FINAL PROJECT REPORT

Multi-Parameter, Remote Groundwater Monitoring with Referencing Using Crossed Optical Fiber Fluorescent Sensor Arrays

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PROJECT SUMMARY

Project Title:	Multi-Parameter, Remote Groundwater Monitoring with Referencing Using Crossed Optical Fiber Fluorescent Sensor Arrays
Project I.D.:	WR06R001
Project Investigator:	*Peter Geissinger*, Associate Professor, Department of Chemistry & Biochemistry, University of Wisconsin-Milwaukee
Research Assistants:	*M. Veronica Rigo*, Graduate Student (Ph.D. program) *Paul E. Henning*, Graduate Student (Ph.D. program) Both: Department of Chemistry & Biochemistry, University of Wisconsin-Milwaukee
Period of Contract:	July 1, 2006 – June 30, 2008

Background/Need: Real-time, in-situ measurements of general chemical and physical properties of groundwater are essential for studying the interactions of aquifers with surrounding soils and minerals. Furthermore, measuring contaminant concentrations continually over extended times and spatially resolved is at the heart of investigations regarding the sources of contamination of groundwater as well as the fate of these contaminants due to transport and chemical reactions. This project focused on the application of our recently developed (and patented) optical fiber sensor platform to groundwater monitoring issues.

Objectives: The goals of this project were to explore real-time, continuous measurement of pH and dissolved oxygen concentration as first sensing applications. For long-term deployment, sensor longevity, and ruggedness were important issues to be considered in the sensor design.

Methods: For measurement of the desired parameters at many locations using only a small number of optical fibers, we create many sensor regions along an optical fiber. At desired locations along the optical fiber, the fiber core is exposed by removing the original fiber cladding and suitable replacement claddings containing sensor molecules are attached. Sensing is based on changes of the fluorescence properties of sensor molecules. Light propagating inside of the fiber interacts with the sensor molecules outside of the fiber through evanescent fields. Fluorosensors located within the range of the evanescent fields are optically excited; subsequent fluorescence emission is captured by the fiber and guided to the detector at the fiber end(s). Spatially resolved readout is possible using pulsed-laser excitation and time-resolved detection; the arrival times of the fluorescence pulses from the respective sensor regions encode their locations. The spatial resolution improves dramatically by using two fibers that form orthogonal fiber-fiber junctions; sensor regions are located between the fibers at these junctions. One fiber carries the excitation light pulses, whereas the second fiber captures the fluorescence pulses and guides them to the detector. Sensor molecules are covalently attached to a porous gel, which replaces the original fiber cladding. This sensor active cladding is covalently attached to the core of the fiber, which is functionalized with a hydrophobic, self-assembled monolayer. In addition to providing attachment sites, this layer protects the fiber core from damage due to hydroxyl penetration. This array constitutes a general, high-spatial resolution sensing platform, whose functionality depends on the chosen sensor molecules.

Results & Discussion: A ruthenium complex was used to measure dissolved oxygen (DO) concentration. Sensing is based on the luminescence quenching due to the presence of oxygen. The dependence of the emission intensity on dissolved oxygen concentration is given by the Stern-Volmer equation. A second sensor region was employed as a reference region, recording excitation laser pulse energies. Signal pulse energies were then divided by reference pulse energies to exclude source fluctuations effects. The sensor was capable of a detection limit of 0.18 ppm DO, which is well below the concentrations found in (most) streams and lakes. The sensor DO concentration resolution was 0.78 ppm at 4 ppm DO concentration and 0.82 ppm at 49 ppm DO concentration. The sensor was kept continuously submerged in (tab) water for more than six month; after this time period the sensor response was at 83% of the initial values, leading to a marginally poorer detection limit and resolution. We also monitored the dependence of the luminescence emission lifetimes on the DO concentration (also given by the Stern-Volmer equation). This measurement is advantageous because it is independent of pulse energies; however, the lifetime measurement is more complicated (signal pulse deconvolution is required for sensor molecules with lifetimes comparable to the excitation pulse widths). We found virtually identical detection limits and sensor resolutions, showing that the use of the sensor reference improves the quality of the pulse-energy-based measurement to that of the lifetime-based measurement.

To improve signal strengths either to allow for lower detection limits or for placing sensors at larger distances from light source and detector, we explored integrating metal nanoparticles into our sensors with the goal of exploiting the phenomenon of metal-enhanced luminescence, which is due to the surface plasmon resonance of the nanoparticles. Initial results using silver nanorods showed a fluorescence enhancement of a factor of 10 for DO measurement using a ruthenium complex as sensor dye.

The dye fluorescein encapsulated in a hydrogel was used for pH sensing. Again, a pulse energy reference sensor region was used to account for fluctuations of the pulse energies of the laser light source. A pH-resolution of better than 0.05 pH-units in the region of the sensor dye's pK_a-value was found. While this sensor performed successfully, the need for improvement of sensor response times (for pH sensing) became clear. We approached this issue by micro-templating the fiber cladding material, this created a porous cladding material that allowed for rapid analyte penetration and, consequently, for fast response times. Response times improved dramatically: 90% of the total signal change occurred within ~2s following a pH change.

Conclusions/Implications/Recommendations: We demonstrated that our fiber sensor platform is suitable for measurements in aqueous environments using pH- and DO-sensing as examples. These measurements were greatly improved by the capability of the platform of providing other sensor regions in close proximity to pH- and DO sensor regions for pulse energy referencing. A novel cladding containing engineered nanochannels improved sensor response times substantially. With these sensor arrays, these measurements can be carried out continuously, in real-time and at many locations!

Related Publications: M. Veronica Rigo, for research conducted within the framework of this project, was recognized with the "Best Oral Presentation Award" at the University of Wisconsin System Laboratory for Surface Studies Symposium (2008), the "Conference Poster Award" at the 2007 International Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies, and a "Poster Prize" at the 2006 American Water Resources Association (Wisconsin Section) Annual Meeting.

Key Words: Remote sensing, optical fibers, fiber sensor arrays, pH, dissolved oxygen

Funding: This work is currently funded by the UW-Milwaukee Research Growth Initiative.

Introduction

The use of optical fiber sensors for monitoring groundwater contaminants was first suggested twenty years ago by T.E. Hirschfeld [1]. Hirschfeld listed a number of arguments in favor of optical fiber sensors for this purpose such as (1) that the insertion of fiber-optic probes into groundwater can be accomplished with small diameter boreholes; (2) that the measurements take place in-situ, avoiding sample contamination due to transport and delayed analysis; and (3) that the monitoring takes place in real time. Measuring contaminant concentrations continually over extended times and spatially resolved is at the heart of investigations regarding the sources of contamination of groundwater as well as the fate of these contaminants due to transport and chemical reactions.

