# An Integrated Optical Biochemical Sensor Fabricated Using Rapid-Prototyping Techniques

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## ABSTRACT

This paper details the design and fabrication of an integrated optical biochemical sensor using a select oxygen-sensitive fluorescent dye, tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate, combined with polymeric waveguides that are fabricated on a glass substrate. The sensor uses evanescent interaction of light confined within the waveguide with the dye that is immobilized on the waveguide surface. Adhesion of the dye to the integrated waveguide surface is accomplished using a unique process of spin-coating/electrostatic layer-by-layer formation. Exposure of the dye molecules to the analyte and subsequent chemical interaction is achieved by directly coupling the fluid channel to the integrated waveguide. A unique fabrication aspect of this sensor is the inherent simplicity of the design, and the resulting rapidity of fabrication, while maintaining a high degree of functionality and flexibility.

**Keywords: Optical, Biosensor, Rapid-Prototyping, SU-8, Evanescent**

## INTRODUCTION AND THEORY

Traditionally, biochemical sensing has been carried out using "extract and evaluate" procedures, where a sample is removed from the system of interest and analyzed to determine the components present, both qualitatively and quantitatively, usually with macroscale equipment in a laboratory situation. This process obviously is time-consuming, limited in application and can be very expensive depending on the difficulty of the extraction process. Sample extraction from within a microsystem would require either alternation of the system design to incorporate a sample exit point, or halting the process and opening the unit to remove the sample material. The latter technique would in most cases require destruction of the microsystem, and both processes will cause severe operational interference. With the advent of numerous microscale systems dedicated to biological separation, processing, handling or sensing, this cumbersome process is simply not feasible.

The need for effective miniaturized sensors has driven a massive research effort towards this end, with systems varying in both principal of operation and morphology. However, despite recent advances in the field of MEMS-based sensors, the fabrication of miniaturized optical biosensors still tends to be a relatively difficult process, limited largely by complicated device fabrication and packaging[1-3]. Optical biosensors are particularly difficult to fabricate, as coupling into microsystem typically requires accurate alignment components, such as micro-positioning stages for end-fire coupling[4-6]. Elements such as grating couplers and V-groove couplers may alleviate some of these difficulties, but are challenging and often impossible to integrate into existing microsystems[7-8].

A simple method to embed an optical sensor in an existing biosensor system is an integrated optical waveguide, which can allow light to be effectively conducted to a select point of interest within the device with minimal interference. Applications for this type of optical sensor vary from micro total-analysis systems (μTAS), chemical-sensing within separation channels or miniaturized bioreactors and artificial tissue culture substrates[9-13]. Quantitative analysis by integrated optical systems is also be possible by using fluorescent intensity sensing with a specific dye that is immobilized at the tip or on the surface of the optical waveguide[14].

Focusing on biochemical sensors, optical sensors have displayed advantageous characteristics, such as ultra low-concentration analyte sensitivity and flexibility , and with fluorescent sensors, high selectivity[15]. Due to their inherent high sensitivity, selectivity and stability, fluorescent sensors have rapidly evolved towards highly accurate, quantitative biochemical sensing[16]. However, to maximize the usage of a fluorescent sensor within a microscale device, two major obstacles must be overcome: immobilization of the dye at the point of interest in the microsystem, and conduction of light to the immobilized dye from illumination sources and back to light-sensitive components.

**1.1 Integrated Waveguides**

Transmission of light over long distances with low loss and acceptable interference has been developed to near perfection using fiber-optic technology, while remaining relatively cost-effective. However, this technology is difficult to incorporate into an established microscale system. The most efficient method to accomplish this task is using integrated waveguides, whereby the waveguides themselves are fabricated as part of the system. The advantage of this approach is lower coupling losses and less interference with the performance of the existing microsystem.

The optimization of integrated waveguide dimensions is critical to the performance of the sensing system, as the interaction area of the waveguide with the microsystem will primarily determine the resolution of an integrated optical system. In this study, an evanescent type interaction is used, whereby the chemical-sensitive dye is immobilized on the surface of the waveguide. A diagram of this interaction is shown in Figure 1.

