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Sensor Application of Poly (Ethylene Glycol) Diacrylate Hydrogels Chemically-Anchored on Polymer Surface

Zhan Gao, Chang-Soo Kim, *Senior Member, IEEE*, and David B. Henthorn

***Abstract*— We demonstrate a device process combining two photopatternable polymers, poly (ethylene glycol) (PEG)-diacrylate-based-hydrogel and epoxy-based photoresist SU-8, to implement a optofluidic bioanalytical platform through a surface anchoring technique. As an exemplary sensor application, optical dissolved oxygen sensors are fabricated and their performance characterized. The PEG-rich hydrogel is used as a matrix material for the immobilization of oxygen-responsive fluorophore, dichlorotris (1, 10-phenanthroline) ruthenium (II) hydrate. This hydrogel is chemically-anchored on the surface of negative-tone photoresist, SU-8, through a free radical reaction in which 1-hydroxycyclohexyl phenyl ketone served as the surface bound photoinitiator. The sensor exhibits a reversible Stern–Volmer response and good storage stability. Cylindrical hydrogel sensing elements are then patterned and anchored within completed SU-8 fluidic channels to serve as the embedded sensing elements in optofluidic platforms. We anticipate that the proposed method has a variety of applications that require the immobilization and patterning of biorecognition agents in hydrophilic matrices within completed polymeric fluidic channel.**

***Index Terms*— Microfluidic sensor, optofluidic sensor, surface modification, SU-8.**

I. Introduction

In THE last decade there has been growing interest in the development of devices which utilize microfluidic channels with dimension of tens to hundreds of micrometers for lab-on-a-chip technologies [1]. To analyze samples more conveniently and seamlessly, optical sensors constructed with sensing elements built within these microfluidic channels have attracted more and more attention. One important application of such optofluidic sensors is in the monitoring of dissolved oxygen, with important uses in chemical, biological and environmental settings. These sensors have many needed advantages, such as no consumption of the oxygen molecules, no need for reference electrodes, and no electrical interference compared with electrochemical oxygen sensors [2]. Most are designed based on a common detection mechanism, which employs the fluorescent quenching behavior of an analyte-responsive fluorophore immobilized in a matrix [3]. As a result, choosing suitable and compatible materials for fabrication of the microfluidic channel (structural material) and entrapment of the fluorophore (matrix material) is crucial. Traditionally, many microfluidic devices have been fabricated with silicon, or if transparent setups are necessary, with glass. Both materials require complex, time-consuming and expensive processes in the fabrication of microfluidic devices. In recent years, negative-tone photoresists have become popular materials as a replacement for glass or silicon substrates due to reduced costs of manufacture. The epoxy-based, photopolymer known as SU-8 has found increasing use as a structural material in microfluidic devices [4]. Besides its special characteristics – ability to produce high aspect ratio microstructures and compatibility with conventional microfabrication techniques [5] – it also has many properties that make it attractive as a structural material for optical sensors. SU-8 is nontoxic and has proper optical and mechanical properties [6], [7] and requires only low temperatures for adhesive bonding in channel fabrication [8], [9]. Additionally, unlike the widely-used microfluidic channel material, polydimethyl siloxane, SU-8 is less permeable to oxygen, an important characteristic for oxygen measurement. The attractiveness of SU-8 photoresist as a structural material in the fabrication of fluidic optical oxygen sensors can be further enhanced if methods are developed to chemically anchor and pattern sensing elements within completed microchannels.

Significant effort has been placed in developing new matrix materials and investigating their performance and compatibility with device structural materials. Issues regarding oxygen permeability, fluorophore solubility within the matrix, and leaching of the fluorophore must be carefully considered. Previous optical oxygen sensor studies have utilized various matrix materials in the immobilization of fluorophore, with the two most widely used materials being sol-gel and silicone rubber [10]. To enhance properties such as biocompatibility and transport of other dissolved species (e.g. glucose in diabetic monitoring), hydrogel materials are often used. The most widely investigated hydrogel materials are based on

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polyethylene glycol (PEG), frequently incorporated through the addition polymerization of a macromonomer such as PEG diacrylate (PEG-DA) [4], [11]–[14].

Although modifications of the surface of SU-8 using PEG polymer layers have been reported, most methods focus on the alteration of surface properties. Wang et al. reported two methods for the photografting of PEG to the surface of SU-8 photoresist [15], [16]. In the first method, PEG chains were grafted from the SU-8 surface through a free radical reaction using the photoacid generator triarylsulfonium hexafluoroantimonate as a photoinitiator [15]. Alternatively, surface exposed epoxy groups of SU-8 photoresist were chemically converted into hydroxyl groups, which then served as initiation sites for the graft polymerization of PEG on SU-8. Both of the steps are catalyzed by cerium (IV) ammonium nitrate in the acid environment [16]. Desai et al. used aminopropyltriethoxysilane as a bridge to couple the PEG monomers to SU-8 surface, where epoxy groups were opened by using concentrated sulfuric acid [17]. The primary focus of these works was to enhance the biocompatibility and wettability properties of SU-8 rather than anchor thick hydrogel structures suitable as a sensing element.

In our previous work, photoinitiator was covalently bonded on specific areas of the SU-8 surface through a hydrogen abstraction reaction, and these molecules later served as chemical anchoring sites for the patterned bulk PEG membranes [18]. This surface initiated polymerization of crosslinked structures serves as the enabling technology to maintain water-swollen hydrogels within a completed fluidic channel. In this report, we prepared polymer channel devices where all internally exposed surfaces are SU-8. This structure allows all surfaces made of the same material to be functionalized at the same time. For example, controlling the hydrophilicity or grafting hydrogel layers over all internal surfaces can be conducted during the same process. A ruthenium complex, dichlorotris (1, 10-phenanthroline) ruthenium (II) hydrated was chosen as the fluorophore for oxygen detection due to its efficient fluorescence, relatively long excited-state lifetime, and significant quenching behavior in the presence of oxygen molecules [3]. Detection characteristics including relative sensitivity, reversibility and long-term stability were investigated and dissolved oxygen content measurement was tested by using a truly integrated optical oxygen SU-8 fluidic sensor.