In our optical fiber sensors (as in Hirschfeld's and other subsequent measurements (see e.g. [2])), sensing is based on changes of molecular fluorescence due to changes in the physical and chemical environment or due to the presence of specific chemical compounds. Fluorescence detection is an inherently sensitive technique, as (in principle) measurements can take place against zero background. Moreover, not only can a change in fluorescence intensity be used for sensing, but also changes in the maximum wavelength of fluorescence emission and changes in the fluorescence lifetime. To detect non-fluorescent compounds, the fibers could be functionalized with indicator molecules whose fluorescence properties change with binding of or interaction with the analyte.

In contrast to Hirschfeld's sensors, which are placed at the end of an optical fiber, we employ many discrete sensor regions along an optical fiber (i.e. quasi-distributed sensing) [3,4] for sensing. Sensor dyes are located in the fiber cladding in a suitable substance replacing the original cladding. The light propagating through the fiber core under total internal reflection conditions can interact with the sensor molecules *outside* of the fiber core through the evanescent fields of the core modes. If a fluorescent molecule is present within the range of the evanescent fields, it can be excited through the light in the fiber core; total internal reflection is attenuated in this case. Subsequently, the fluorescence emitted by this molecule can be captured by the fiber and propagate to the fiber ends *under guided conditions*! Spatially resolved readout is accomplished using pulsed laser excitation and time-resolved detection: a laser pulse is coupled into the fiber core and triggers a fluorescent pulse at a sensor region (see Figure 1). This fluorescence pulse, after being captured by the fiber, travels back to the front-end of the fiber; from the time delay τ_d between the exciting pulse and the fluorescence pulse the location L of the emitting fluorophore can be calculated according to $L = \left(c/2n_\text{co}\right)\tau_\text{d}$, where c and n_co are the speed of light in vacuum and the refractive index of the fiber core, respectively. Each sensor region on the fiber returns fluorescence pulses with its own characteristic time delay: the measured signal

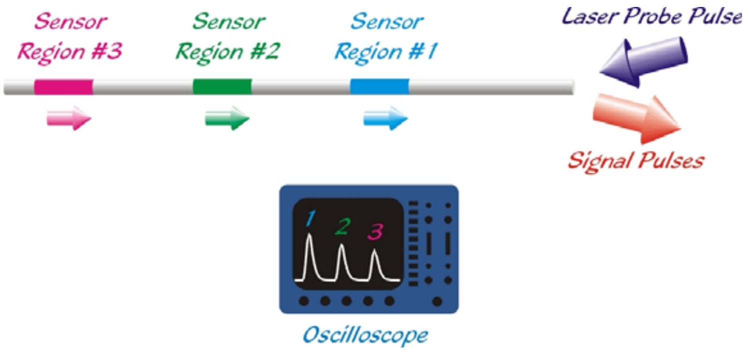


Figure 1: *Optical time-of-flight detection for spatially resolved readout of many sensor regions supported by a single fiber. The time delay between excitation pulse and returning fluorescence pulse encodes the locating of the emitting sensor (see text).*

is a pulse train that encodes the location of all emitting sensors. This technique is known as Optical Time Domain Reflectometry [5], or, more appropriately for fluorescence based measurements, Optical Time-of Flight Detection (OTOF) [6].

This project focused on the application of our recently developed (and patented) optical fiber sensor platform (see below for details) to groundwater monitoring issues. The goal was to implement two fairly standard measurement tasks, namely pH and dissolved oxygen concentration, which still are very relevant measurements given that on optical fibers the measurements can take place continuously, in real-time, and at many remote locations. Moreover, measurements in demanding environments such as groundwater are frequently plagued by biofouling, which is the deposition of organic matter on the submerged probes. This issue limits the time a sensor can be deployed. Therefore, we studied ways to protect the fiber sensor from fouling and from damage from hydroxyls entering the fiber core through microcracks (causing optical transmission losses to increase).

Procedures and Methods

Optical Setup: The spatial resolution, i.e. the minimum separation of adjacent sensor regions on a fiber, is determined by the fluorescence lifetimes of the sensor molecules. For standard sensor molecules, the spatial resolution is of the order of 2.5m or larger. To allow for point monitoring of multiple parameters, we developed an optical fiber sensor array [7-9], which is based on the evanescent coupling between two optical fibers forming orthogonal fiber-fiber junctions. The sensor molecules are placed at this fiber-fiber junction. A laser pulse traveling through the first fiber excites the sensor molecules at the junction through the evanescent fields; the subsequent fluorescence pulse is captured – again through evanescent fields – by the second fiber and guided to the detector. The merits are illustrated when multiple fiber-fiber junctions are considered (see Figure 2): along the excitation fiber, which carries the excitation laser pulse, the sensor regions may be spaced by as little as a few millimeters; however, the fluorescence pulses entering the second fiber are delayed with respect to each other on the way to the detector using delay coils, restoring full time- and, hence, spatial resolution.

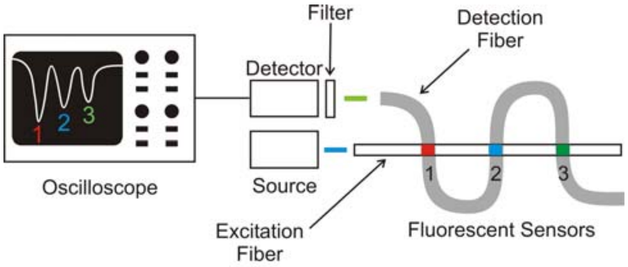


Figure 2: *Fiber-fiber coupling scheme for improved spatial resolution (see text). Sensor regions are located at the fiber-fiber junctions.*

A dye laser pumped by a pulsed nitrogen laser (pulse width 0.6 ns) was used to provide to excited the sensor molecules; various photomultiplier tubes (PMT) were used to detect their fluorescence. The PMT output was recorded by fast oscilloscope (bandwidth 1 GHz).

Sensing Mechanisms: Dissolved oxygen (DO) sensing is based on the luminescence quenching of a ruthenium complex. A collision of an oxygen molecule with the ruthenium complex while the latter is in its electronic excited state results in energy transfer from ruthenium to oxygen. Thus, the ruthenium complex returns to its electronic ground state non-radiatively, which results in a decrease of the luminescence signal. This process is described by the Stern-Volmer equation

$$\frac{\tilde{I}_0}{\tilde{I}} = 1 + K_{SV} DO ,$$

where $\tilde{I}_0$ and $\tilde{I}$ are the integrated ruthenium luminescence intensities in absence and presence of O_2, respectively, DO is the dissolved oxygen concentration, and K_{SV} is the Stern-Volmer quenching constant. In many cases, this equation also holds true for the luminescence lifetimes τ_0 and τ absence and presence of O_2, i.e.

$$\frac{\tau_0}{\tau} = 1 + K_{SV} DO\,.$$

pH-sensing is based on the protonation/deprotonation reaction of a dye (in our case fluorescein) as the pH changes. Protonation/deprotonation changes the luminescence properties of the dye. pH-sensing is most sensitive in the pH-range about the pK_a-value (which indicates 50% protonation).