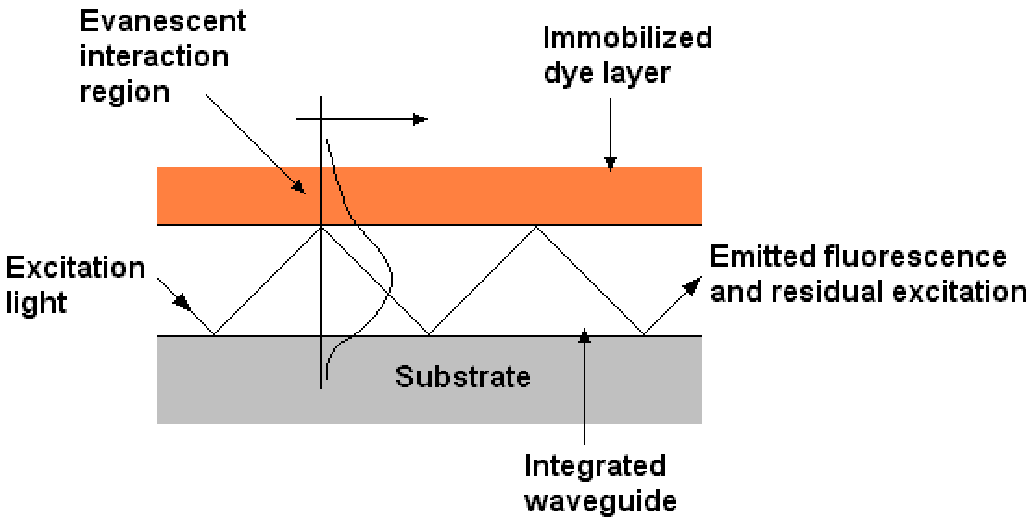


Figure 1 Evanescent wave interaction

As light is conducted down the integrated waveguide, the transverse energy field extends past the waveguide core boundaries into the surrounding substrate and dye layer. The evanescent energy field excites the dye molecules resulting in fluorescence emission and this emitted energy is superimposed on the transmitted light signal. Thus, the emitted signal from the waveguide contains components of the residual excitation signal and the fluorescent emission. Using a simple filtering system, one can quantitatively extract the spectra of the fluorescent dye. Changes in the chemical nature of the immobilized dye can result in a change in fluorescent emission intensity and output spectra, allowing one to determine if specific interactions are occurring at the waveguide surface. However, for the waveguide sensor to operate effectively, the waveguide must confine the light within the waveguide by total internal reflection, while allowing transmission of a substantial portion – at least 80 percent for short waveguide structures – of the light at both excitation and emission frequencies. The material chosen for this study possesses a relatively high refractive index of 1.80 as compared to the glass substrate of 1.46, and transmits over 94% of light above 400nm.

**1.2 Polymeric Waveguides**

The majority of advances in the field of integrated optical systems have been driven by telecommunications, and numerous innovative integrated waveguide systems have been developed to this end[17-21]. However, these systems operate in the infrared range of the electromagnetic spectrum, usually at 1300 or 1510nm. These wavelengths are not typically useful for fluorescent-based optical biosensors, as the majority of available fluorescent probes require excitation in the near-ultraviolet and visible (400-700nm) range, and also emit fluorescence in the same range. Thus a truly versatile waveguide system for integration into a biological microsystem should be capable of conducting light in this wavelength range. Silica-based waveguides provide excellent transmitting characteristics, but suffer two major drawbacks: silica is difficult to fabricate on an existing substrate surface of thickness greater than 5µm, and even heavily doped silica has a relatively low refractive index, making mode confinement difficult. Ultimately, the difficulty in both fabrication and coupling into silica-based integrated waveguides makes this material impractical to integrate into some microsystems.

Polymeric waveguides may provide an expedient solution to this problem. Polymers can generally be applied in relatively thick layers (50-150µm) and are easily patterned using methods such as photopolymerization or micromolding[22-27]. The relatively thick layers facilitates simpler coupling, and also allows multiple modes to travel down the guides, an advantage important to evanescent sensing systems. Additionally, many polymers are capable of transmitting light in the UV-Visible region, making them suitable candidates for fluorescence-based biosensing systems.

Following the requirement for a truly versatile optical biosensing system, the polymer chosen as the integrated waveguide core for this study is SU-8, an Epon®-based negative photoresist (Microchem) that has shown remarkable versatility in microfluidic and micromechanical systems[28-34]. SU-8 is capable of producing large-aspect ratio structures with very smooth sidewalls, making it an attractive material for channel waveguides. The photoresist transmittance falls primarily in the UV-visible region, and has a relatively high refractive index of 1.80, facilitating superior mode confinement. Additionally, SU-8 is patterned with established photolithography systems, using uncomplicated processing techniques, and thus is capable of successful integration into established microsystems. Finally, the fully polymerized resist is very stable in organic solvents, allowing it to be used in adverse chemical and biochemical systems.

