

An Integrated Optical Glucose Sensor Fabricated Using PDMS Waveguides on a PDMS Substrate

David A. Chang-Yen and Bruce K. Gale

Department of Mechanical Engineering, University of Utah, Salt Lake City, UT 84112

ABSTRACT

This paper describes the design and fabrication of an integrated optical glucose sensing system using the combination of the oxygen sensitive dye tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate and glucose oxidase. Layer-by-layer self-assembly is used to immobilize the dye/enzyme system onto the surface of the waveguides. Changes in the enzyme/dye system as it interacts with the surrounding environment are monitored using end-face interaction with light injected into waveguides. The waveguides are thermally-defined monolithic polydimethylsiloxane (PDMS) waveguide system, fabricated on a PDMS substrate. The method of waveguide fabrication is a radical departure from conventional microscale waveguide systems, and offers unique opportunities for integration of this sensor into existing microfluidic systems.

Keywords: Optical, PDMS, waveguide, sensor

1. INTRODUCTION

Microfluidic systems, particularly for diagnostic purposes have created a demand for novel sensing strategies that are capable of interfacing seamlessly into the existing microsystems.¹⁻¹² The sensors should not interfere with other functions of the microsystem, and still maintain requirements for sensitivity and selectivity. Biocompatibility requirements must also be fulfilled when the sensor is used in biological applications. The use of multiple materials in these systems can complicate the design process; systems fabricated from a single material are favored. Finally, even if a sensor fulfills these basic requirements, processing interference can hamper or even prevent successful integration in the microsystems. SU-8 is a prime example, as it requires the use of the hazardous developing chemical propylene glycol monomethyl ether acetate (PGMEA).¹³ While SU-8 optical sensors designed for biochemical applications have been manufactured and tested successfully, the need for a simpler and more biocompatible sensing system motivates the change to a different waveguide material.¹⁴⁻¹⁵

2. BACKGROUND

Recent advances in microfabrication technology and chemical synthesis have allowed more specific detection of increasingly smaller concentrations of biological and chemical agents.¹⁶⁻¹⁸ However, a number of serious limitations exist in each of these systems. Systems generally sacrifice portability and simplicity for reduced detection limits and improved specificity. Biologically based sensors show great promise in fulfilling this task, but are hampered by characteristically low output signals and components that degrade by extended environmental exposure.¹⁹⁻²¹ The ability to fabricate components of miniature sensors on a nanometer scale may overcome these limitations and allow researchers to control reactions between sensors and samples, but many conventional thin-film fabrication techniques such as plasma-enhanced chemical vapor deposition (PECVD) are not compatible with biologically derived sensor components. Processes such as Langmuir-Blodgett deposition are more compatible with biosensor development, but are complex, slow, and expensive, requiring sophisticated facilities and equipment.

The process of layer-by-layer self-assembly is a simple solution to this problem. Sensing materials can be engineered to adsorb to desired substrates in a very controlled manner, facilitating the manufacture of chemical and biological sensors for biochemicals of interest. Fluorescent dyes are of particular interest, as they demonstrate high sensitivity, and an inherent selectivity.²² The combination of nanotechnology with microscale optical and fluidic systems

can facilitate the production of a simple, inexpensive nerve agent sensing system that is also adaptable towards any of the aforementioned applications.

Despite the attractiveness of fluorescence-based enzymatic sensing systems, they usually fail in commercial application for a number of reasons: bulkiness and delicateness of the instruments render them impractical for in-the-field use; difficulty in manufacture of the systems while maintaining device sensitivity and overall quality drive up the price of the instruments to unrealistic levels; inability to completely miniaturize the system limit the potential of the system especially towards medical implant scenarios. The latter factor also renders these sensors incapable of integrating into existing microfluidic systems without either considerable modification to the microsystem, or complicated packaging. To contend with all of the limiting issues, a microfabricated system must be relatively simple to fabricate and robust, yet maintain required levels of sensitivity at the same time. Use of existing microsystem fabrication processes would also allow the integration of the sensor fabrication process into current microsystems. Biocompatibility or the ability to render the device and packaging compatible in biological situations can also be a deciding factor in determining system viability. The use of polymer-based systems makes solution of all these problems possible, and also lends to increased manufacturability and low cost, especially where established polymer systems such as PDMS and SU-8 are used with little or no modification.