II. Experimental

A. Materials

PEG-DA with an average molecular weight of 575, 1-hydroxycyclohexyl phenyl ketone (HCPK) photoinitiator, dichlorotris (1, 10-phenanthroline) ruthenium (II) hydrated 98%, and toluene were purchased from Sigma-Aldrich (St. Louis, MO). Acetone, methanol and isopropanol were obtained from Fisher Scientific (Fair lawn, NJ). The negative-tone photoresist SU-8 (formulation 2050) and SU-8 developer were purchased from MicroChem (Newton, MA). 700 μm thick 4 inch borofloat glass wafers were obtained from University Wafer (South Boston, MA).

B. SU-8 Substrate Films

Negative photomasks for 1 cm $\times$ 1 cm square SU-8 patterns were designed using the Microsoft Visio software and printed on Mylar sheets. These sheets were attached to transparent square glass plates to form photomasks for the subsequent photolithography step.

A 4 inch glass wafer was cleaned using acetone, methanol, and then D.I. water for 3 minutes, followed by drying with air for 30 seconds. Next, the glass wafer was dehydrated on a hotplate at 150 °C for 10 minutes. A 75 μm thick SU-8 film was formed on the glass wafer by spin-coating at 500 rpm for 10 seconds, followed by 2000 rpm for 30 seconds using a single wafer spin processor (WS-400E-6NPP/LITE, Laurell Technologies Corporation). Directly after the spin-coating procedure, the samples were put onto the hotplate at 65 °C for 3 minutes and then at 95 °C for 9 min in the softbake step. The SU-8 patterned films were prepared through exposure to a collimated UV light source ($\sim$5.5 mW/cm^2 at 365 nm) for 60 seconds. The total exposure energy dose was 330 mJ/cm2. After irradiation, the postbake step was carried out on a hotplate at 65 °C for 1 hour. After slowly cooling to room temperature, the patterned SU-8 sample was soaked in SU-8 developer for 7 minutes and then rinsed with isopropanol to remove any unreacted SU-8 residue. Finally, the sample was dried under vacuum for 12 hours.

C. Oxygen-Sensitive Hydrogel Test Structure

The glass wafers with SU-8 coatings were immersed in a 5 % (w/w) HCPK in ethanol solution for 10 minutes and later placed in nitrogen atmosphere to prevent any oxygen quenching effects. The photoinitiator was covalently bound to the SU-8 surface through 30 minutes irradiation by uncollimated UV light ($\sim$26.5 mW/cm^2 at 365 nm) via a hydrogen abstraction technique, as detailed previously [18]. Once irradiated, the photoinitiated SU-8 film coated glass wafers were immersed in ethanol overnight to remove any unbound photoinitiators and dried in a vacuum oven for 12 hours. The glass wafer was later cut into 1.5 cm $\times$ 1.5 cm small pieces, each of them with one intact initiated SU-8 pattern on the top surface.

Previously published work showed that 40% water content is optimal for the diffusion of small molecules through the PEG-DA matrix [14]. Thus, the recipe, used by this group to form PEG-based oxygen sensor, was adopted here [12], [14]. This ruthenium complex was chosen due to its solubility in water and resistance to photobleaching during photopolymerization, allowing for the formation of hydrogels with good transport properties in aqueous media, all while using traditional photolithographic processes. The ruthenium complex solution was made in a 4:1 (v:v) mixture of methanol and toluene. PEG-DA was dissolved in deionized water at 60% (w/w) prior to polymerization, with the large water content being used to produce gels with an open structure to facilitate analyte diffusion. Finally, the ruthenium complex solution was added to the diluted PEG-DA solution to make a 1:10 (v:v) mixture. This mixture was bubbled with nitrogen gas for 30 minutes before polymerization to remove oxygen

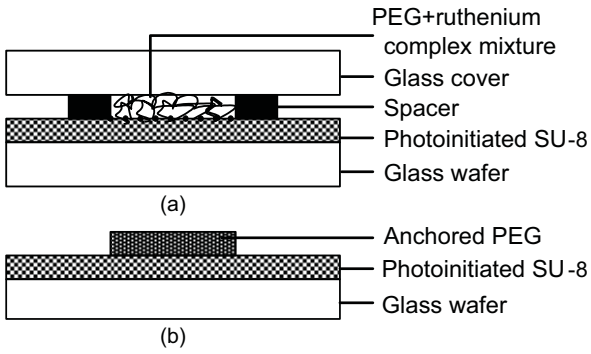


Fig. 1. Preparation of test structure of oxygen-sensitive hydrogel anchored on SU-8 surface. (a) Ruthenium complex and PEG-DA monomer mixture filled out the space of glass cover/spacer/SU-8 assembly. (b) PEG-DA membrane was chemically-anchored on the photoinitiated SU-8 surface after UV exposure.

molecules to eliminate the oxygen quenching effect on PEG-DA cross-linking.

As shown in Fig. 1 (a), voidspace was created by using 175 μm thick black insulation tapes as a spacer layer, leaving an SU-8 area of 0.5 cm $\times$ 0.5 cm. Immediately after the ruthenium mixture was saturated with nitrogen, 0.01 ml of PEG-DA/ruthenium mixture was allowed to fill the space between the glass cover and the photoinitiated SU-8 film. A glass cover was secured tightly on the top of the sample to avoid any oxygen quenching effect on polymerization. The excess liquid around the edge of the tape was cleaned with Kimwipes.

This glass cover/PEG-DA/SU-8 assembly was placed on the sample stage of a mask aligner (CA-800, Cobilt, Computervision) and exposed to UV light ($\sim$5.5 mW/cm^2 at 365 nm) for 30 minutes. After the irradiation, the assembly was pulled apart, leaving the ruthenium loaded PEG-DA covalently bound to the SU-8 film [shown in Fig. 1 (b)]. The sensor was placed in DI water for 12 hours before fluorescence intensity measurements were made to allow for the removal of any unentrapped ruthenium complex or unreacted monomers.