Sensor Fabrication: To create a sensor region, the fiber core has to be exposed. First the outer layers of commercially available fibers were removed with mechanical fiber strippers. The most effective way of removing the fiber cladding was by burning in a flame. Fiber core images recorded with a scanning electron microscope verified that most of the cladding is removed with the technique and that the fiber core is undamaged.

The next step is to functionalize the exposed fiber core with a self-assembled, hydrophobic monolayer. This layer protects the fiber core from hydroxyl penetration and creates a reactive surface that is used to covalently attach sensor-active fiber claddings. The latter encapsulate the sensor molecules. Alternatively, sensor molecules may be covalently attached to these claddings, as long as the attachment does not negatively affect their fluorescence properties. Figure 3 shows the steps for the fabrication of the DO sensor. Coating solutions were created by mixing the cladding material (poly(ethylene glycol) diacrylate 575 (PEG-DA)), a crosslinking agent and a photoinitiator. This solution had a refractive index of 1.3814 at 20° C, thus maintaining the light-guiding properties of the optical fiber. Separate solutions of dichlorotris (1, 10-phenanthroline) ruthenium (II) (RuPhen - the oxygen sensor dye) and Rhodamine 110 (Rh110 - the intensity reference dye,) in ethanol were made. Each fluorophore solution was added to the PEG-DA 575 solution to make a 1:10 (v/v) mixture. Exposure to UV radiation (wavelength 365 nm) initiated crosslinking and attachment to the monolayer on the fiber core. The pH-sensor was created in a similar fashion. The commercially available dye fluorescein was derivatized to allow for covalent attachment to PEG-DA.

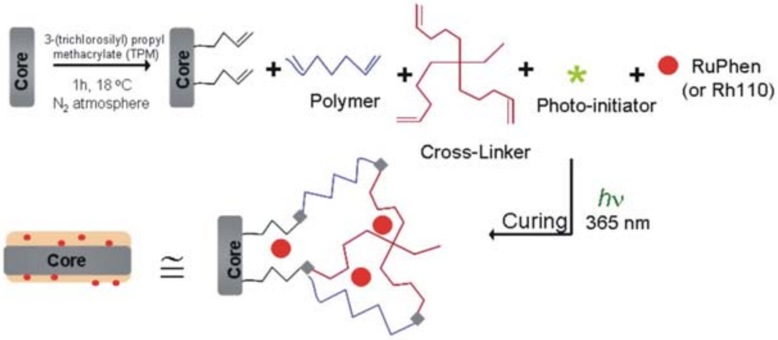


Figure 3: *DO sensor fabrication scheme (for details see text).*

Results & Discussion

Leaching: We tested the degree of encapsulation of RuPhen and Rh110 in the cladding material. Based on the molecular size of Rh110 and RuPhen the best matrix candidates are PEG-DA with a molecular weight of 575 or/and PEG-MA with a molecular weight of 1000. The higher the polymer molecular weight, the more hydrophobic and porous it becomes. The fiber was submerged in water and the degree of dye leakage out of the cladding was monitored by monitoring the change the fluorescence intensity every ten minutes for two days. We found that an increase in the dye leaching occurs as the matrix molecular weight increases. This can be understood in terms of the increasing porosity and decreasing hydrophobicity of the matrix as the molecular weight increases. Addition of the crosslinker and photopolymerization greatly reduced the dye leakage; after an initial loss of dye of ~ 20%, the fluorescence signal remained constant. However, as crosslinking decreases the matrix porosity, the sensor response times may be affected. Clearly, when possible, covalent attachment is preferable, as shown with the dye used for pH sensing.

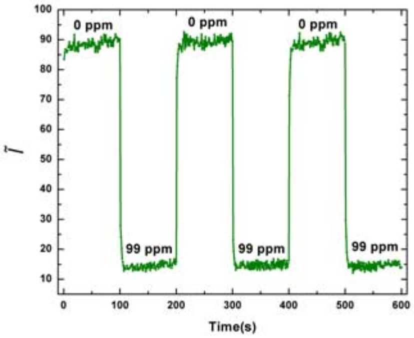


Figure 4: *DO sensor response time in N_2 and O_2 saturated media.*

Sensor Response Times: Figure 4 shows the response of the DO sensor to repeated changes between a nitrogen and oxygen environment by bubbling nitrogen through the water in the cell containing the fiber sensors. The calculated transition times are of the order of 5 seconds; however we believe that the actual sensor response times are shorter, because changing between oxygenated and deoxygenated environments is not instantaneous due to the filling times of the attached tubing and due to the time required for the diffusion of oxygen to and away from the sensor sites. Thus, the given response times constitute an upper limit.

The response times of the pH-sensor, however, were disappointing: upon change of pH from 4.0 to 6.4, the fluorescence signal increased only gradually; for full equilibration, two hours were required in a static solution (i.e. no stirring - see Figure 5)! A different approach was required to dramatically improve the sensor response times: we decided to employ a nano/microsphere templating approach to engineer nano/microscale channels into the cladding material for improved analyte penetration. Polystyrene spheres (diameter 950 nm) were mixed with the matrix polymer, crosslinking agent, and photoinitiator. This slurry was placed onto the fiber core. After brief exposure to UV radiation, the slurry hardened. Subsequently, the polystyrene beads were removed with toluene, leaving behind a network of micro- and nanoscale channels (see Figure 7), which allow for easy analyte penetration to the sensor molecules. Figure 6 shows the response of the pH-sensor in a nano/microtemplated cladding to a series of pH-changes. It can be seen that there is now an initial fast response to pH-changes; most of the total signal change occurs during this time period! Full equilibration is reached after 15 minutes.