The use of both of these polymers for both microoptical and microfluidic applications has prompted their use in this project.^{4,12,23-24} Using existing facilities such as photolithographic patterning, plasma surface treatments and innovative polymer casting methods, a viable yet simple optical arrangement that is capable of interfacing with nanoscale, biologically based, self-assembled layers and commercially available optical transducers has been developed. A chemical sensing arrangement using an oxygen-sensitive dye and the enzyme glucose oxidase was combined with PDMS-based waveguides to create an appropriate biosensor model.

2.1 PDMS Waveguides

Design of the integrated optics morphology, interfacing and packaging in this work is based upon previous work by Chang-Yen and coworkers.¹³ In this study, the waveguide material used instead of SU-8 is the material polydimethylsiloxane (PDMS), also commonly known as silicone. This material presented several advantages over SU-8 as a waveguide material: simple fabrication process, higher optical transmittance and increased robustness. However, microscale PDMS structures have traditionally been fabricated using peel-off molds.²⁵⁻²⁶ Since the molded waveguides must have a lower refractive index than their substrate, a low refractive index (~1.45), flexible material that is capable of bonding to PDMS but not the mold is required. It was determined that the material that best fit these parameters was PDMS itself, necessitating a difference in refractive index between the waveguiding PDMS and the substrate PDMS.

PDMS can be obtained in a non-crosslinked form under the trade name SYLGARD[®] 184 (Dow Corning), as a two-part resin and crosslinker. Setting of the elastomer is achieved by mixing the two components in 10:1 ratio by weight respectively. The chemical crosslinking reaction takes place spontaneously at room temperature over an approximately 48-hour period. This setting time can be drastically shortened by applying heat to the setting PDMS. Since the crosslinking effect appeared to be greatly increased by heating, one of our postulations was that the refractive index of PDMS would increase with baking time and temperature. To determine if this hypothesis was true, a series of PDMS on glass samples were heat treated at temperatures varying from 50-150°C, for periods varying from 30 to 60 minutes. The refractive indices for each of the samples were determined using an ellipsometer (R. A. Woolam Company) and compared to a control sample that had been allowed to cure at room temperature over a period of 48 hours. The results indicated that PDMS baked at 150°C for one hour would increase its refractive index by 0.02 (1.45 to 1.47 at 450nm and 1.41 to 1.43 at 610nm) compared to PDMS that had been cured at room temperature. The concept of PDMS waveguides on a PDMS substrate was thus based upon these results.

2.2 Dye/Enzyme Immobilization

For the PDMS waveguides to operate as a glucose sensor, a combination of glucose oxidase and a ruthenium-based oxygen sensitive dye were used to transduce the glucose concentration into an optical signal. Increases in glucose concentration caused a proportionally increased consumption of ambient oxygen, which was detected by a reduction in oxygen quenching of the ruthenium dye fluorescence. To interface the enzyme and dye with the optical system, end-face excitation and fluorescence sensing was chosen, in which the dye/enzyme system was immobilized onto a series of "vanes" that were placed at 45° in the light path. The fluorescent emission was collected using a similar waveguide as used for excitation, although the emission waveguide was placed at 90° to the excitation. This arrangement maximized the interaction of the light with the dye while reducing the amount of excitation light feeding back to the spectrometer.

However, since the vanes were only separated by 40 μ m, a dye/enzyme immobilization technique was needed that would not block the channels yet maximize the dye density.