D. SU-8 Channel With Embedded Hydrogel Sensing Element

A transparent glass microscope slide used as glass substrate was cleaned in acetone, methanol and D.I. water for 3 minutes respectively and dried in air for 30 seconds after each step. It was then dehydrated on a hotplate at 200 °C for at least 15 minutes immediately prior to use. 4 ml SU-8 2050 was spin-coated on the dehydrated glass slide at 500 rpm for 10 seconds, followed by 2000 rpm for 30 seconds to produce a 75 μm thick SU-8 layer. This SU-8 layer was soft-baked on a hotplate at 65 °C for 3 minutes followed by holding at 95 °C for 9 minutes and then put on a mask aligner (Karl Suss MA6, Suss MicroTech) stage to cool down to room temperature. This SU-8 layer was then exposed to UV light (5.7 mW/cm^2, 365 nm) for 56 seconds and hard-baked on a hotplate at 95 °C for 9 min. The second SU-8 layer was spin-coated on the first one at 500 rpm for 10 seconds, followed by 1000 rpm for 30 second to produce a 125 μm thick film. The second, thicker SU-8 layer was soft-baked on a hot plate at 95 °C for 10 min and then exposed to UV light with an appropriate mask for 88 seconds. After UV flood exposure step, this sample was post-baked using the same receipt as for the soft-bake step. The channel structure was developed in propylene glycol methyl ether acetate (PGMEA) for 9 minutes and then rinsed with isopropanol to remove any unreacted SU-8 residue.

A second glass microscope slide was used as a cover. The slide was cleaned as previously described, followed by dehydration on a hotplate at 200 °C for at least 15 minutes before the spin-coating step. 4 ml SU-8 2007, which was diluted from SU-8 2050 with SU-8 thinner, was casted on the dehydrated glass slide and spin-coated at 500 rpm for 10 seconds, followed by 2000 rpm for 30 seconds. A 20 μm thick, uncrosslinked SU-8 layer was formed on the glass slide after 4 min soft-baking step at 95 °C. After the sample cooled down to room temperature, a drill (Model 750, Dremel) was used to create two holes to be used as fluidic inlet and outlet ports.

The glass substrate bearing the SU-8 channel structure and the glass cover with uncrosslinked SU-8 layer were placed on a hotplate at 65 °C for 5 minutes. The glass transition temperature of uncrosslinked SU-8 is approximately 55 °C [8]. If the bonding temperature is too low, it is difficult for SU-8 layers to make intimate contract with each other, and poor bonding results. On the other hand, if the bonding temperature is higher than the glass transition temperature (e.g. >75 °C), the bonded SU-8 channel may be blocked due to softening of the SU-8 and flow into the channel structure. In our work, the optimum bonding temperature was found to be 65 °C, slightly higher than glass transition temperature of SU-8. It is considered that at that temperature, the SU-8 layer is pliable and makes significant contact. As the glass slides were contacted, external pressure was applied to help to achieve better contact and to eliminate air bubbles since the bonding process was not carried out in vacuum environment. After the sample had cooled to room temperature, 52 seconds of UV flood exposure was conducted through the transparent glass slide, followed by 4 minutes of post-baking at 95 °C. This solidified the uncrosslinked SU-8 layer, producing a transparent sealed SU-8 channel structure.

A 5 % (w/w) HCPK in ethanol solution, bubbled with N_2 gas for 30 minutes, was injected into the channel after bonding process and then exposed to UV light (5.7 mW/cm^2, 365 nm) for 30 minutes (as shown in Fig. 2). The initiator, HCPK was grafted on the channel wall surface and severed as the active site for anchoring the PEG matrix material. The SU-8 channel was then washed thoroughly using ethanol to remove any unreacted photoinitiator remaining. To fabricate a 1 mm diameter, chemically-anchored hydrogel structure within the SU-8 channel, the channel was first filled with ruthenium complex-PEG mixture solution which had been saturated with nitrogen. The optimal ruthenium complex concentration, found through the previous experiment, was used in this task. The precursor-filled channel was then exposed to UV light (365 nm, 5.7 mW/cm^2) for 30 minutes through a designed photomask. Areas exposed to UV light formed densely crosslinked hydrogel networks, entrapping the ruthenium

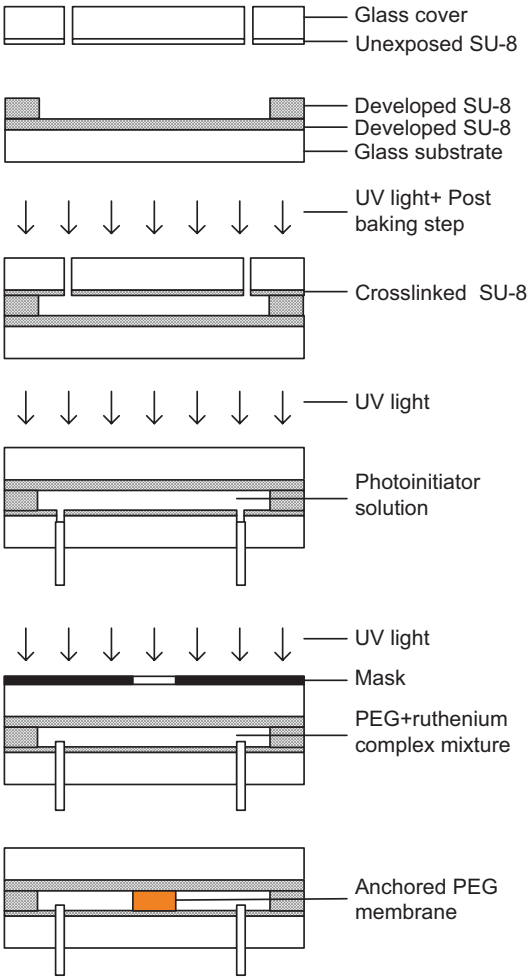


Fig. 2. Fabrication process scheme of optofluidic dissolved oxygen sensor with embedded sensing element within completed channel structure; the channel (height 125 μm, width 2 mm, and length 3 cm) and the sensing element (diameter 1 mm) were designed rather large in this proof-of-concept stage.

complex. Following polymerization, unreacted macromonomer and unentrapped ruthenium complex were washed out through injections of copious amount of D.I. water. In order to obtain initial stability before oxygen concentration measurements were made, the completed channel and sensing elements were flooded with D.I. water and exposed to LED light source for 12 hours. This procedure was designed to enhance leaching of any unentrapped complex.