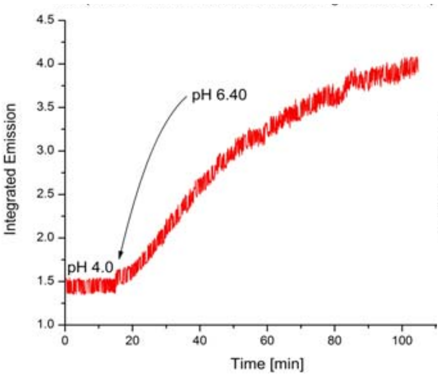


Figure 5: *Sensor response time to a pH-change from 4.0 to 6.4.*

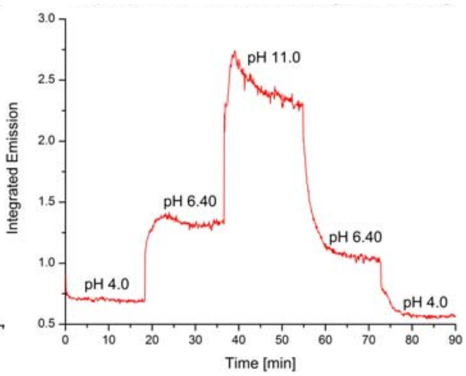


Figure 6: *Response of the pH-sensor in a microtemplated cladding.*

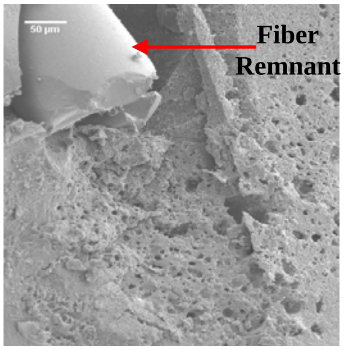


Figure 7: *SEM image of a templated fiber junction.*

Calibration Curves: The sensor response to a series of DO concentrations was determined from the ratio of the fluorescence intensity of RuPhen to that of Rh110; this ratio is denoted $\tilde{I}$. The total sensor sensitivity can be expressed by the overall quenching response to dissolved oxygen (Q_{DO}) given by

$$Q_{DO} = \frac{\tilde{I}_{N2} - \tilde{I}_{O2}}{\tilde{I}_{N2}} \times 100\% \ ,$$

where $\tilde{I}_{N2}$ and $\tilde{I}_{O2}$ represent referenced intensities in fully deoxygenated and fully oxygenated water, respectively. The higher the Q_{DO}-value, the better is the sensor sensitivity. For sensor molecules encapsulated and/or attached to polymers, the Q_{DO}-value is dependent on the polymer material composition. While hydrogel films demonstrated a good performance for sensing oxygen gas, their performance for DO sensing was expected to be different. Oxygen has a relatively low solubility in water (0.00425 g in 100 g of water at 21° C, 1 atm), which implies that there is a reduced number of oxygen molecules in air-equilibrated water compared with air under ambient conditions. Tailoring the matrix composition alleviates this problem and increases Q_{DO}. The chemical fabrication of the PEG-DA hydrogels allows for manipulating the physicochemical properties of this material by varying the amount or type of reactant used in its synthesis. A less hydrophilic film should enhance the DO sensing process by reducing water solubility in the matrix, causing the partitioning of oxygen out of the solution to the matrix, thus increasing the accessibility of oxygen molecules to RuPhen sensor molecules. In particular, addition of the crosslinker increases the hydrophobicity of the PEG-DA 575 hydrogel film, which leads to an enhancement in the quenching response, resulting in a value of Q_{DO}= 83% (see Figure 8). It is important to mention that the addition of the crosslinker increases the refractive index of the hydrogel cladding. Thus, there is a limit to the amount of crosslinker that may be added, because the refractive index of the hydrogel clad n_{clad} has to remain smaller than that of the core, i.e. $n_{\text{clad}} < n_{\text{core}}$, to preserve the guiding conditions of the optical fiber.

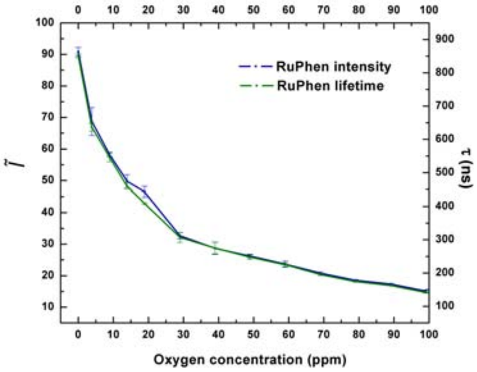


Figure 8: *Referenced luminescence intensity (blue, left axis) and lifetime (green, right axis of the RuPhen sensor for different DO concentrations [ppm].*

Figure 8 shows the referenced RuPhen luminescence intensity and excited state lifetime vs. DO concentration (the sensor and reference were submerged in water). Both quantities decrease with increasing oxygen concentration in accordance with the Stern-Volmer equation. To better illustrate this, both quantities were plotted as $\tilde{I}_0/\tilde{I} - 1$ and $\tau_0/\tau - 1$ vs. DO concentration in accordance with the Stern-Volmer equation (see Figure 9). The dash-dotted line represents the best linear fit generated by the least-squared method. The sensor has a good linearity over the whole range of DO concentration tested, which may indicate negligible matrix heterogeneity effects. The good

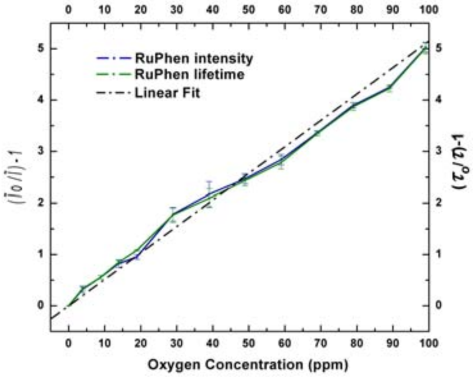


Figure 9: *Stern-Volmer plot of the referenced intensity and lifetime for the DO sensor in the aqueous phase.*

Q_{DO} value and the linearity in the Stern-Volmer plot may result from the structure and hydrophobic nature of the film used in the sensor preparation.

The sensor sensitivity, i.e. the change of the luminescence intensity per unit DO concentration change, depends on DO concentration; as the Stern-Volmer equation shows, the sensor luminescence intensity *I* depends on DO concentration as DO^{-1}. Thus, the sensor resolution, R, which is the smallest measurable DO concentration change, also depends on DO concentration. It is important to note that the sensor sensitivity and resolution increase with decreasing DO concentration. Therefore, the sensor performs best at the lowest DO concentrations. We found at 4 ppm DO concentration a resolution of *R*= 0.78 ppm, whereas at 49 ppm DO concentration the resolution was R= 0.82ppm. The DO detection limit was found to be 0.18 ppm.

The calibration curve for the pH sensor in a nano/microtemplated cladding was established by placing the fiber-fiber junctions into a series of buffer solutions. Although the emission intensity of the fluorescein sensor dye was usually stable, the use of an intensity reference improved the sensor accuracy. In this case, the reference signal was detected by directing part of the laser pulses towards a photodiode detector, before the laser pulses were coupled in the fiber. Figure 10 shows the calibration curve (emission intensity divided reference intensity vs. pH) with a sigmoidal fit curve, which also allowed for the extraction of the pK_a value, which was found to be pK_a = 7.07 ±0.04. Hysteresis effects were small, i.e. there were minor signal differences for measurement sequences from low to high pH vs. sequences from high to low pH.