### 1.3 Layer-by-Layer Spin Formation

For the integrated waveguide system to operate as a fluorescent sensing system, the fluorophore must be either immobilized at the tip of the waveguide, or along the waveguide surface. The latter technique uses a phenomenon known as evanescent wave interaction, where the light's electrical field extends through the surface of the waveguide core into the surrounding material. Interactions such as fluorophore excitation with materials immobilized on the surface of the waveguide can thus take place. To immobilize the dye, a combination of spin-coating coupled with electrostatic interlayer attraction and interpolyelectrolyte formation was used. This technique is a variation of layer-by-layer formation by electrostatic self-assembly of alternately charged polyions.

Electrostatic layer-by-layer (ELBL) self-assembly technique is based upon the electrostatic attraction of ionically charged sites on a polymeric molecule to other charged species. These molecules, known collectively as *polyions*, exist in two forms, polycations, and polyanions, in reference to the positive or negative charges that exist along their chain structure. Adsorption of the first polyion layer takes place when the charged sites of the first polyion are electrostatically attracted to oppositely charged sites on the substrate surface. However, the number of charged sites on the polyion far outweighs the number on the substrate surface, and the remaining sites project away from the surface. This phenomenon of incomplete neutralization is essential to further adsorption and multilayer formation[35]. The remaining polyion charged sites induce a net surface charge, reversing the overall surface charge, and preventing any further polyion adsorption. Thus, a monolayer of polyions is formed on the substrate surface. A polyion of opposite charge to the first can then be applied to and be adsorbed to the surface, also being incompletely neutralized, and again reversing the surface charge. The process of incomplete neutralization and surface charge inversion can be performed indefinitely, thus permitting the formation of polyion multilayers.

Entrapment of dye molecules within the polyion multilayers can be achieved by pre-mixing the dye with an oppositely polyion, to form stable inter-molecular bonds between them, with excess charges on the polyions allowing continued surface adsorption and multilayer formation[16]. This technique, known as interpolyelectrolyte formation is shown in Figure 2.

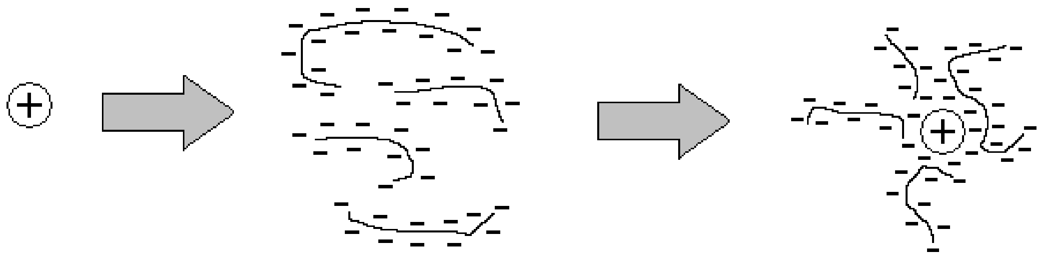

Figure 2 Interpolyelectrolyte formation

Interpolyelectrolyte complexes have net charges similar to the polyions used to form them, and are similar in form to a branched polymeric molecule. To ensure that the trapped molecule does not completely neutralize the polyion, a polyion that is long enough to retain excess charged sites after complex formation must be chosen. The entire complex thus has the ability to adsorb to an oppositely charged surface, leaving excess charged sites on its outer surface.

However, for surface adsorption to take place, the substrate must also possess a surface charge opposite to that of the molecules to be adsorbed. The waveguide material SU-8 in polymerized form does not possess the charged sites necessary to initiate self-assembly, and thus the electrostatic-self assembly process was augmented by spinning the polyions onto the surface of the substrate, and annealing them to the surface by heating. To provide an initial surface charge on the SU-8, the substrate was immersed in concentrated $H_2SO_4$ for a short period, inducing the formation of charged surface groups, as described further.