E. Instrumentation

All fluorescence intensity measurements were done with a spectrofluorometer (USB2000-FLG, Ocean Optics, Dunedin, FL) and recorded with the SpectraSuite software, as shown in Fig. 3. The excitation and emission wavelengths of ruthenium complex, dichlorotris (1, 10 – phenanthroline) ruthenium (II) hydrate were 470 nm and 608 nm respectively. A blue LED (LS450, Ocean Optics) was used as the excitation light source. It was connected to the spectrometer by using a reflection probe (R400-7-UV-VIS, Ocean Optics) with three ends. This probe consisted of six 400 μm illumination fibers and one

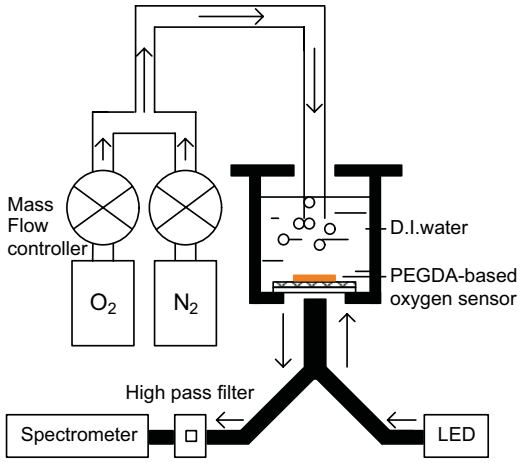


Fig. 3. Experimental setup with spectroscopic equipment and controlled gas composition for fluorescent oxygen detection. A reflection fiber probe was used to illuminate the excitation light and detect the emission light.

400 μm read fiber to illuminate the PEG-DA matrix and to transmit light to the spectrometer, respectively. The signals captured by the read fiber were high-pass filtered (>540 nm) before being passed to the spectrometer.

The PEG-DA matrix with incorporated ruthenium complex was placed in a container filled with 20 ml D.I. water. Mass flow controllers (Laminar Technologies, San Jose, CA) were used to introduce oxygen and nitrogen gases into the container at prescribed percentages and a fixed volumetric flow rate of 1000 sccm. Fluorescence signal from the sensing element was recorded with the spectrometer as a function of oxygen percentage to determine a standard calibration. The entire measurement platform was wrapped with a black cloth to eliminate the interference from ambient light.

III. Results and Discussions

A. Verification of Chemical Anchoring Scheme

To determine whether the photoinitiator HCPK played a role in initiating the polymerization reaction, a set of experiments comparing two groups of three SU-8 samples were carried out. The first set of materials was fabricated with surface bound photoinitiator while the second and third groups featured pristine SU-8 surfaces. All fabrication followed the procedures detailed in Fig. 1. All samples were soaked for one day in D.I. water after polymerization. PEG-DA membranes formed on the surface of the first group remained attached when swollen in water, while materials that were formed without the photoinitiator treatment were easily washed off. It was found that this surface bound initiator technique led to films which adhered indefinitely on the SU-8 material. The thickness of the PEG-DA hydrogel material was measured using a stylus profilometer (Alpha-step 200, Tencor Instrument) and was found to be approximately 175 μm in the hydrated state, the same thickness as the spacer layer used in fabrication.

B. Effect of Photoinitiation Process and Initial Photostability

Highly reactive radicals are generated during the aforementioned polymerization process and it is therefore feasible that degradation of the ruthenium complex may occur.

TABLE I
FLUORESCENCE INTENSITY BEFORE AND AFTER UV POLYMERIZATION

		Before (RFU*)	After (RFU*)	Percentage of Fluorescence Intensity Retention
First batch	Mean	1.60E + 08	1.59E + 08	99%
(n = 6)	Standard deviation	7.00E + 06	1.00E + 06	
Second batch	Mean	1.59E + 08	1.53E + 08	96%
(n = 6)	Standard Deviation	6.00E + 06	1.00E + 06	
Third Batch	Mean	1.60E + 08	1.58E + 08	98%
(n = 6)	Standard Deviation	8.00E + 06	1.00E + 06	

*Relative fluorescence units: photodetector signal of spectrometer (Beckman Coulter, DTX 880) responding to fluorescence at 625 nm.

Damage to the complex would result in alteration of the fluorophore's chemical structure and subsequent optical properties. To examine the photostability of the ruthenium complex during the polymerization step, a series of comparison experiments were conducted. A precursor mixture was prepared by mixing 20 mg ruthenium complex, 1.0 ml PEG-DA macromonomers, 0.67 ml D.I. water, 0.03 ml toluene, and 0.13 ml methanol. A trace amount of HCPK photoinitiator was added to this mixture to trigger the polymerization reaction. This ruthenium mixture was pipetted into 6 wells in a 96 well microplate. Fluorescent intensity was measured before and after polymerization and the mean values are shown in Table 1. This comparison experiment was repeated three times. Results from Table 1 show fluorescent intensity retention of 98% (with minimal 96% and maximum 99%) after polymerization, demonstrating the photostability of the sensing dye through the free radical reaction.

C. Spectral Response and Initial Stability

The excitation/emission spectrum of dichlorotris (1, 10–phenanthroline) ruthenium (II) in a chemically-anchored PEG-DA-based membrane in D.I. water is shown in Fig. 4. The excitation wavelength used is 470 nm, with a peak emission wavelength at 608 nm. An initial decrease in fluorescent emission intensity occurred when a freshly prepared sensing membrane was continuously exposed to the LED excitation source. It is considered that this intensity reduction is caused by the photobleaching of the ruthenium complex to become inactive from the newly formed material with continuous irradiation. Also, it appears that some of the loaded ruthenium complex leaches out from the hydrogel. This initial intensity, however, stabilized after 12 hours of irradiation. For this reason, every hydrogel membrane was soaked in D.I. water and exposed to continuous LED light illumination for 12 hours before the first fluorescent intensity measurements were conducted. This dialysis procedure is also expected to remove unreacted monomer or insufficiently reacted polymeric materials.

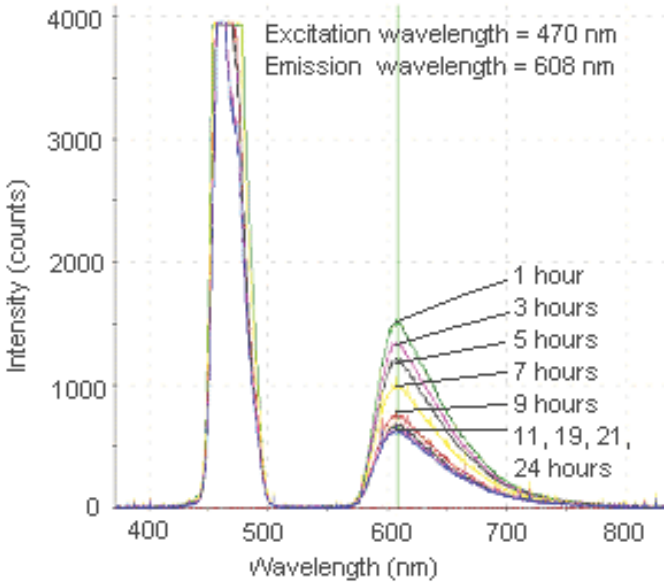


Fig. 4. Initial stability of the fluorescent emission from ruthenium complex after incorporation in chemically-anchored PEG-DA membrane in D.I. water.