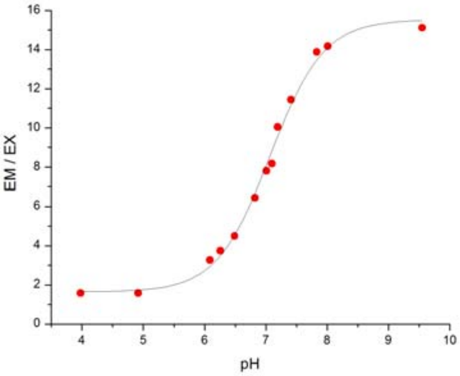


Figure 10: *pH-sensor calibration curve (see text for details).*

From the calibration curve (Figure 10), it is obvious that the sensor is most sensitive (i.e. has the largest signal change for a unit pH change) in the pH region around the pK_a value. Consequently, the pH resolution is highest in this pH region as well; we determined a pH resolution of 0.03 pH units within the range of pKa ± 0.5 and a pH resolution of 0.07 pH units within the range of pKa ± 1.0.

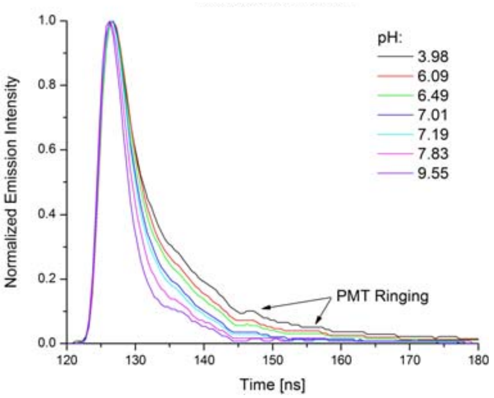


Figure 11: *Fluorescein emission pulse shapes for different pH values.*

In addition to emission intensity changes, fluorescein also exhibits a decrease of the fluorescence lifetimes with increasing pH. Figure 11 shows a series of signal pulses vs. time for different pH values, showing that fluorescence lifetime measurements can serve as alternative, intensity-independent measurement method for pH. As the fluorescence lifetimes (~ 10 ns) – which are much shorter than those of RuPhen – are close to the laser pulse width, deconvolution of the measured signal pulse shapes is required to extract the correct fluorescence lifetimes. This is complicated by the PMT ringing (see Figure 11) at the tails of the emission pulses. PMT ringing is an artifact inherent in PMTs. However, we are currently implementing time-correlated single photon counting measurements, which are insensitive to this artifact and allow for detection of the lowest light levels.

Sensor Reversibility, Repeatability, Longevity: We used the cycle-purging method to check the reversibility of RuPhen DO sensor physically entrapped in the PEG-DA 575 hydrogel matrix. The referenced sensor intensity $\tilde{I}$ was plotted during several oxygenation/deoxygenation cycles (Figure 12). Upon switching from nitrogen-saturated to oxygen-saturated solution, the referenced intensity $\tilde{I}$ decreased with an increase of DO concentration as expected. This process was repeated multiples times with no significant change in $\tilde{I}$ between the initial nitrogen-saturated solution, indicating complete reversibility and repeatability of the sensor.

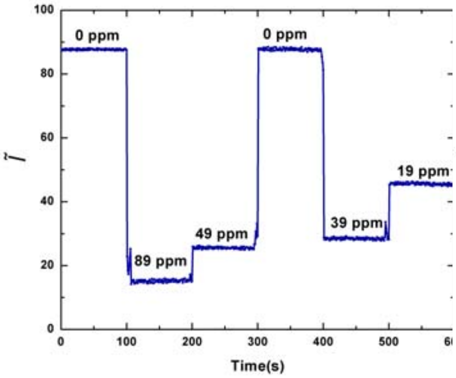


Figure 12: *Reversibility of the DO sensor in the PEG-based hydrogel for alternating DO concentrations.*

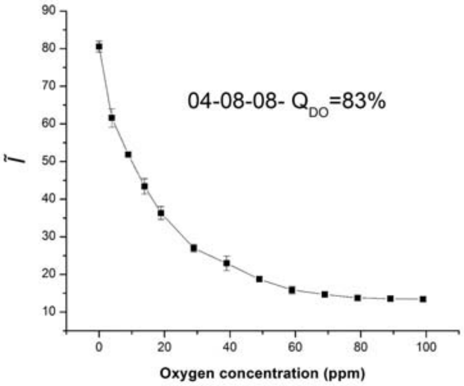


Figure 13: *DO sensor - referenced intensities vs. DO concentration after six months submerged in water.*

The DO sensor was submerged continuously in (laboratory) water for more than six months. Figure 13 shows the referenced emission intensities vs. DO concentrations after this time period. There was no performance degradation, as shown by the unchanged Q_{DO}-value of 83%!

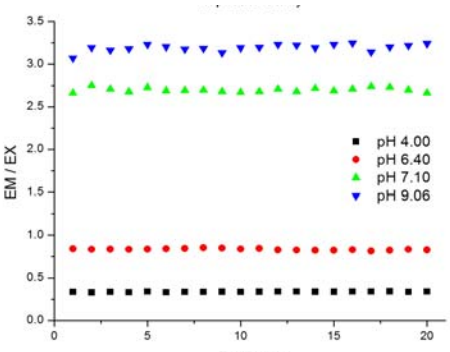


Figure 14: *pH sensor repeatability. Referenced emission intensity vs. number of replicate measurements.*

The pH-sensor was also tested for repeatability. Twenty replicate measurements were made for four different pH-values (see Figure 14). The sensor proved to produce stable results. The dye fluorescein is known for being photolabile, that is, it tends to decompose after prolonged exposure to resonant light. While most energy relaxation takes place via photon emission (which provide our sensor signal), the occasional non-radiative relaxation does occur, which means that excitation energy is released as heat. We observed this effect in earlier measurements with physically entrapped fluorescein molecules, which have to store this energy internally, which in turn could lead to decomposition. Covalent attachment of fluorescein to the hydrogel matrix, however, appears to improve the photostability of fluorescein; the excitation energy absorbed by fluorescein when optically excited can be released as heat to the hydrogel matrix through the attachment bond. Further measurements are needed to quantify the improvement of the sensor stability due to the covalent attachment.

