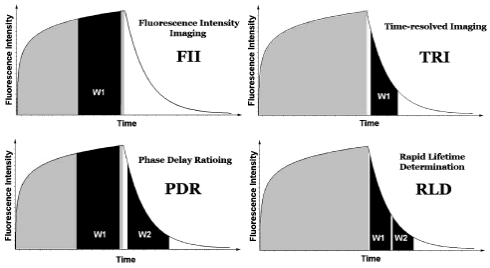
426. **Fluorescence Quenching of the Europium Tetracycline Hydrogen Peroxide Complex by Copper(II) and other Metal Ions**, C. Cano-Raya, M. D. Fernández Ramos, L. F. Capitán Vallvey, O. S. Wolfbeis, M. Schaeferling; *Appl. Spectrosc.* **(2005)**, *59*, 1209. DOI: 10.1366/000370205774430945. IF: 1.9.

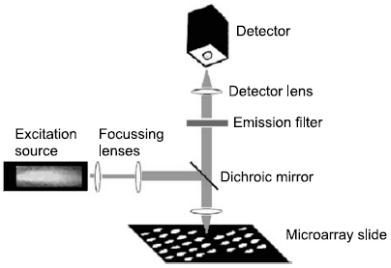
Abstract: The europium-tetracycline complex [Eu(Tc)] on addition of hydrogen peroxide (HP) undergoes a 15-fold increase in luminescence intensity. Luminescence is quenched by Cu^{2+}, Fe^{3+}, Ag^{+}, Al^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+}. The stropngest quenching (both static and dynamic) is induced byCu^{2+}, and these processes were quantified by means of their quenching constants. Stern-Volmer plots were also derived from lifetime imaging measurements accomplished by the Rapid Lifetime Determination (RLD) technique based on microwell plate assays, and also by the Time-Correlated Single Photon Counting technique (TCSPC). A time-resolved fluorescent method for determination of copper is presented. The response to copper is linear up to 1.6 µM, providing a detection limit of 0.2 µM. The figure shows grey-scale images of solutions of the [Eu(Tc)(HP)] complex in a microwell plate exposed to different concentrations of Cu(II). Data were acquired by the rapid lifetime determination (RLD) method.



424. *Review.* **Fluorescence Analysis in Microarray Technology**, S. Nagl, M. Schaeferling, O. S. Wolfbeis; *Microchim. Acta* **2005**, *151*, 1-21. DOI: 10.1007/s00604-005-0393-9. IF: 1.6.

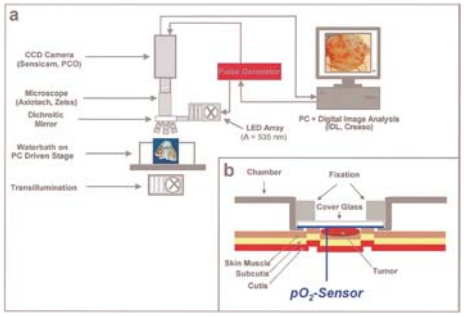
Abstract: We review the most common methods and the state-of-the-art of all areas in microarray fluorescence analysis. Starting with an overview on microarray formats with a focus on (a) their demands on the readout, (b) common organic fluorescent stains, (c) the use of semiconductor nanocrystals (quantum dots), polymer and silica nanoparticles and (d) of fluorescent proteins as labels. Ways to enhance the intrinsically low signal on biochips have become increasingly important as they offer a sound approach towards the detection of low concentration sample content. The three main categories are presented, viz. amplification using DNA, enzymes, and dendrimers. As much diversity as on the microarrays themselve can be found at the detection device. Standard optical microarray detectors, and non-standard methods using fluorescence anisotropy, fluorescence lifetime imaging (FLIM) and fluorescence resonance energy transfer (FRET), and their advantages and dis-advantages are discussed. The figures shows common methods for intensity or lifetime based imaging (left), and a typical optical arrangement (right).





421. **In Vivo Phosphorescence Imaging of pO_2 Using Planar Oxygen Sensors.** P. Babilas, G. Liebsch, V. Schacht, I. Klimant, O. S. Wolfbeis, R.-M. Szeimies, C. Abels, *Microcirculation (Philadelphia)* (**2005**), *12*, 477-487. DOI: 10.1080/10739680591003314 . IF: 2.4.