D. Effect of Ruthenium Concentration on Relative Sensitivity

The oxygen detection by fluorescence quenching can be described by the Stern-Volmer equation [3]:

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = \frac{\Phi_0}{\Phi} = 1 + K_{SV}[O_2] \quad (1)$$

$$K_{SV} = k\tau_0 \quad (2)$$

where I_0 and $I(\tau, \Phi)$ are the fluorescent intensities (alternatively the fluorescent lifetime, or phase shift of the fluorophore emission) in the absence and presence of the dissolved oxygen molecules, respectively; $[O_2]$ is defined as percent oxygen saturation (i.e. a sample of 50% percent oxygen saturation refers to D.I. water that has been saturated with a gas comprised of 50% oxygen and 50% nitrogen by mole at atmosphere pressure), K_{SV} is the Stern-Volmer quenching constant, and k is the quencher rate coefficient. Alternatively, one may correlate the fluorescence lifetime, τ, or the phase shift, Φ, to oxygen concentration under time-dependent emission experiments.

Under isothermal and isobaric conditions, K_{SV} is considered to be constant, and the relationship between relative sensitivity, I_0 / I, and $[O_2]$ is linear. However, as shown in Fig. 5 (a), the Stern-Volmer plot shows deviation from this linear relation over the full range from 0% to 100% saturation. This is a common feature of immobilized ruthenium complex and several explanations are available. The multisites model and the non-linear solubility model are two common models used to account for this phenomenon [3]. In most cases, these two models were introduced to fit the analysis of fluorescence quenching-based sensors in gas phase. Since percent oxygen saturation in the aqueous environment is proportional to the oxygen gas portion in the gas phase above the D.I. water, it is considered that these two models can be used to explain our non-linear Stern-Volmer relationship. First, according to the multisites model, the fluorophore dye has two or more quenchable sites, each with its own characteristic

quenching constant. The model developed based on two or more quenching constants is different from the Stern-Volmer equation with only one quenching constant, leading to the downward curve. Second, in the non-linear solubility model, it is assumed that fluorophore dye only has a single quenchable site, which detects the average dissolved oxygen concentration. The downward curvature is, however, attributed to the heterogeneity of solid matrix, which will set some obstacles at the quenching binding sites, followed by the lower local quenching constant in the high oxygen level. In our work, both of them are considered as possible reasons for the deviation from the linearity due to the multisites of ruthenium complex and heterogeneity of crosslinked network structure of PEG hydrogel.

The last plausible reason is the clustering or aggregation of the ruthenium complex itself within the matrix material. The common chloride and perchlorate salts of the ruthenium complex are hydrophilic and thus cannot disperse very well in hydrophobic polymer matrices, like sol-gel or silicone rubber. Ruthenium complex will aggregate together in the matrix and block the path of oxygen molecules towards the quenching sites, thus causing the deviation. In our work, this is not an accountable reason since uniform distribution of ruthenium complex at low concentration is expected due to the hydrophilic property of PEG-DA matrix, which was further confirmed by the confocal brightfield image of the PEG hydrogel with encapsulated ruthenium complex at low concentration taken by another group [14]. Nevertheless, the possibility of clustering still may be considered when the ruthenium complex concentration is excessive.

To determine the optimum amount of ruthenium complex loading in the PEG-DA hydrogel, several different concentrations were tried. Figs. 5(a) and 5(b) are the Stern-Volmer plots of various ruthenium-complex concentrations and the re-plot of the sensitivity data points (Io/I) at 100% oxygen saturation in Fig. 5(a), respectively. The linearity of all curves in Fig. 5 (a) was rather similar and none of them was clearly distinguishable among others. Similarly, all sensitivity values in Fig. 5(b) were statistically quite equivalent, although a slight increase of the average values was observed with respect to the ruthenium loading. Therefore, within the range of ruthenium loading we have tested, it did not appear to significantly matter in terms of the relative sensitivity (Io/I) while the absolute intensities (Io and I) were affected. Consequently, 2.81 mM was found to be the optimum loading for later experiment to keep the high absolute intensity, while minimizing the clustering issue. Depending on the future applications, it will be necessary to tailor the target dynamic range with a better linearity (e.g. a narrow range of 0–20% for biological applications or a wider range of 0–100% for industrial applications, etc.).

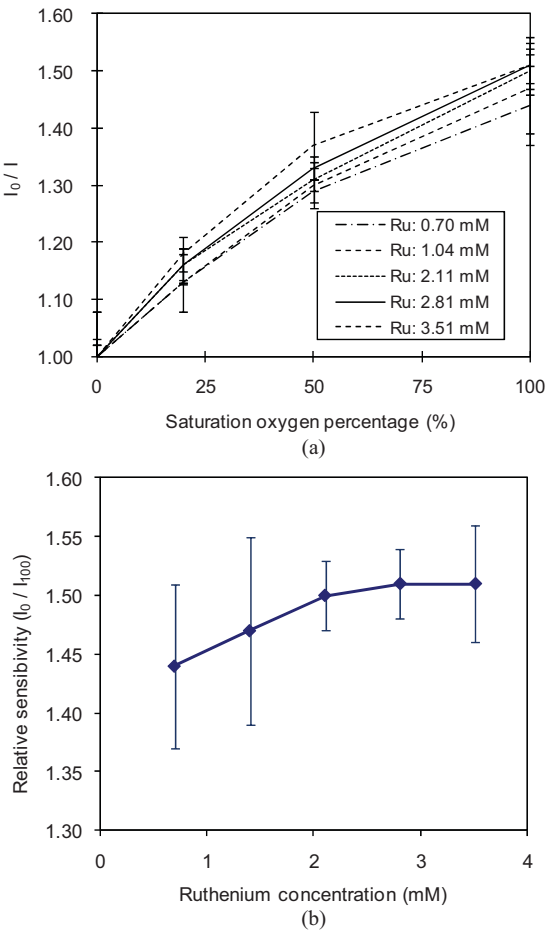
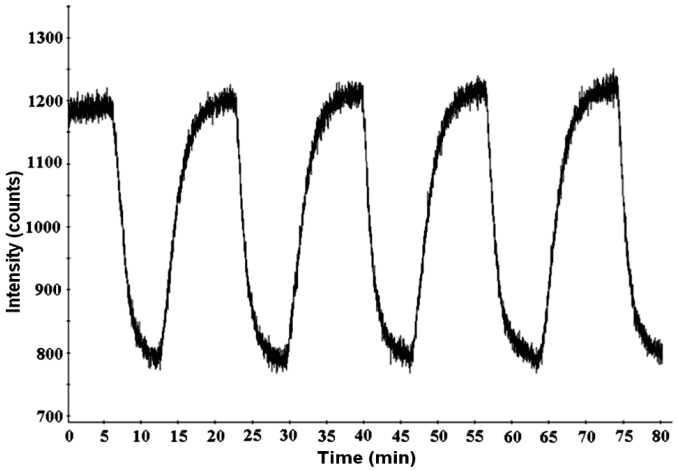