## METHODOLOGY

### 2.1 SU-8 Surface Modification

The waveguide core material SU-8 is an electrostatically neutral material at the required environmental pH of 7.6. To induce a surface charge, the fabricated waveguides were soaked for 3-5 seconds in 95% $H_2SO_4$ at 85°C. This treatment generated negatively charged phenol and glycol groups on the surface of the waveguide, thus facilitating polyion-surface adhesion. However, since extended immersion in hot $H_2SO_4$ dissolves SU-8, insufficient charged surface groups were formed for efficient self-assembly, and thus spin-coating of the polyions was required to form the multilayer film on the waveguide surface.

### 2.2 Materials

The fluorophore used for this study was the oxygen-sensitive dye tris(2,2'-bipyridyl dichlororuthenium) hexahydrate (Ru(bpy)), purchased from Aldrich Chemical Company in crystalline form and used in solution mixed with the polyanion poly(sodium styrenesulfonate) (PSS) at concentration of 0.57mg/mL. The polycation used was the chemical poly(diallyl dimethlyammomium) chloride (PDDA), and the initial polyion layer was composed of the polycation poly(ethlyenimine) (PEI). The specifications for the polyions used were as follows: PEI of molecular weight 70,000 at 1.5mg/mL, PSS of molecular weight 500,000 at 3mg/mL, and PDDA of molecular weight 70,000 at 2mg/mL (Sigma-Aldrich Chemical). All the polyions used in this study were strongly charged at pH 6-8[37]. The chemical structures for the dye and polyions used are given in Figure 5.

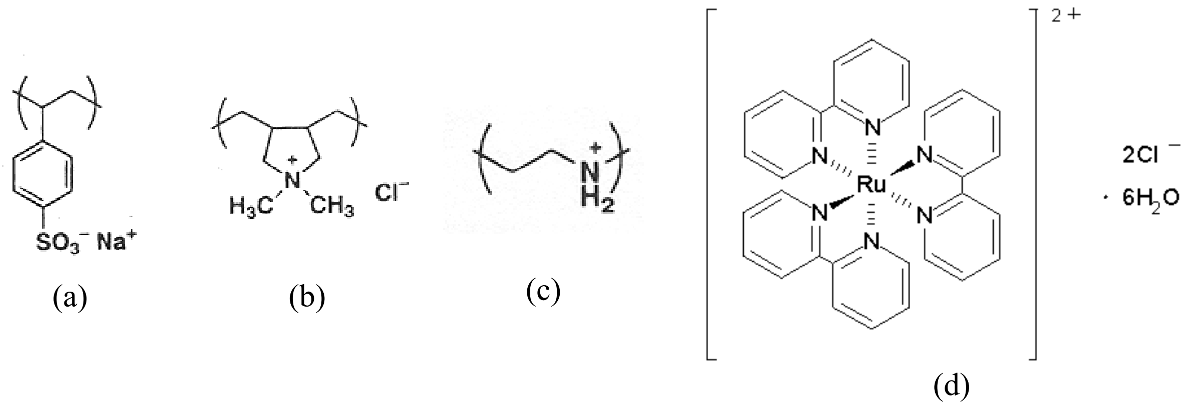

Figure 5 Structural images of polyion and dye molecules (a) poly(sodium styrenesulfonate) (b) poly(diallyl dimethlyammomium)chloride (c) poly(ethlyenimine) (d) tris(2,2'-bipyridyl dichlororuthenium) hexahydrate

The initial five layers that were applied to the waveguides consisted of PEI, followed by a pair of PSS/PDDA bilayers. The purpose of these preparatory layers was to overlap charge non-uniformities on the waveguide, thus providing a uniform surface charge for the successive dye-impregnated polyion multilayers. Twenty functional bilayers were applied to the surface of the waveguides, for a total of 45 monolayers.

### 2.3 Multilayer Spin-Coat Formation

The spin-coating process was adopted from work by Chiarelli and coworkers [38], and is fundamentally different from self-assembly. Monolayer formation by self-assembly uses the electrostatic attraction between the alternately charged polyion layers to adsorb polyion material from solution onto the substrate solution to form a solid film. However, since the surface charge of the $H_2SO_4$-treated SU-8 was insufficient to cause adsorption, the polyion solutions were each spun onto the surface of the substrate at 200rpm for 60 seconds, then heated for 1 minute at 105°C to anneal the film. Annealing improved interlayer adhesion by increasing the interpenetration between the films, augmenting the stability of the multilayer film. The electrostatic attraction between the polyion monolayers thus takes a secondary role in the film formation as the interlayer stabilizing force instead of the formation mechanism.