Layer-by-layer self-assembly fulfills this requirement, as the individual layer thicknesses are uniform and predictable. Also, this method is capable of producing very thin yet dense layers that have strong fluorescent responses. To produce the required combination and concentrations of dye within the system, a technique known as interpolyelectrolyte formation was used, whereby the dye was mixed with an oppositely charged polyion solution to produce a complex in solution with charges similar to its respective polyion. The enzyme, being large enough to act as a polyion, was layered alternately with an oppositely charged polyion on top of the adsorbed dye complex layers. This technique was adapted directly from Fang and coworkers.²⁷

3. METHODOLOGY

3.1 Fabrication Overview

The sensor fabrication procedure was developed using a combination of previous work involving fabrication of an oxygen sensor using SU-8 waveguides¹³ and the recently-discovered approach of creating PDMS optical waveguides upon a PDMS substrate, where the refractive index of the waveguide and substrate are defined using different polymer curing processes. The PDMS patterns are created using photolithographically created SU-8 molds, and the fluidic interfacing system is also PDMS-based, making the completed device without external packaging essentially monolithic. Surface modification of the PDMS is also performed to facilitate electrostatic layer-by-layer self-assembly, and a custom self-assembly procedure is used to immobilize the dye/enzyme system on the waveguide surfaces.

3.2 SU-8 Mold Fabrication

This stage of the fabrication process, though usually overlooked posed sufficient difficulties to warrant further elucidation. The SU-8 layer that the mold was patterned from was spun onto 3-inch silicon wafers to 125 μ m to match the cladding diameter of the fiber optics used. SU-8-50 from Microchem was used as received, although the recommended soft-baking time of 30 minutes at 95°C was altered to 2 ½ hours to remove as much of the solvent as possible before exposure. SU-8 on wafers that were baked for only 30 minutes buckled upon post-exposure baking, whereas the longer soft-baked wafers produced smooth resist patterns. Following the development of the wafers, they were coated with a dilute detergent solution to prevent sticking of the PDMS to the mold. Treatment of the SU-8 molds with a fluorinating compound proved unsuccessful, and the detergent solution proved to be the most reliable method to prevent sticking.

3.3 PDMS Molding

The waveguide fabrication was accomplished in two basic steps: filling of the SU-8 mold with PDMS and baking, and then waveguide substrate casting. This fabrication process is demonstrated in Figure 1.