Sensor Specificity: The DO response sensor was tested in present of different anions, cations reducing and oxidizing agents as well as in the presence of proteins to determine the possible interferences to the DO measurement. The results are shown in Figure 15. The DO sensor

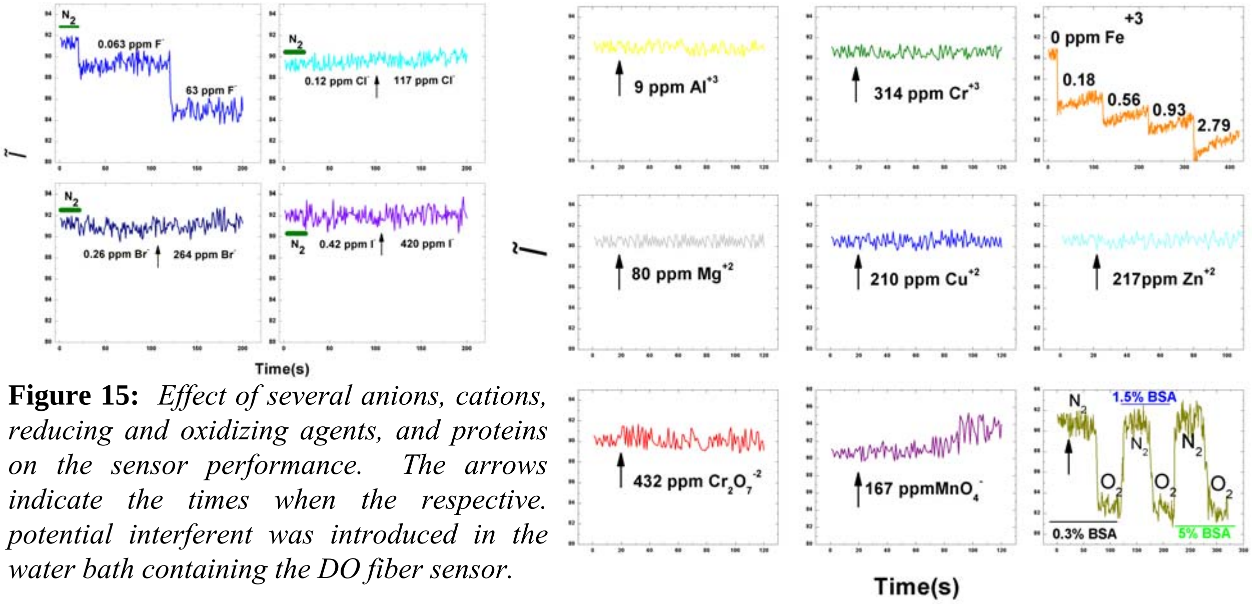


Figure 15: *Effect of several anions, cations, reducing and oxidizing agents, and proteins on the sensor performance. The arrows indicate the times when the respective. potential interferent was introduced in the water bath containing the DO fiber sensor.*

response was affected by the presence of F$^-$ and Fe^{+3}. Non-covalent interactions between the fluorescent sensor and native proteins (Bovine Serum Albumin) were investigated, which is important in cellular environments. These plots also show that the encapsulation of RuPhen in a biocompatible material reduces dye toxicity and minimizes biofouling.

The response of the pH sensor was tested for a possible dependence on the ionic strength of the surrounding aqueous solution. Calibration curves for two different ionic strengths were obtained (see Figure 16). We found that the pH measurement is in fact independent of ionic strength. Thus, the pH measurement does not have to be corrected for ionic strength; future arrays will contain separate sensor regions for measuring ionic strength.

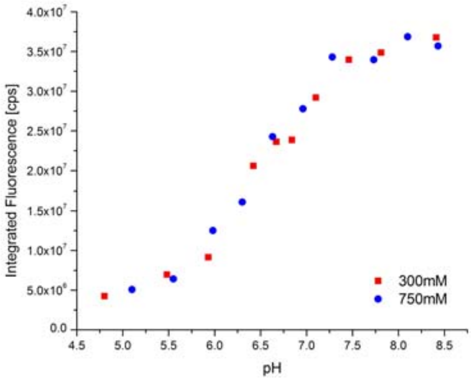


Figure 16: *pH sensor calibration curves for two values of solution ionic strength. No effect can be seen.*

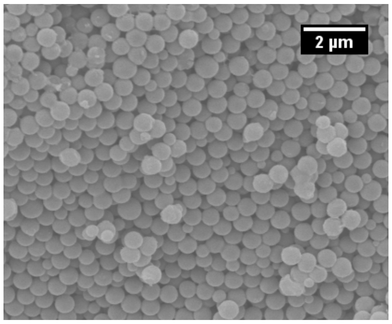


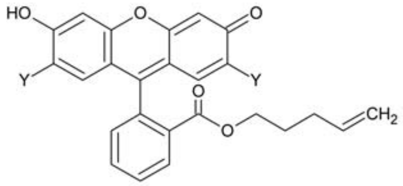
Figure 17: *Polystyrene nano-sphere templates synthesized in our lab for sensor cladding fabrication with nanochannels.*

Ongoing Research and Future Work:

<u>(a) Cladding materials with engineered channels for rapid analyte penetration</u>

Because of the dramatic improvements in the sensor response times when using a cladding with engineered micro- and nanochannels, we will continue to improve the templating strategy. Starting point are the polystyrene spheres. While the first set was acquired commercially, we have begun to synthesize our own spheres (see Figure 17). This allows us to systematically vary sphere diameter and also sphere diameter distributions. It is anticipated that a broader distribution of bead diameters leads to more continuous channel networks, thus improving analyte penetration and, consequently, sensor response times. We will also use different polymers to form the cladding "scaffold" once the spheres have been removed. Moreover, we will develop strategies for covalently attaching other fluorescent sensor dyes to the walls of the nanochannels for improved analyte access, and, consequently, for improved sensor responses.

(b) pH-sensor improvement



Y	pK_a
H	6.4
Cl	5.0
F	4.7
Ethyl	6.8
Hexyl	8.4

Figure 18: *Fluorescein derivatives and their pK_a values.*

To extend the range of pH values that can be measured with our fiber sensor arrays, we have begun synthesizing derivatives of fluorescein with different pK_a values. This is accomplished by varying one single side group (see Figure 18). Thus, by creating several sensors regions (which is easily accomplished within our fiber sensor platform), a significantly wider pH range may be probed. All of these derivatives can be covalently attached to avoid dye leaching and to increase photostability.

(c) Dissolved oxygen sensor improvement

The oxygen-quenching process – i.e. the mechanism used for oxygen sensing – is temperature dependent. Thus, the sensor response must be corrected for temperature effects to allow for quantitative remote DO concentration measurements in environments experiencing wide temperature variations. We recently began testing a fluorescent dye as temperature sensor. The dye is enclosed in an oxygen impermeable polymer nanoparticle to exclude any effect of oxygen on this sensor. The temperature sensor will be added to our fiber sensor arrays. Furthermore, we are planning to add sensor regions that monitor the concentration of substances that were identified as interferents of the DO measurement. With these sensor responses, the DO response can be corrected for increased accuracy.