Following the complete multilayer formation process, the substrate was immersed in buffered water at pH 7.6 to observe if any dye desorption took place. After three hours, a small amount of dye was observed in the water, with no further desorption after six hours.

### 2.4 Waveguide Fabrication and Fiber-Optic Coupling

A unique approach to the fabrication of the waveguides and optical interfaces was used for this study, by combining the two manufacturing steps into a single process. The monolithic nature of the integrated optics also lends to increased robustness. Additionally designed into this single process was the self-aligning ability of the interfacing fiber optics with the integrated waveguides, allowing the device optics to be assembled by hand with minimal difficulties[36]. Finally, the inherent simplicity of the merged process greatly decreased the project complexity, thus improving the device yield, while facilitating the rapid fabrication of large numbers of the optical elements. The SU-8 waveguides/couplers were produced using a single lithographic mask on a 1" x 1.5" microscope slide (Fisher Scientific), and coupled with 50µm core-125µm cladding multimode optical fiber (Thorlabs). A photograph of the SU-8 waveguides on the glass substrate is shown in Figure 3, and a diagram of the waveguide/coupling system is shown in Figure 4. The tapered optical fiber interfaces allowed rapid assembly of the system using no specialized alignment stages – the optical fibers were inserted into the tapered and self-guided to the integrated waveguide interface region. To fix the fibers in position, a small amount of SU-8 was used as an adhesive.