Objective: Oxygen-dependent quenching of luminescence of metal porphyrin complexes has been used to image the pO2 distribution over tumor and normal tissue.
Methods: An exptl. setup is described using a platinum(II)–octaethyl–porphyrin immobilized in a polystyrene matrix as transparent planar sensor.
Results: Sensitivity over a broad range is high at low pO2 values (±0.2 mm Hg at 0 mm Hg; ±1.5 mm Hg at 160mmHg pO2). Due to intrinsically referencing via lifetime encoding there was no modification of the sensor response in vivo in the dorsal skinfold chamber model with amelanotic melanoma (AMEL-3) in awake hamsters when compared to the in vitro calibration. pO2 measurements over normal tissue (25.8 ± 5.1 mm Hg) and tumor tissue (9.2 ± 5.1 mm Hg) were in excellent agreement with previous results obtained in this model using a surface multiwire electrode.
Conclusions: Using the presented method the surface pO2 distribution can be mapped with a high temporal resolution of approximately 100 ms and a spatial resolution of at least 25 µm. Moreover, the transparent sensor allows the simultaneous visualization of the underlying microvasculature.

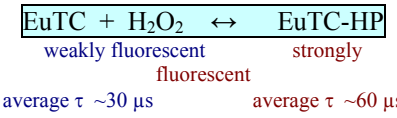


420. **Time-Resolved Fluorescent Chirality Sensing and Imaging of Malate in Aqueous Solution.** Z. Lin, M. Wu, O. S. Wolfbeis; *Chirality* (**2005**), *17*, 464–469. DOI: 10.1002/chir.20185. IF: 2.2.

Abstract: Chiral discrimination of malates in aqueous solutions at near-neutral pH is achieved through fluorescence measurement and imaging using the europium(III)–tetracycline complex (EuTc) as a fluorescent probe. The method is based on the significantly different fluorescence properties of the ternary complexes (Eu–Tc–malate) formed between EuTc and the enantiomeric malates. The enantiomeric excess (ee) of chiral malates can be quantified by both steady-state and time-resolved fluorescence, using either a conventional fluorescence microplate reader or fluorescence imaging. It offers a facile and sensitive method for high-throughput chiral discrimination.

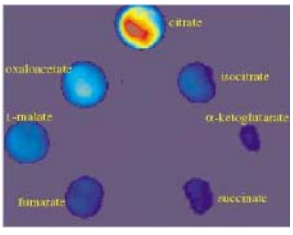
412. **Fluorescence Imaging of the Activity of Glucose Oxidase Using a Hydrogen Peroxide Sensitive Europium Probe**, M. Wu, Z. Lin, M. Schaeferling, A. Duerkop, O. S. Wolfbeis; *Anal. Biochem.* **2005**, *340*, 66-73. DOI: 10.1016/j.ab.2005.01.050. IF: 3.0.

Abstract: A method for optical imaging of the activity of glucose oxidase (GOx) using a fluorescent europium(III) tetracycline probe (EuTC) for hydrogen peroxide is presented. A decay time in the microsecond range and the large Stokes shift of 210 nm of the probe facilitate intensity-based, time-resolved, and decay-time-based imaging of glucose oxidase. Four methods for imaging the activity of GOx were compared, and rapid lifetime determination imaging was found to be the best in giving a linear range from 0.32 to 2.7 mUnit/mL. The detection limit is 1.7 ng/mL. Fluorescent imaging of the activity of GOx is considered to be a useful tool for GOx-based immunoassays with potential for high-throughput screening, immobilization studies, and biosensor array technologies.



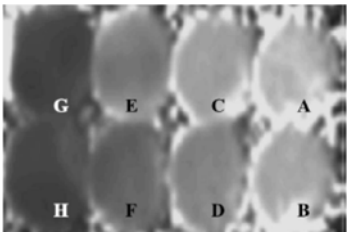
400. **Fluorescent Imaging of Citrate and Other Intermediates in the Citric Acid Cycle,** Z. Lin, M. Wu, M. Schaeferling, O. S. Wolfbeis; *Angew. Chem. Intl. Ed.* **2004**, *43*, 1735 –1738. DOI: 10.1002/anie.200353169. IF: 10.3.