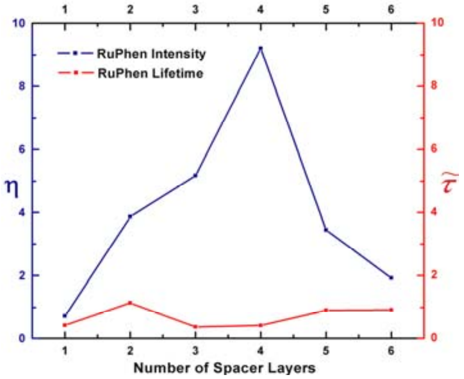


Figure 19: *Luminescence enhancement factor η (blue) for RuPhen vs. number of spacer layers between RuPhen and silver nanorods.*

Finally, using the DO sensor as example, we began implementing a strategy of increasing the luminescence of sensor dyes. This is accomplished by adding to sensor regions metal nanoparticles at well-defined distances (~ 8-10 nm) from the sensor dyes. When the free electrons of the metal nanoparticles are excited at their surface plasmon resonance frequency, the electromagnetic field at the sensor dyes is enhanced. This leads to enhanced absorption and/or enhanced emission of a dye molecule, if the nanoparticle sizes are chosen appropriately. We constructed a DO sensor fiber-fiber junction that meets these requirements; we found that silver nanorods separated by four spacer layers from RuPhen (the thickness of one spacer layer is 2 nm), produced an almost ten-fold luminescence enhancement of RuPhen! This is shown in more detail in Figure 19, where the luminescence enhancement factor η is plotted vs. the number of spacer layers; for four spacer layers there is a clear maximum (blue curve) in the luminescent enhancement. This emission enhancement should translate into lower detection limits and/or the ability of placing sensor farther along the fiber from the light source and detector. We will continue this promising line of investigation with the goal of further improving the enhancement factor for different nanoparticles shapes and sizes and for other sensor dyes.

(d) Sensor platform improvement

We will continue to develop the fiber sensor platform for sensing of many parameters in aqueous environments. The next steps are the addition of sensor regions for temperature and ionic strength measurements (see above). Furthermore, we are currently assembling a highly sensitive detection scheme (time correlated single photon counting) that allows for detection single photon and precisely measure their arrival times at the detector. This promises much improved detection limits and precise determination of luminescence lifetimes.

Conclusions and Recommendations

We demonstrated the suitability of our fiber sensor platform for use in aqueous environments. The performance of a dissolved oxygen sensor and a pH sensor were carefully evaluated with regard to detection limits, resolution, interferents, repeatability, and longevity. The response time of the pH sensor was improved dramatically using engineered cladding materials containing nano- and microscale channels for improved analyte penetration. The “scaffold” material around these channels was for optimized for longevity in aqueous environments; the dissolved oxygen fiber sensor was submerged continuously in water for six months and showed not performance degradation. These are very promising results for future long-term deployment of fiber sensor arrays into ground and surface waters. Further improvement of our sensor platform is ongoing.

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APPENDIX A:

AWARDS

M. Veronica Rigo, for research conducted within the framework of this project, was recognized with the "Best Oral Presentation Award" at the University of Wisconsin System Laboratory for Surface Studies Symposium (2008), the "Conference Poster Award" at the 2007 International Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies, and a "Poster Prize" at the 2006 American Water Resources Association (Wisconsin Section) Annual Meeting.

PRESENTATIONS

Invited Talks

Department of Chemistry Colloquium
University of Wisconsin-Oshkosh
Oshkosh, WI, U.S.A., November 2, 2007
The "Lighter" Side of Remote Sensing: Distributed Optical Fiber Sensing using Fiber-Grid Arrays and Metal-Enhancement Effects
Peter Geissinger

Science and Math Student Club
University of Wisconsin-Washington County
West Bend, WI, U.S.A., October 24, 2007
The "Lighter" Side of Communication: Connecting the World with Optical Fibers
Peter Geissinger

Research Seminar
Intelligent Optical Systems, Inc.
Torrance, CA, U.S.A., June 19, 2007
High Spatial Resolution Optical Fiber Sensor Arrays
Peter Geissinger

Department of Chemistry Colloquium
University of Wisconsin-Whitewater
Whitewater, WI, U.S.A., February 23, 2007
The "Lighter" Side of Remote Sensing: Distributed Optical Fiber Sensing using Fiber-Grid Arrays and Metal-Enhancement Effects
Peter Geissinger

. The Science Bag Public Lecture Series
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., January 5, 12, 14, 19, 26, 2007
The "Lighter" Side of Communication: Connecting the World with Optical Fibers
Peter Geissinger and Robert J. Olsson

Department of Chemistry Colloquium
University of Missouri – Columbia
Columbia, MO, U.S.A., October 6, 2006

The "Lighter" Side of Remote Sensing: Distributed Optical Fiber Sensing using Fiber-Grid Arrays, Metal-Enhancement Effects, and …Combinatorial Chemistry?
<u>Peter Geissinger</u>

Contributed Talks

FACSS 2008: 35th Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies
Federation of Analytical Chemistry and Spectroscopy Societies
Reno, NV, U.S.A., September 28-October 2, 2008
Fabrication of Functional Plasmonic Nanoparticles through Layer-by-Layer Assembly on Optical Fiber for Real-Time and Spatially-Resolved Oxygen Measurement
<u>M. Veronica Rigo</u> and Peter Geissinger

Laboratory for Surface Studies Summer Symposium
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., August 21, 2008
Plasmonic Optical Fiber Sensors
<u>M. Veronica Rigo</u> and Peter Geissinger

FACSS 2007: 34th Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies
Federation of Analytical Chemistry and Spectroscopy Societies
Memphis, TN, U.S.A., October 9-13, 2007
Fabrication of Porous Optical Fiber Claddings for Crossed-Fiber Sensor Arrays Using Microsphere Templating
<u>Paul E. Henning</u>, M. Veronica Rigo, and Peter Geissinger

American Water Resources Association – Wisconsin Section Annual Meeting
Wisconsin Dells, WI, U.S.A., March 1-2, 2007
Optical Sensing of Dissolved Oxygen and Temperature in Aqueous Environments
<u>M. Veronica Rigo</u>, Robert J. Olsson, and Peter Geissinger

Poster Presentations

FACSS 2008: 35th Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies
Federation of Analytical Chemistry and Spectroscopy Societies
Reno, NV, U.S.A., September 28-October 2, 2008
Fabrication of Porous Cladding Materials for Remote Sensing with Crossed-Fiber Sensor Arrays Using Microsphere Templating
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

Laboratory for Surface Studies Summer Symposium
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., August 21, 2008
Absorptivity of a Dye-Doped Hydrogel Matrix for Optical Fiber Sensor Applications
Megan Schultz and Peter Geissinger

Laboratory for Surface Studies Summer Symposium
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., August 21, 2008
Fabrication of Porous Cladding Materials Using Microsphere Templating for Remote Sensing with Crossed-Fiber Sensor Arrays
Paul E. Henning and Peter Geissinger

Chicago/Wisconsin/Central Illinois Rubber Group Meeting
American Chemical Society- Rubber Division
Lake Geneva, WI, U.S.A., May 13, 2008
Plasmon-Controlled Fluorescent Optical-Fiber Sensor for Ultrasensitive and Real Time Detection of Oxygen in Gas and Aqueous Media
M. Veronica Rigo and Peter Geissinger