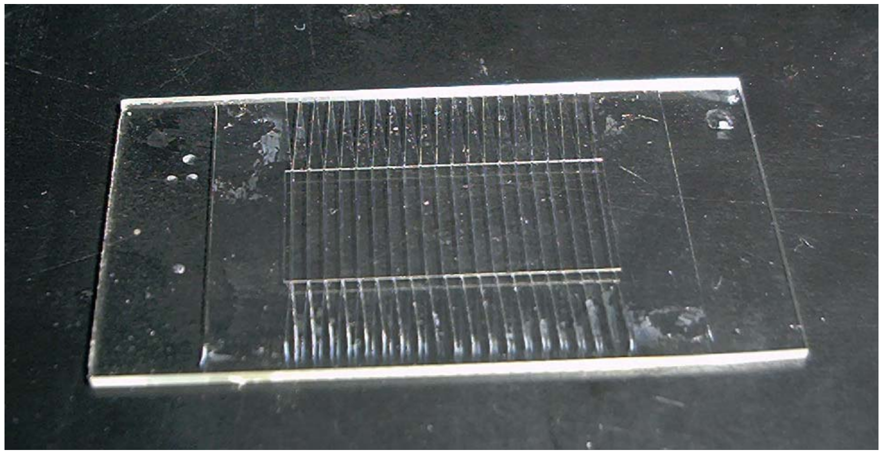

Figure 3 SU-8 waveguides on glass substrate

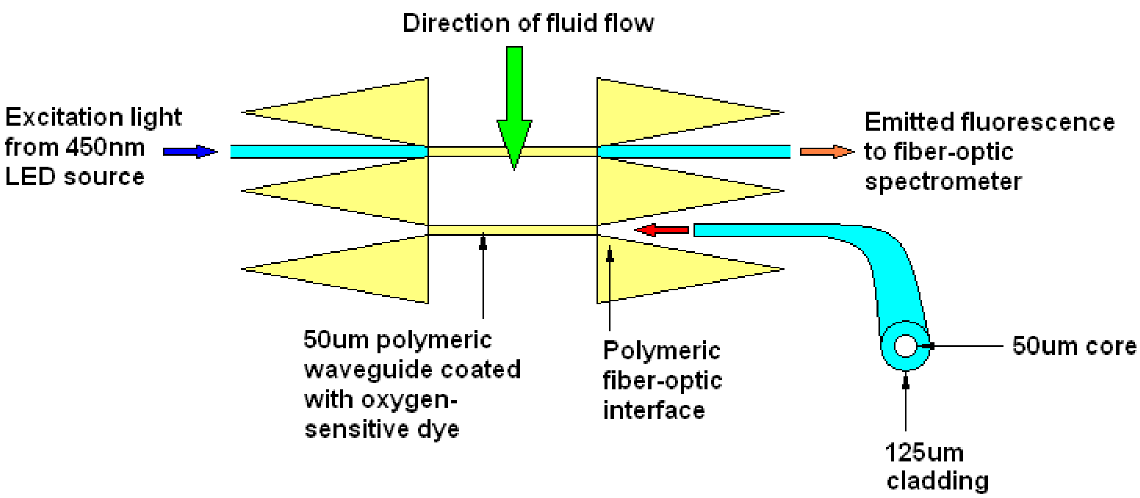


Figure 4 Waveguide/coupling system

### 2.5 Fluid Coupling

Continuing with the precept of simple and rapid device fabrication, the microfluidic channel was constructed from poly(dimethyl siloxane) (PDMS) using a single-stage casting process. Fluid inlet and outlet ports were integrated into the cast, further shortening the device construction time. The waveguide/flow cell was assembled into a controlled dissolved oxygen apparatus for sensitivity testing, as shown in Figure 6.

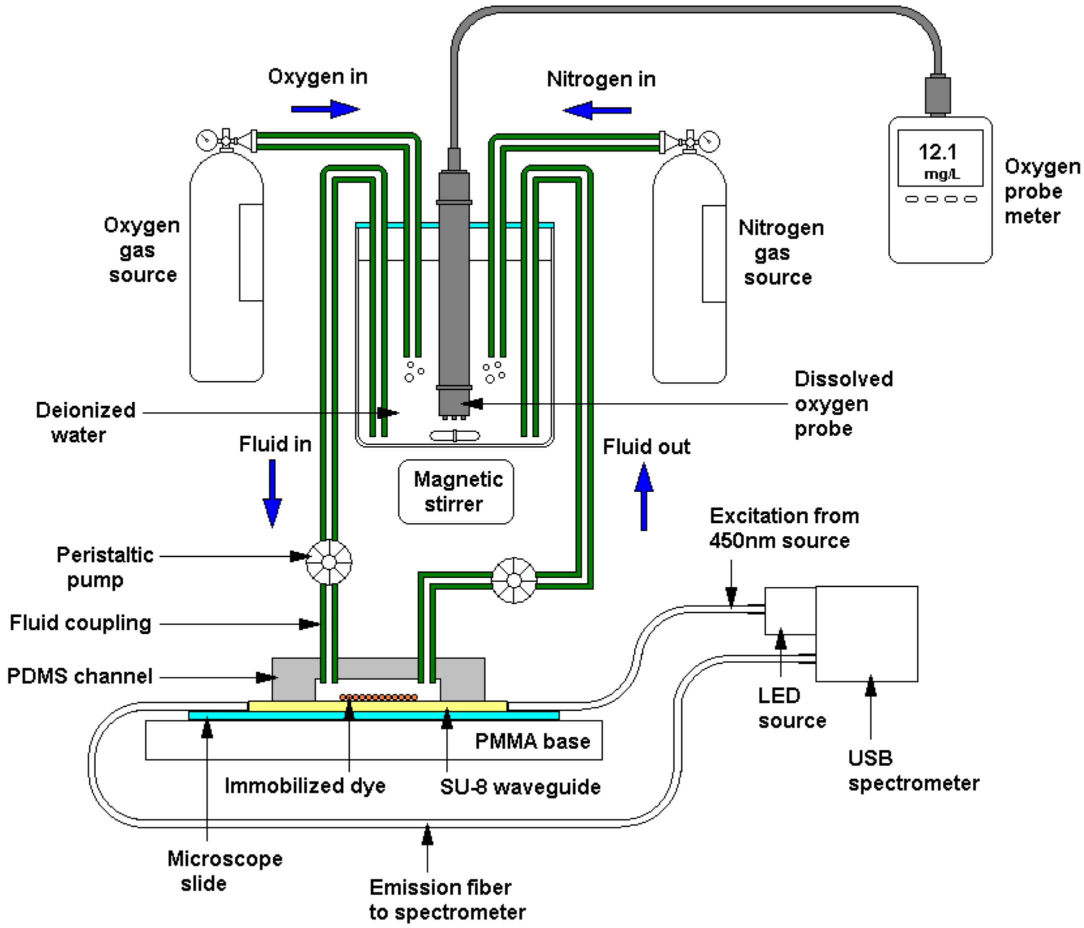


Figure 6 Schematic of controlled dissolved oxygen apparatus

The dissolved oxygen concentration in water buffered at pH 7.6 was varied from 0.8 to 24.8mg/L by controlling the ratio of bubbled oxygen and nitrogen into the mixing chamber, which was monitored with a Traceable® dissolved oxygen probe (VWR Scinetific). Once the dissolved oxygen concentration had stabilized to a set value, the dye was excited by the 450nm LED source, and the emitted fluorescence was detected by the USB spectrometer (Oceanoptics), using a five second integration period with ten averaged samples and a 30 point boxcar average. The fluorescence signal strength was extracted from the output spectra, normalized and correlated with the known dissolved oxygen concentrations to produce a calibration plot.