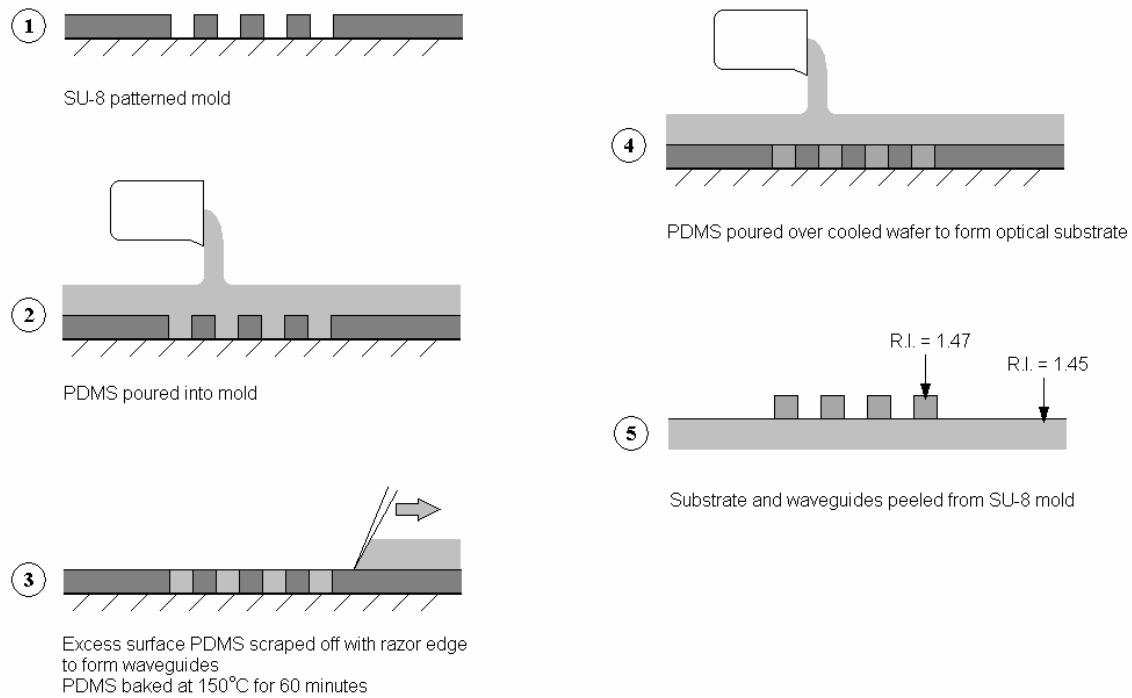


Figure 1. PDMS waveguide fabrication process

3.4 PDMS Surface Treatment

The surface of PDMS in its natural state is hydrophobic, and is not suitable for electrostatic self-assembly. Treatment with a low power RF-induced oxygen plasma will oxidize the PDMS and create a thin layer of silicon dioxide on the surface. Although this SiO_2 layer is negatively charged and capable of supporting electrostatic self-assembly, it is short-lived, lasting approximately two hours for a PDMS surface treated with a 150W RF oxygen plasma at 100mTorr for 60 seconds. This reversion to the hydrophobic state is caused by migration of low-molecular weight uncrosslinked PDMS chains within the polymerized structure to the treated surface. To counter this problem, a solution combining techniques developed by Genzer and coworkers²⁸ and Colic²⁹ was used, whereby the surface of the PDMS was stretched by 33%, treated with an oxygen plasma, soaked in 1% γ -aminotrimethoxysilane (Dow Chemicals), and then allowed to return to its natural size. The contraction of the PDMS surface caused grafted sites to be crowded together, preventing migrating free polymer chains from reaching the surface. This surface treatment process is shown in Figure 2.