Fig. 5. Optical properties of oxygen sensitive PEG-DA membranes on SU-8 surface. (a) Stern–Volmer plots for materials comprised of various ruthenium concentrations in PEG-DA membranes. (b) Relative sensitivity (I0/I100) as a function of ruthenium complex concentration in PEG-DA membrane ($n = 3$, error bar = standard deviation).

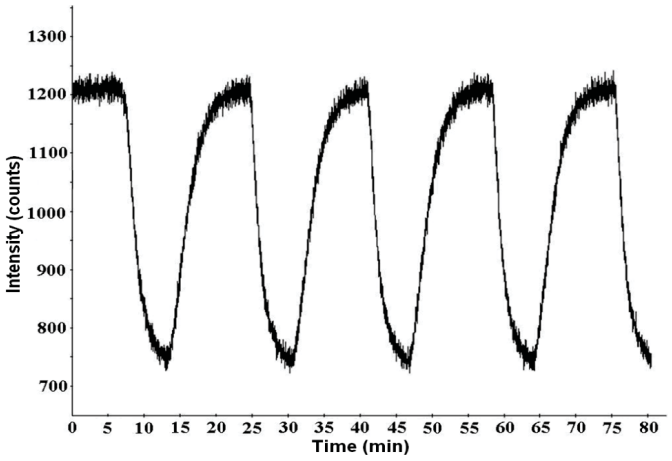
E. Response Time and Reversibility

The response time and reversibility of the oxygen sensitive membrane fabricated with optimal ruthenium complex concentration of 2.81 mM was tested by alternating saturation oxygen percentages. Before conducting the measurements, the sensor was immersed in D.I. water for 12 hours and exposed to the LED light source, as described previously. As shown in Fig. 6 (a), the signal changes were fully reversible, and the response time was consistent. This result demonstrates the sensor to be highly reproducible with an excellent reversible response to aqueous dissolved oxygen. The results also indicate that PEG-DA is a good candidate for optical sensor applications as a matrix material for fluorophore immobilization.

Fig. 6 (a) also shows the typical response curve of the ruthenium complex. The response time from nitrogen to oxygen saturated D.I. water was about 5 minutes (90% response from 0% to 100% oxygen), whereas the shift from oxygen to nitrogen saturated water took about 10 minutes (90% response from 100% to 0% oxygen). The response time is slower than desired, but is likely easily improved after analyzing the underlying reasons. The response time is the result of two parts in this setup of Fig. 3. First, a finite amount of time is required for dissolution of oxygen into the D.I. test water, a process that is dependent on the gas bubbling rate and water volume. In our experimental setup, a water volume of 20 ml was used. Smaller volumes of water (microliter range or less) in the test cell would speed this process and will be utilized when the device is fully operational in microfabricated form. Secondly,



(a)



(b)

Fig. 6. Response time and reversibility of fluorescence intensity changes (peak wavelength 608 nm) of PEG-DA membrane upon switching between nitrogen and oxygen saturated D.I. water repeatedly (an average of ten data points was read 10 min after switching each sample to obtain all Stern–Volmer plots from these typical time responses). (a) Sample stored in D.I. wafer for 1 day. (b) Sample stored in D.I. water for 2 months.

time is required for the transport of oxygen from the fluid bulk into the sensing membrane. This process is limited by the diffusional rate of the oxygen molecule and the shape and chemical structure of hydrogel matrix. Increasing the ratio of surface area-to-volume will lead to the faster response time. Basically two approaches are available to increase the rate of oxygen transport – decreasing the volume (width and/or thickness) of sensing elements and increasing the hydrogel mesh size. All of these are feasible and easy manipulations for PEG-DA matrix. However, some potential drawbacks exist as well. For instance, increasing the mesh size of the hydrogel will enhance mass transfer, but the more open network may entrap less fluorophore, with a subsequent decrease in total fluorescent intensity.

A follow-up study was completed after storing the PEG-DA matrix in D.I. water for a period of two months. The response time and reversibility of the stored sensor were investigated again. Fig. 6 (b) shows good reversibility on a typical response curve and a similar response time. No delamination or degradation of PEG-DA membrane appeared

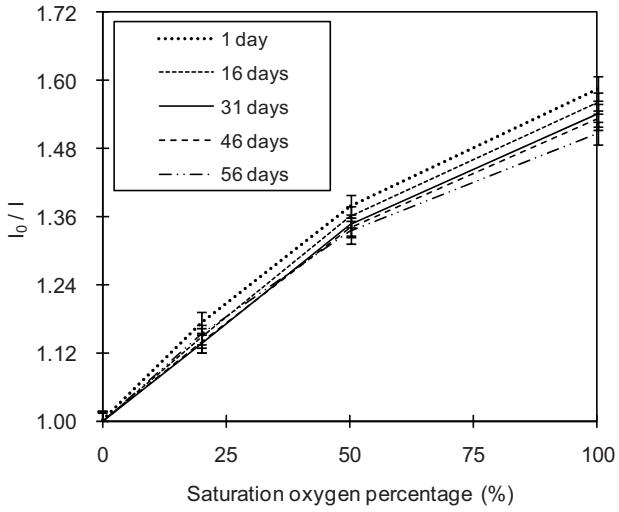


Fig. 7. Stern–Volmer plot showing long-term stability of ruthenium complex incorporated in chemically-anchored PEG-DA membrane on SU-8 surface ($n = 3$, error bar = standard deviation).

during the experiment. Therefore, the PEG-DA matrix has successfully been used to entrap the ruthenium complex and it was strongly bound to the SU-8 surface through our simple photoinitiation steps.