Abstract: Citrate and other intermediates of the Krebs cycle (isocitrate, ketoglutarate, succinate, fumarate, malate, oxaloacetate) can be sensed and imaged by time-resolved fluorescence spectroscopy using the Eu^{3+}-tetracycline complex as a fluorescent probe. Time-resolutions enables discrimination between different intermediates, and enzymatic conversions are not needed for making the species susceptible to imaging. The complexes with different intermediates can be discriminated via their different "fluorescence" decay times.



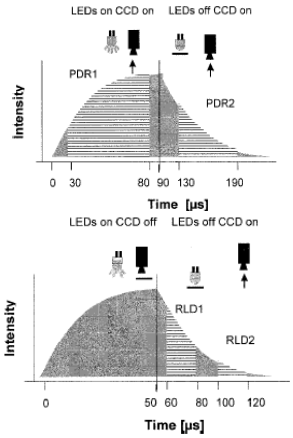
399. **Time-Resolved Fluorescent Imaging of Glucose**, M. Schaeferling, M. Wu, O. S. Wolfbeis; *J. Fluoresc.* **2004**, *14*, 561-568. DOI: 10.1023/B:JOFL.0000039343.02843.12. IF: 2.6.

Abstract: A method for the fluorescent imaging of glucose is described that is based on the detection of enzymatically produced hydrogen peroxide, using the europium(III) tetracycline complex as the fluorescent probe incorporated into a hydrophilic polymer layer. Coadsorption of glucose oxidase (GOx) makes these sensor layers respond to the hydrogen peroxide produced by the GOx-assisted oxidation of glucose. These hydrogels are integrated into a 96-microwell for a parallel and simultaneous detection. Glucose is visualized by means of time resolved luminescence lifetime imaging. Unlike in previous methods, the determination of H_2O_2 does not require the addition of peroxidase or a catalyst to form a fluorescent product. The lifetime-based images obtained are compared to conventional fluorescence intensity-based methods with respect to sensitivity and the dynamic range of the sensor layer. The Figure shows grey-scale ratiometric fluorescence images of sensor membrane spots placed in a microwell plate following exposure to various concentrations of glucose (from 0 to 1 M).



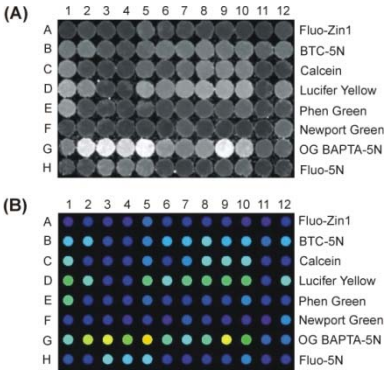
392. **Time-Resolved Luminescence Imaging of Hydrogen Peroxide Using Europium-Tetracycline Sensor Membranes in a Microwell Format**, M. Schaeferling, M. Wu, J. Enderlein, H. Bauer, O. S. Wolfbeis; *Appl. Spectrosc.* **57** (2003) 1386-1392. DOI: 10.1366/000370203322554554. IF: 1.9.

Abstract: We demonstrate an optical imaging scheme for hydrogen peroxide in a microwell-based format using the europium(III) tetracycline complex as the fluorescent probe, which is incorporated into a poly(acrylonitrile)-co-poly(acrylamide) polymer matrix. The resulting sensor membranes are integrated into a 96-microwell plate. Hydrogen peroxide can be visualized by means of time-resolved imaging. The imaging system consists of a fast, gated CCD camera and a pulsed array of 96 LEDs. Fluorescence lifetime images were acquired by both rapid lifetime detection and phase delay rationing, and compared with intensity-based methods with respect to sensitivity and dynamic range. The response time of the sensor is comparatively high, typically in the range of 10 to 20 min, but the response is reversible. The largest signal changes are observed at pH 6.5 – 7.5. Below is a schematic representation of the time gates in the PDR imaging method (left), and in the RLD imaging method (right). In the PDR scheme, the luminescence is measured first in the excitation period (PDR1), and then in the emission period (PDR2). In the RLD scheme, both time gates (RLD1 and RLD2) are detected during the emission period.