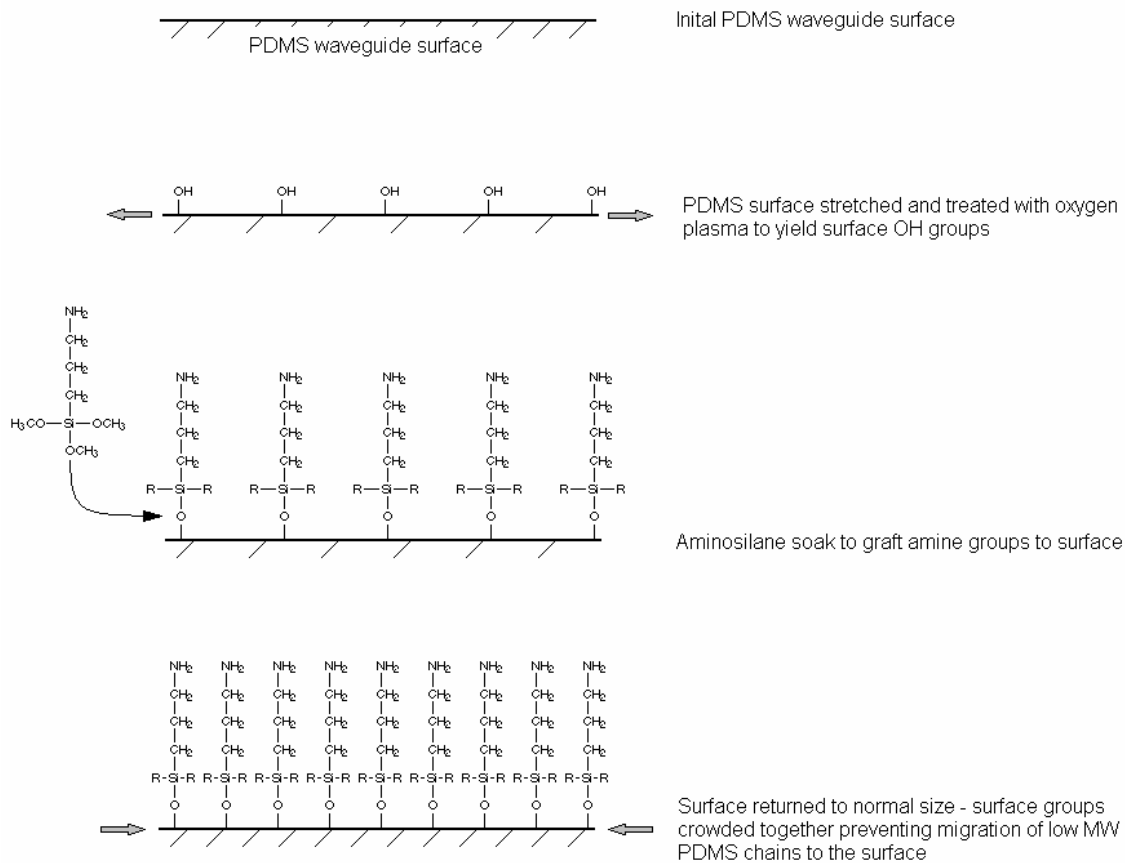


Figure 2. PDMS waveguide surface treatment

3.5 Fluidic and Optic Assembly

The surface-treated PDMS waveguides were connected to an LED source that injected light into the waveguides at a 450nm peak wavelength output. The oxygen-sensitive ruthenium dye absorbs strongly at this wavelength, and emits light at a peak wavelength of 610nm. Silica fibers with a core of 105 μ m and a cladding of 10 μ m for a total outside diameter of 125 μ m were inserted into the PDMS interface and glued in position using PDMS. This eliminated the extra reflection interface at the insertion point usually caused by trapped air between the PDMS waveguide and silica fiber. To remove as much of the 450nm excitation light from the emitted signal, a Kodak Wratten long-pass filter, centered at 530nm (Edmund Optics) was placed before the spectrometer using a fiber coupler. Once the fiber had been inserted and glued (Figure 3), a PDMS cover slab was cured at room temperature and clamped over the waveguide region with the dye/enzyme coating. This channel isolated the fluidic system from the waveguides. Holes were bored in the cover PDMS slab down to the fluid input and exit ports using a 20 gauge needle modified for coring. Unmodified 20 gauge needles were then inserted into the holes, and the contraction of the PDMS cover slab around the needle shafts provided the fluid seal. 1/16" tubing was attached to the needles using luer adaptors (Scivex) to provide a simple, controlled fluid path.