F. Long-Term Storage Stability

Every fifteen days, this fluorescent oxygen sensor was taken out from the D.I. water storage container and subjected to fluorescent measurements. The data in Fig. 7 demonstrate that the sensor is stable within the error of the laboratory test system. The relative sensitivity of the sensor decreased about 6% from the original sensitivity during the two month period. The fluorophore exhibits good long-term stability when ruthenium complex was physically incorporated in a covalently bound PEG-DA matrix. No noticeable physical shape change or delamination of the PEG-DA matrix from the SU-8 surface was observed after two months storage. It indicates that our photoinduced chemical anchoring technique can be used to covalently bond the thick PEG-DA matrix to SU-8 successfully, without negative effects on the structure of the matrix.

It is commonly known that the instability issue of leaching and photobleaching can be circumvented by measuring the Φ and τ, which are virtually independent of external perturbations. According to the work done by other groups with these time-resolved measurements, the relative sensitivity of optical oxygen sensor decreased about 5% over three months [19]. Better long-term stability results can be expected if the lifetime or phase shift of ruthenium complex entrapped in PEG-DA matrix was measured.

G. Dissolved Oxygen Measurements Within SU-8 Channel

Three sealed SU-8 channels with patterned and embedded optical oxygen-sensing elements were fabricated and tested with sequential injections of water samples containing various amounts of oxygen. Prior to the experiments,

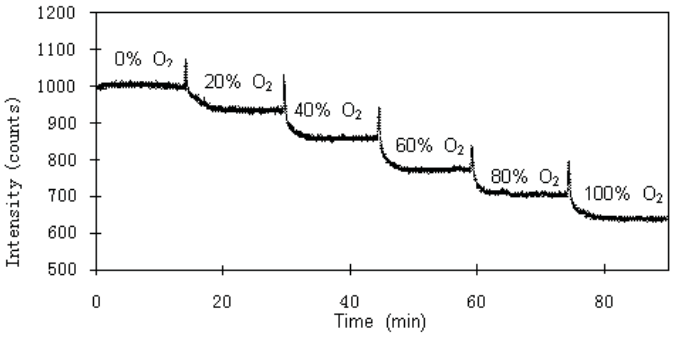


Fig. 8. Typical time response of the optical fluidic sensor with different oxygen concentrations.

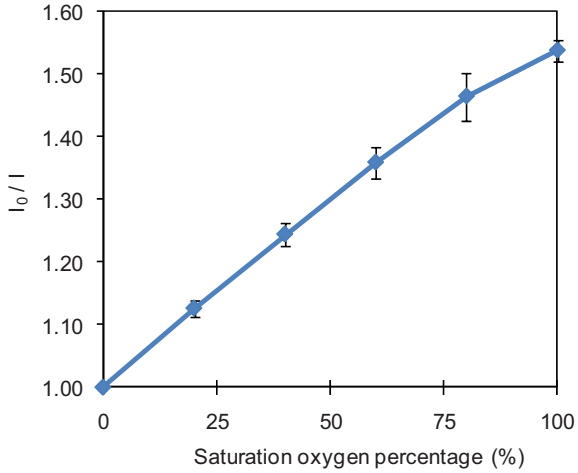


Fig. 9. Relative sensitivity (I_0/I) as a function of saturation oxygen percentage in PEG-DA element embedded in SU-8 fluidic channel ($n = 6$, error bar = standard deviation)

the SU-8 channel was first filled with nitrogen-saturated D.I. water for 30 minutes to ensure the fluorescent intensity (I_0) of sensing element was maximal. After this, test solutions were injected into the SU-8 channel sequentially, working from low oxygen levels to higher ones. During the oxygen measurements, 10 ml D. I. water was saturated by bubbling nitrogen gas and oxygen gas at different ratios in a separate glass container for 15 minutes. After saturation, the test solution was transferred with a 5 ml syringe and injected into the SU-8 channel. Immediately after injecting each solution, the inlet and outlet ports were capped with fittings to minimize the influence of ambient air. As shown in Fig. 8, the fluorescence intensity at the emission wavelength, 608 nm, decreased as the percentage of dissolved oxygen increased. Six intensity measurements were recorded for each oxygen level. It is considered that the spikes are the mechanical artifacts each time when the sample solution was injected through tubing. The Stern-Volmer relationship, plotted in Fig. 9, shows a non-linear tendency similar to that described in the previous section. The results indicate that the optical sensing element retains its sensitivity even when integrated within a sealed SU-8 channel.

During the initial stability step and the oxygen concentration experiment, cylindrical hydrogel structure remained in the middle of the SU-8 channel without physical shape change, migration, or delamination. This clearly demonstrates that PEG-rich hydrogel was covalently immobilized on the planar SU-8 surface within the sealed fluidic channel by utilizing our simple, novel surface modification technique. The size of sensing elements can be further reduced. Currently, traditional lithography with a photomask placed on top of the device is done as in Fig. 2, which may result in a low pattern transfer fidelity caused by UV interference through thick glass and SU-8 layers. A technique is available such as built-in "in-device" photomasks [20], [21] to allow selective formation of "in-channel" sensing elements utilizing patterned reflective or absorbance (anti-reflective) layers. Smaller sensing elements, however, inevitably require more sensitive photodetectors because the absolute emission intensities responding to target analytes becomes low. Therefore, this technique has a strong potential application where fabrication of sensing elements is desired after the channel has been bonded and sealed. Feasibility of integration of bioanalytical elements or polymer films, especially if they are sensitive to the harsh conditions required for bonding process, is enhanced.

IV. Conclusion

Surface bound PEG-rich hydrogel matrices containing an oxygen-sensitive fluorophore were synthesized for potential application in fluidic channels fabricated from the photoresist SU-8. The PEG-DA matrix was chemically-anchored to the SU-8 surface through the use of a surface-bound photoinitiator which acted as a site for chain growth. These densely-crosslinked polymeric networks were suitable structures for the physical entrapment of the oxygen-sensitive ruthenium complex with fluorophore leaching and photobleaching occurring only in the initial washing steps. The reported hydrogel matrix exhibited fluorescence quenching behavior, described in part by the Stern-Volmer relationship. Long-term storage stability and reversibility were also successfully demonstrated, illustrating that surface-bound PEG-rich matrices are good candidates for the immobilization of fluorophores for a long-term use.