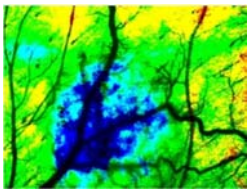


386. **Cross-Reactive Metal Ion Sensor Array in a Microtiterplate Format**, T. Mayr, C. Igel, G. Liebsch, I. Klimant, O.S. Wolfbeis; *Anal. Chem.* **75** (2003) 4389-4396. DOI: 10.1021/ac020774t. IF: 5.7.

Abstract: A cross-reactive array in a micro titer plate (MTP) format is described that is based on a versatile and highly flexible scheme. It makes use of rather unspecific metal ions probes having almost identical fluorescence spectra, thus enabling (a) interrogation at identical analytical wavelengths, and (b) imaging of the probes contained in the wells of the MTP using a CCD camera and an array of blue-light-emitting diodes as a light source. The response of the indicators in the presence of 5 divalent cations generated a characteristic pattern that was analyzed by chemometry. The fluorescence intensity of the indicators was transfered into a time-dependent parameter applying a scheme called *dual lifetime referencing*. In this method, the fluorescence decay profile of the indicator is referenced against the phosphorescence of an inert reference dye. The pictures obtained form the basis for evaluation by pattern recognition algorithms. Support vector machines are capable of predicting the presence of significant concns. of metal ions with high accuracy.

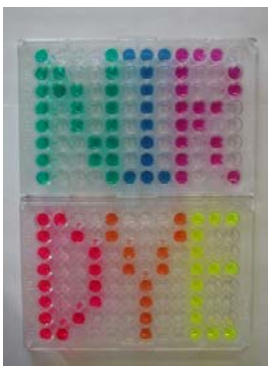


381. **Effects of Light Fractionation and Different Fluence Rates on Photodynamic Therapy with 5-Aminolaevulinic Acid in-vivo**, P. Babilas, V. Schacht, G. Liebsch, O. S. Wolfbeis, M. Landthaler, R. M. Szeimies; *Brit. J. Cancer* **88** (2003) 1462-1469. DOI: 10.1038/sj.bjc.6600910. DOI: 10.1038/sj.bjc.6600910. IF: 4.5. *Abstract:* Photodynamic therapy (PDT) was performed on hamsters with amelanotic melanoma. Prior to, and up to 24 h after PDT tissue, the pO_2 was measured using luminescence lifetime imaging. The efficacy of PDT was evaluated by measuring the tumor vol.ume of amelanotic melanoma cells grown subcutaneously in the back of the hamsters. Only high-dose PDT resulted in a significant decrease of pO_2, while low-dose PDT failed to induce a significant decrease.The image shows the distribution of oxygen in tumorous skin, with a larger underoxygenation in the blue (= tumorous) areas before PDT. This area disappears following successful PDT.



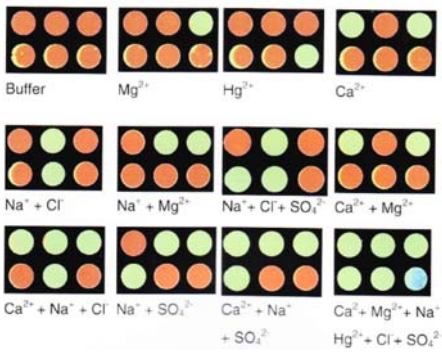
374. *Book Chapter.* **Advanced Luminescent Labels, Probes and Beads, and Their Application to Luminescence Bioassay and Imaging**, O. S. Wolfbeis, M. Boehmer, A. Duerkop, J. Enderlein, M. Gruber, I. Klimant, Ch. Krause, J. Kuerner, G. Liebsch, Zh. Lin, B. Oswald, M. Wu; *Springer Series in Fluorescence Spectroscopy,* vol. 2 (R. Kraayenhof, A. J. W. G. Visser, H. C. Gerritsen, eds.); Springer Verlag, Berlin-Heidelberg, 2002; pp. 3-42. DOI: 10.1007/978-3-642-56067-5_1.