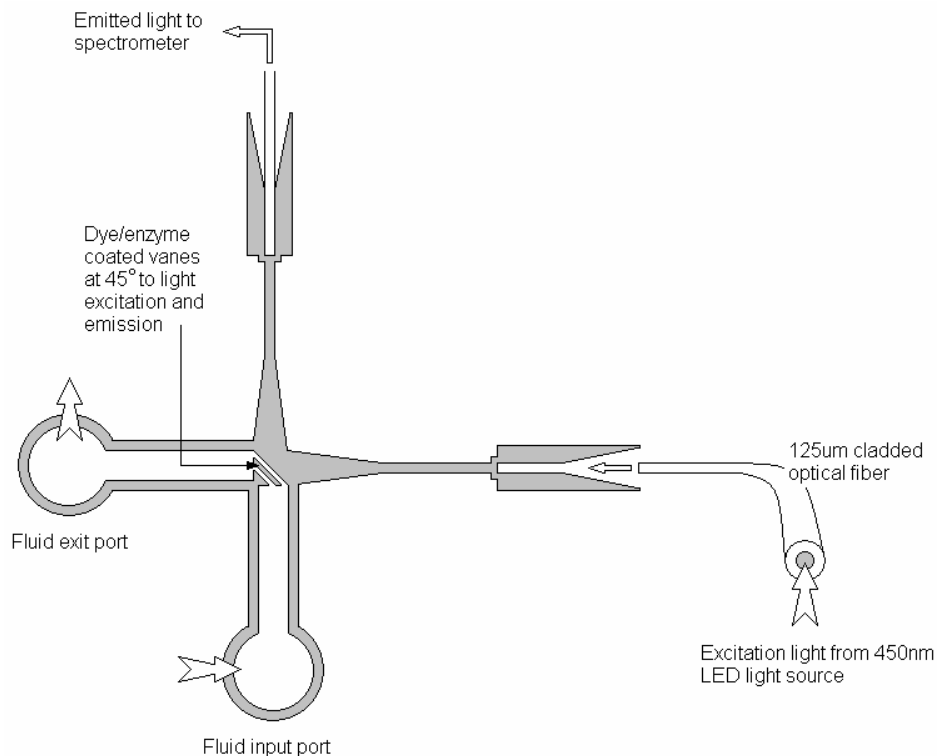


Figure 3. PDMS waveguide optical interface

3.6 Dye/Enzyme Layer-by-Layer Self-Assembly Process

The dye/enzyme layering procedure is shown in Figure 4. Glucose oxidase from *Aspergillus niger* was used in solution at 2mg/mL. Its alternating polycation poly(ethylenimine) (PEI) was used in solution at 1.5mg/mL. The oxygen-sensitive dye tris(2,2'-bipyridyl dichlororuthenium) hexahydrate (Ru(bpy)) at 1.14mg/mL was mixed into the polyanion poly(sodium styrenesulfonate) (PSS) at 3mg/mL. Its alternating polycation poly(diallyl dimethylammomium) chloride (PDDA) was used in solution at 2mg/mL. All solutions were created at a pH of 7.6. All chemicals were acquired from the Sigma-Aldrich Company, and solutions were created using HPLC-grade water (J.T. Baker).

To coat the vane surfaces, each layering solution was alternately injected into the fluid channel and allowed to sit stagnant for 20 minutes for self-assembly to occur. Between each layering solution, the vanes were rinsed for 2 minutes using a wash of water buffered at pH 7.6 to remove any excess polyion solution. A total of 20 dye/polyion and 15 enzyme/polyion bilayers were applied to the waveguide surface, beginning with Ru(bpy)-PSS.