## RESULTS

The normalized fluorescence-oxygen concentration response calibration plot at the fluorescence wavelength of 615.27nm is shown in Figure 7.

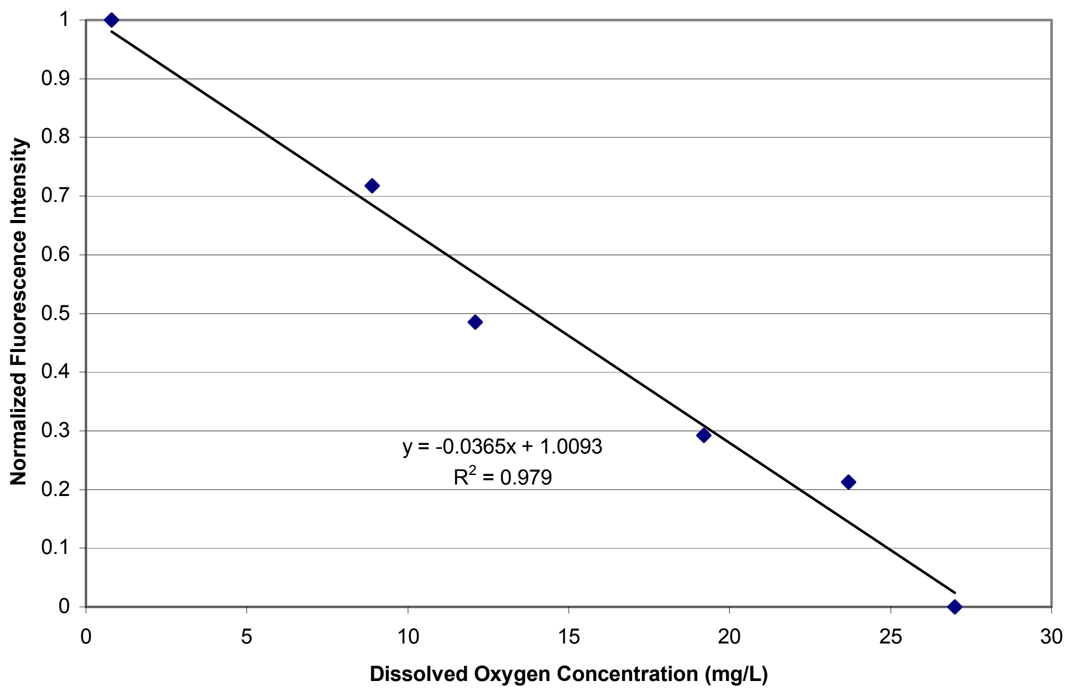


Figure 7 Normalized fluorescence-oxygen concentration response calibration plot

The calibration plot shows a strong correlation between the changing oxygen concentration and the fluorescence emissions intensity, indicative of successful oxygen sensing by the system. This relationship is predicted by the Stern-Volmer relationship, which associates the dissolved oxygen concentration with the normalized fluorescence intensity by a linear relationship[39]. The range of oxygen concentration used for this study was relatively large, which allows this sensing technique to be used for a wide variety of oxygen-sensing applications. Additionally, due to the large visible transmission range of SU-8, any fluorophore that excites using a wavelength longer than 400nm is capable of operating with this system.

## CONCLUSIONS

A polymeric integrated optical evanescent waveguide sensor for dissolved oxygen was fabricated using a single stage lithographic process. Dye immobilization on the waveguide surface was achieved using a combined spin assembly/layer-by-layer deposition with interpolyelectrolyte formation. The assembled waveguide sensor successfully demonstrated oxygen sensitivity over a range of 0.8 to 24.8mg/L with a strongly linear response. Possible applications for this type of optical sensor vary from micro total-analysis systems (μTAS), chemical-sensing within separation channels or miniaturized bioreactors and artificial tissue culture substrates.

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