An optical oxygen fluidic channel sensor was fabricated and integrated with PEG-rich matrix after bonding process by utilizing our novel surface modification technique. Good performance of dissolved oxygen content measurement was demonstrated and no physical shape change or migration of the PEG-rich hydrogel was detected. To our knowledge, this work is the first report of a method which can be used to integrate PEG-based hydrogel structures after completion of the channel bonding process. This merit enhances the potential applications of PEG-based hydrogels as matrix materials for integrated BioMEMS and bioanalytical elements, addressing concerns of activity loss due to the high temperatures and/or pressures required for bonding.

References

[1] G. M. Whitesides, "The origins and the future of microfluidic," *Nature*, vol. 442, pp. 368–373, Jul. 2006.
[2] O. S. Wolfbeis, "Materials for fluorescence-based optical chemical sensors," *J. Mater. Chem.*, vol. 15, pp. 2657–2669, Apr. 2005.

[3] A. Mills, "Optical oxygen sensors: Utilizing the luminescence of platinum metals complexes," *Platinum Met. Rev.*, vol. 41, pp. 115–127, 1997.
[4] H. Lorenz, M. Despont, N. Fahmi, N. L. Bianca, P. Renaud, and M. J. Vettiger, "SU-8: A low cost negative resist for MEMS," *J. Micromech. Microeng.*, vol. 7, no. 3, pp. 121–124, 1997.
[5] P. Abgrall, V. Conedera, H. Camon, A. M. Gue, and N. T. Nguyen, "SU-8 as a structural material for labs-on-chips and microelectromechanical system," *Electrophoresist*, vol. 28, no. 4, pp. 4539–4551, Dec. 2007.
[6] G. Kotzar, M. Freas, P. Abel, A. Fleischman, S. Roy, C. Zorman, J. M. Moran, and J. Melzak, "Evaluation of MEMS materials of construction for implantable medical devices," *Biomaterials*, vol. 23, no. 13, pp. 2737–2750, 2002.
[7] V. Seidemann, J. Rabe, M. Feldmann, and S. Buttgenbach, "SU8-micromechanical structures with in situ fabricated movable parts," *Microsyst. Technol.*, vol. 8, nos. 4–5, pp. 348–350, 2002.
[8] K. B. Mogensen, J. El-Ali, A. Wolff, and J. P. Kutter, "Integration of polymer waveguides for optical detection of microfabricated chemical analysis systems," *Appl. Opt.*, vol. 42, no. 19, pp. 4072–4079, 2003.
[9] R. J. Jackman, T. M. Floyd, R. Ghodssi, M. A. Schmidt, and K. F. Jensen, "Microfluidic systems with on-line UV detection fabricated in photodefinable epoxy," *J. Micromech. Microeng.*, vol. 11, no. 3, pp. 263–269, 2001.
[10] O. S. Wolfbeis, "Materials for fluorescence-based optical chemical sensors," *J. Mater. Chem.*, vol. 15, pp. 2657–2669, Apr. 2005.
[11] N. A. Peppas, *Hydrogel in Medicine and Pharmacy*, vol. 1, 1st ed. Boca Raton, FL: CRC Press, 1986, p. 96.
[12] D. P. O'Neal, M. A. Meledeo, J. R. Davis, B. L. Ibey, V. A. Gant, M. V. Pishko, and G. L. Coté, "Oxygen sensor based on the fluorescence quenching of a ruthenium complex immobilized in a biocompatible poly(ethylene glycol) hydrogel," *IEEE Sens. J.*, vol. 4, no. 6, pp. 728–734, Dec. 2004.
[13] S. Lee, B. L. Ibey, G. L. Coté, and M. V. Pishko, "Measurement of pH and dissolved oxygen within cell culture media using a hydrogel microarray sensor," *Sens. Actuators B*, vol. 128, no. 2, pp. 388–398, 2008.
[14] R. J. Russell, A. C. Axel, K. L. Shields, and M. V. Pishko, "Mass transfer in rapidly photopolymerized poly(ethylene glycol) hydrogels used for chemical sensing," *Polymer*, vol. 42, no. 11, pp. 4893–4901, 2001.
[15] Y. L. Wang, M. Bachman, C. E. Sims, G. P. Li, and N. L. Allbritton, "Simple photografting method to chemically modify and micropattern the surface of SU-8 photoresist," *Langmuir*, vol. 22, no. 6, pp. 2719–2725, 2006.
[16] Y. L. Wang, J. H. Pai, H. H. Lai, C. E. Sims, M. Bachman, G. P. Li, and N. L. Allbritton, "Surface graft polymerization of SU-8 for bio-MEMS application," *J. Micromech. Microeng.*, vol. 17, no. 7, pp. 1371–1380, 2007.
[17] S. L. Tao, K. C. Popat, J. J. Norman, and T. A. Desai, "Surface modification of SU-8 for enhanced bioflunctionality and nonfouling properties," *Langmuir*, vol. 24, no. 6, pp. 2631–2636, 2008.
[18] Z. Gao, D. B. Henthorn, and C.-S. Kim, "Enhanced wettability of an SU-8 photoresist through a photografting procedure for bioanalytical device applications," *J. Micromech. Microeng.*, vol. 18, no. 4, pp. 045013-1–045013-7, 2008.
[19] C. McDonagh, C. Kolle, A. K. McEvoy, D. L. Dowling, A. A. Cafolla, S. J. Cullen, and B. D. MacCraith, "Phase fluorometric dissolved oxygen sensor," *Sens. Actuators B*, vol. 74, nos. 1–3, pp. 124–130, 2001.
[20] B. E. J. Alderman, C. M. Mann, D. P. Steenson, and J. M. Chamberlain, "Microfabrication of channels using an embedded mask in negative resist," *J. Micromech. Microeng.*, vol. 11, no. 6, pp. 703–705, 2001.
[21] Y.-J. Chuang, F.-G. Tseng, J.-H. Cheng, and W.-K. Lin, "A novel fabrication method of embedded micro-channels by using thick-film photoresists," *Sens. Actuators A*, vol. 103, pp. 64–69, Jan. 2003.

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