Abstract: An overview is given on our recent activities in the following areas: (1) general logics for designing fluorescent probes and labels; (2) new diode laser-excitable probes for non-covalent protein detection; (3) diode laser-compatible amine-reactive labels; (4) diode laser-assisted fluorescent single molecule detection of dyes and labeled proteins; (5) new labels for flow cytometric determination of HSA; (6) new DNA labels; (7) fluorescence resonance energy transfer gene assays; (8) reactive ruthenium ligand complexes as markers for bioassays; (9) diode laser-excitable fluorescent polymer beads; (10) polyaniline-coated nanobeads as pH probes; (11) phosphorescent poly(acrylonitrile) nanospheres as markers for optical assays; (12) competititve binding of streptavidin to biotinylated nanobeads as studied by resonance energy transfer; (13) nanobeads as reference dyes in luminescent lifetime imaging using DLR; (14) phosphorescent nanospheres for use in time-resolved multiplexed bioassays; (15) beads dyed with a europium-based label and excitable with the 405-nm diode laser; and (16) a europium(III)-based



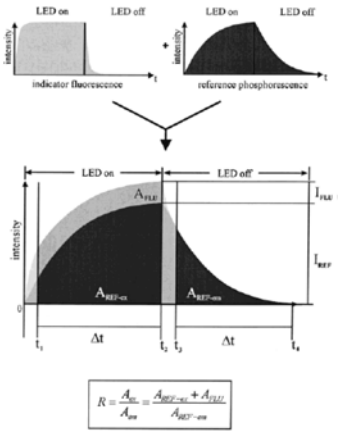
probe for use in oxidase-associated reactions.

363. **Multi-Ion Imaging Using Fluorescent Sensors in a Microtiterplate Array Format.** T. Mayr, G. Liebsch, I. Klimant, O. S. Wolfbeis; *Analyst* **127** (2002) 201-203. DOI: 10.1039/b1110776. IF: 3.2.



Abstract: A novel type of sensor array destined for water analyses is described. The sensor delivers simple on/off patterns of complex ion mixtures. Fluorescent indicators for Ca^{2+}, Na^+, Mg^{2+}, SO_4^{2-}, Cl^- and Hg^{2+} were arranged in microtiterplates and the analytical information was imaged with a CCD camera within microseconds using an intrinsically referencing method.

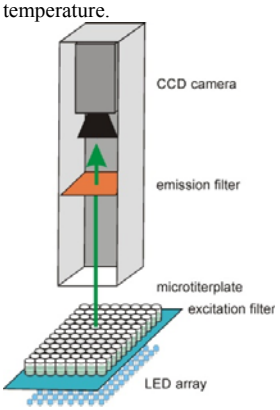
355. **Fluorescent Imaging of pH with Optical Sensors Using Time Domain Dual Lifetime Referencing**, G. Liebsch, I. Klimant, Ch. Krause, O. S. Wolfbeis; *Anal. Chem.* **73** (2001) 4354-4363. DOI: 10.1021/ac0100852. IF: 5.7.