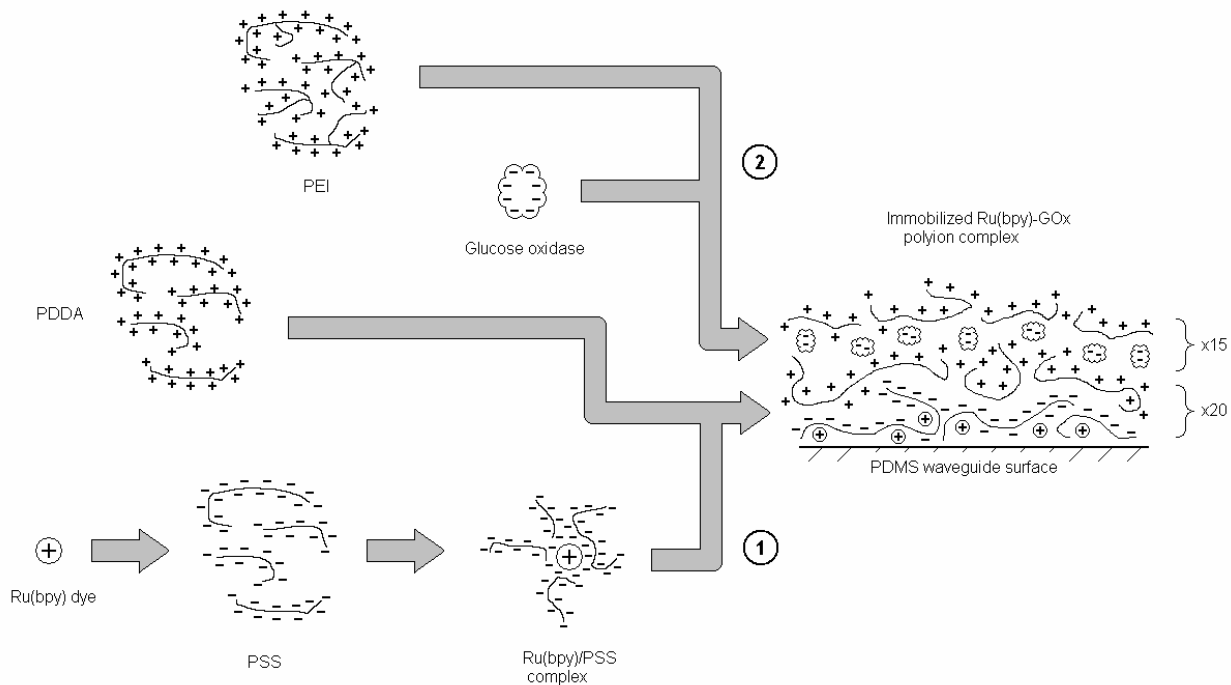


Figure 4. Interpolyelectrolyte formation and layering

3.7 Sensitivity Testing

The completed waveguide/fluidic system with connecting fluid tubing is shown in Figures 5A and 5B. In Figure 5A, the excitation and emission fibers are connected to the left and top interfaces, and the fluid connection needles are connected to the right and bottom fluid ports. The angled interface is visible to the center of the image, although the fluid vanes did not demold during the casting process due to inconsistencies in the photolithographic exposure of the SU-8. The complete system showing the syringe needle and fiber optic connections is shown in Figure 5B. Upon completion of the assembly process, the LED excitation source and fiber-optic spectrometer (Oceanoptics) were connected to each end of the waveguides. The spectrometer integration time was set at 5000ms, with a 40-point spectral average and 20-sample average. Two modes of testing were performed: first comparing response to ambient air and 100 percent nitrogen to determine the sensitivity of the dye alone; second comparing response to water and 40mM glucose solutions to determine the sensitivity of both the dye and enzyme.

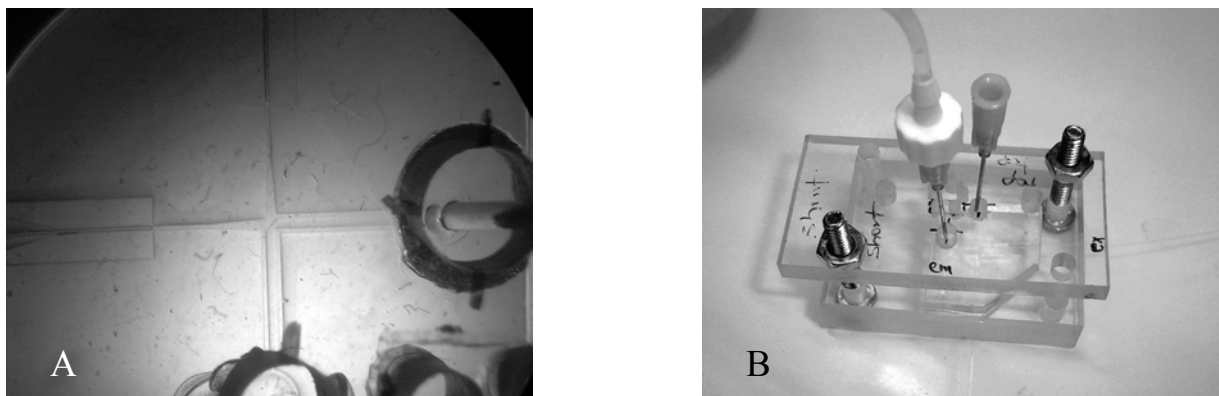


Figure 5. Waveguide/fluidic system

4. RESULTS

4.1 Waveguide Quality

Experimental spectra taken before dye/enzyme layering without using the long-pass emission filter showed a relatively strong intensity, although mechanical compression of the assembled system did reduce the overall output intensity. Using conventional PDMS bonding techniques, such as RIE surface activation, can eliminate the requirement for the clamping apparatus used and remove any stresses present on the waveguide structure. Overall, the physical waveguide quality was observed to be excellent, with very low surface roughness and no observed light scattering from within the core.