Chicago/Wisconsin/Central Illinois Rubber Group Meeting
American Chemical Society- Rubber Division
Lake Geneva, WI, U.S.A., May 13, 2008
Fabrication of Porous Cladding Materials for Crossed-Fiber Sensor Arrays Using Microsphere Templating
Paul E. Henning and Peter Geissinger

University of Wisconsin-Milwaukee
Department of Chemistry Student Award Day Research Poster Session
Milwaukee, WI, U.S.A., May 8, 2008
Plasmon-Controlled Fluorescent Optical-Fiber Sensor for Ultrasensitive and Real Time Detection of Oxygen in Gas and Aqueous Media
M. Veronica Rigo and Peter Geissinger

University of Wisconsin-Milwaukee
Department of Chemistry Student Award Day Research Poster Session
Milwaukee, WI, U.S.A., May 8, 2008
Fabrication of Porous Cladding Materials for Crossed-Fiber Sensor Arrays Using Microsphere Templating
Paul E. Henning and Peter Geissinger

American Water Resources Association – Wisconsin Section Annual Meeting
Brookfield, WI, U.S.A., March 6-7, 2008
Optical Fiber Sensors for Real-Time, Remote, and Spatially Resolved Oxygen Sensing
M. Veronica Rigo and Peter Geissinger

American Water Resources Association – Wisconsin Section Annual Meeting
Brookfield, WI, U.S.A., March 6-7, 2008
Rugged, Highly-Porous Fiber Claddings for Remote Sensing in Aqueous Environments
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

Pittcon 2008 – 59th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy
New Orleans, LA, U.S.A., March 2-7, 2008
Fabrication of Porous Cladding Materials for Crossed-Fiber Sensor Arrays Using Microsphere Templating
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

Pittcon 2008 – 59th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy
New Orleans, LA, U.S.A., March 2-7, 2008
Metal-Enhanced Fluorescent Optical Fiber Platform for Real-Time Oxygen Sensing
M. Veronica Rigo and Peter Geissinger

Pittcon 2008 – 59th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy
New Orleans, LA, U.S.A., March 2-7, 2008
Multi-Fluorophore Optical Fiber Sensors for Real Time Oxygen Measurement
M. Veronica Rigo, Robert J. Olsson, and Peter Geissinger

SETAC 2007 28th Annual North American Meeting
Society of Environmental Toxicology and Chemistry
Milwaukee, WI, U.S.A., November 11-15, 2007
Optical Fiber Platform for Real-Time, Remote, and Spatially Resolved Sensing of Oxygen
M. Veronica Rigo and Peter Geissinger

SETAC 2007: 28th Annual North American Meeting
Society of Environmental Toxicology and Chemistry
Milwaukee, WI, U.S.A., November 11-15, 2007
Optical Fiber Sensors with Microsphere-Templated, Porous Fiber Claddings for Remote Measurement in Aqueous Environments
Paul E. Henning, M. Veronica Rigo and Peter Geissinger

University of Wisconsin System Chemistry Faculties Meeting
University of Wisconsin-Green Bay
Green Bay, WI, U.S.A., October 20-21, 2007
Using Microsphere Templating to Create Porous Optical Fiber Claddings for Crossed-Fiber Sensor Arrays
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

FACSS 2007: 34th Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies
Federation of Analytical Chemistry and Spectroscopy Societies
Memphis, TN, U.S.A., October 9-13, 2007
Surface Plasmon Resonance Optical Fiber Platform for Real-Time Oxygen Sensing
M. Veronica Rigo and Peter Geissinger

Laboratory for Surface Studies Summer Symposium
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., August 28, 2007
Using Microsphere Templating to Create Porous Optical Fiber Claddings for Crossed-Fiber Sensor Arrays
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

Laboratory for Surface Studies Summer Symposium
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., August 28, 2007
Localized Surface Plasmon Resonance of Nanostructures for Sensor Applications
M. Veronica Rigo and Peter Geissinger

University of Wisconsin-Milwaukee
Department of Chemistry Student Award Day Research Poster Session
Milwaukee, WI, U.S.A., May 3, 2007
Metal-Enhanced-Fluorescence Optical Fiber Oxygen Sensor Using Silver Nanostructures
M. Veronica Rigo and Peter Geissinger

University of Wisconsin-Milwaukee
Department of Chemistry Student Award Day Research Poster Session
Milwaukee, WI, U.S.A., May 3, 2007
Development of Porous Fiber Claddings by Microsphere Templating
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

2007 National Meeting
American Chemical Society
Chicago, IL, U.S.A., March 25-29, 2007
Fourier Transform Analysis for Linear, Periodic Arrays
Peter Geissinger and Alan W. Schwabacher

2007 National Meeting
American Chemical Society
Chicago, IL, U.S.A., March 25-29, 2007
Application of Microsphere Templating to Create Porous Optical Fiber Claddings for Crossed-Fiber Sensor Arrays
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

American Water Resources Association – Wisconsin Section Annual Meeting
Wisconsin Dells, WI, U.S.A., March 1-2, 2007
Optical Fiber Sensors with Microsphere-Templated, Porous Fiber Claddings for Remote Measurement in Aqueous Environments
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

Pittcon 2007 – 58th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy
Chicago, IL, U.S.A., February 25 – March 2, 2007
Application of Microsphere Templating to Create Porous Optical Fiber Claddings for Crossed-Fiber Sensor Arrays
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

Pittcon 2007 – 58th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy
Chicago, IL, U.S.A., February 25 – March 2, 2007
Fiber Optic Surface Plasmon Resonance Sensor for Oxygen Measurement
M. Veronica Rigo and Peter Geissinger

41st Midwest Regional Meeting
American Chemical Society
Quincy, IL, U.S.A., October 25-27, 2006
Nanosphere Templating for Rigid, Porous Optical Fiber Claddings for Use in Large Crossed-Fiber Sensor Arrays
Paul E. Henning, M. Veronica Rigo, and Peter Geissinger

41st Midwest Regional Meeting
American Chemical Society
Quincy, IL, U.S.A., October 25-27, 2006
Improving Detection Limits in Optical Fiber Sensor Arrays through Metal Enhanced Fluorescence
M. Veronica Rigo and Peter Geissinger

Laboratory for Surface Studies Summer Symposium
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., August 18, 2006
Ratiometric Sensing Using Dual-Emissive Dyes and Dye Mixtures in a High-Spatial Resolution Optical Fiber Sensor Array
Paul E. Henning, Robert J. Olsson, and Peter Geissinger

Laboratory for Surface Studies Summer Symposium
University of Wisconsin-Milwaukee
Milwaukee, WI, U.S.A., August 18, 2006
Fiber Optic Sensor for Oxygen Measurement in Gas and Aqueous Phase
M. Veronica Rigo, Robert J. Olsson, and Peter Geissinger