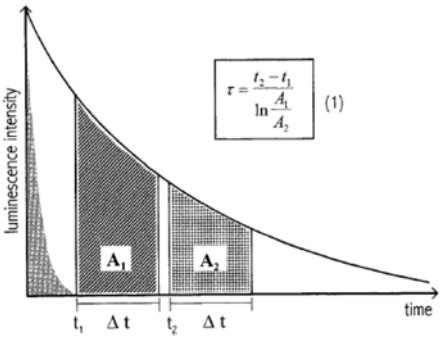


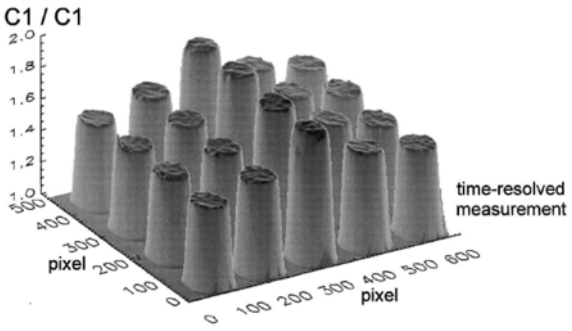
Abstract: We present a referenced scheme for fluorescence intensity measurements which is useful for imaging applications. It is based on the conversion of the fluorescence intensity information into a time dependent parameter. A phosphorescent dye is added, in the form of approx. 10 µm particles to the sample containing the pH-sensitive fluorescent indicator. Both the ref-dye and the pH probe are excited simultaneously by a blue LED, and an overall luminescence is measured. In the time-resolved imaging method presented here, two images taken at different time gates were recorded with a CCD-camera. The first image is recorded during excitation and reflects the luminescence signal of both the fluorophore (pH) and the phosphor (reference). The second image which is measured after a certain delay (after switching off the light source), is solely caused by the long-lived phosphorescent dye. Since the intensity of the fluorophore contains the information on pH, whereas phosphorescence is pH-independent, the ratio of the images displays a referenced intensity distribution that reflects the pH at each picture element (pixel). The scheme is useful for LED light sources and CCD cameras that can be gated with square pulses in the microsecond range. The fundamentals and potential of this new method - to which we refer to as t*ime domain dual lifetime referencing* - are demonstrated.

338. **Luminescence Lifetime Imaging of Oxygen, pH, and Carbon Dioxide Distribution Using Optical Sensors.** G. Liebsch, I. Klimant, B. Frank, G. Holst, O. S. Wolfbeis; *Appl. Spectrosc.* **54** (2000) 548-559. DOI: 10.1366/0003702001949726. IF: 1.9.

Abstract: We present a modular system for time-resolved two-dimensional luminescence lifetime imaging of planar optical chemical sensors. It is based on a fast gateable CCD camera without image intensifier and a pulsable LED array as a light source. A software was developed for data acquisition with a maximum of parameter variability and for background suppression (see the figure below). This allows the operation of the system even under daylight. Optical sensors showing analyte specific changes of their luminescence decay time were tested and used for sensing pO_2, pCO_2, pH values, and temperature. The luminophores employed are either Pt^{2+}-porphyrins or Ru^{2+}-polypyridyl complexes, contained in polymer films, and can be efficiently excited by blue LEDs. The decay times of the sensor films vary from 70 µs for the Pt^{2+}-porphyrins to several 100 ns for the Ru^{2+}-complexes. In a typical application, 7-mm diameter spots of the respective optical sensor films were placed at the bottom of the wells of microtiterplates. Thus, every well represents a separate calibration chamber with an integrated sensor element. Both luminescence intensity-based and time-resolved images of the sensor spots were evaluated and compared. The combination of optical sensor technology with time-resolved imaging allows for the determination of the distribution of chemical or physical parameters in heterogeneous systems and is therefore a powerful tool for screening and mapping applications. The Figures show the experimental setup, the principle of rapid lifetime determination (RLD), and a typical image obtained with ratiometric data (that supress background light) for microplate wells filled with solutions of different temperature.

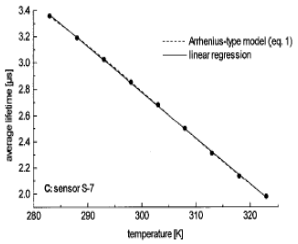






330. **Luminescence Lifetime Temperature Sensing Based on Sol-Gels and Poly(acrylonitrile)s Dyed with Ruthenium Metal Ligand Complexes.** G. Liebsch, I. Klimant, O. S. Wolfbeis; *Adv. Mater.* **11** (1999) 1296-1299. DOI: 10.1002/(SICI)1521-4095(199910)11:15<1296::AID-ADMA1296>3.0.CO;2-B

Abstract: Temperature (*T*)-sensitive luminescent materials are obtained by embedding the Ru(II)-tris-1,10-phenanthroline complex into a poly(acrylonitrile) (PAN) or a densified sol-gel matrix. Both the luminescence intensity and the luminescence decay time are strongly affected by *T*, while the gas-impermeable matrices prevent dynamic quenching by oxygen or other quenchers. *T* sensors can be prepared in the form of transparent planar films and coatings, or as a powder. The *T* dependence was established in the 10 to 40 °C range and the films are demonstrated to be useful for *T*'s up to 100 °C. The materials respond completely reversibly, are highly luminescent, have fast response and possess lifetimes in the microsecond time regime. They can be used as a *T*-sensitive paint for single-point measurements, for imaging or for internal *T* compensation in optical chemical sensors.



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