4.2 Sensor Response

The normalized fluorescence spectra comparing ambient air to pure nitrogen are shown in Figure 6. Despite low experimental fluorescence intensities, a definite increase in fluorescence intensity especially in the 610-630nm range can be seen.

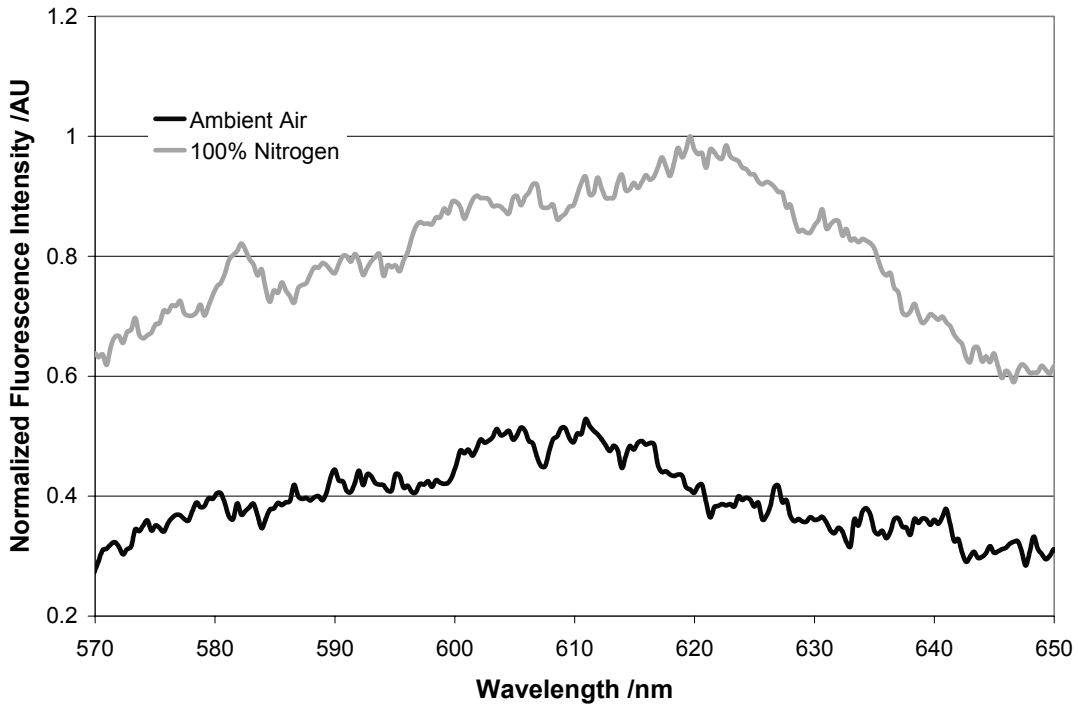


Figure 6. Oxygen concentration response spectra of the immobilized dye

The system was also examined using a flood 450nm light source, and although the overall fluorescence intensity was very low, visible low-level fluorescence was observed along the inside surfaces of the entire channel region. This observation confirmed the success of the surface modification by the aminosilane and subsequent self-assembly of the interpolyelectrolyte complex, as well as the capability of the PDMS waveguides to efficiently conduct low-level light intensities. However, since the fluorescence intensity at the detector was and the signal-to-noise ratio were both low, we allowed the spectrometer sufficient time to stabilize and averaged of 6 consecutive readings for the gas testing and 10 consecutive readings for the glucose testing. This procedure yielded significant differences in glucose concentration readings to produce a glucose sensitivity plot, shown in Figure 7. Due to time limitations, only a single glucose concentration test was possible, but the difference in intensity with concentration is apparent.

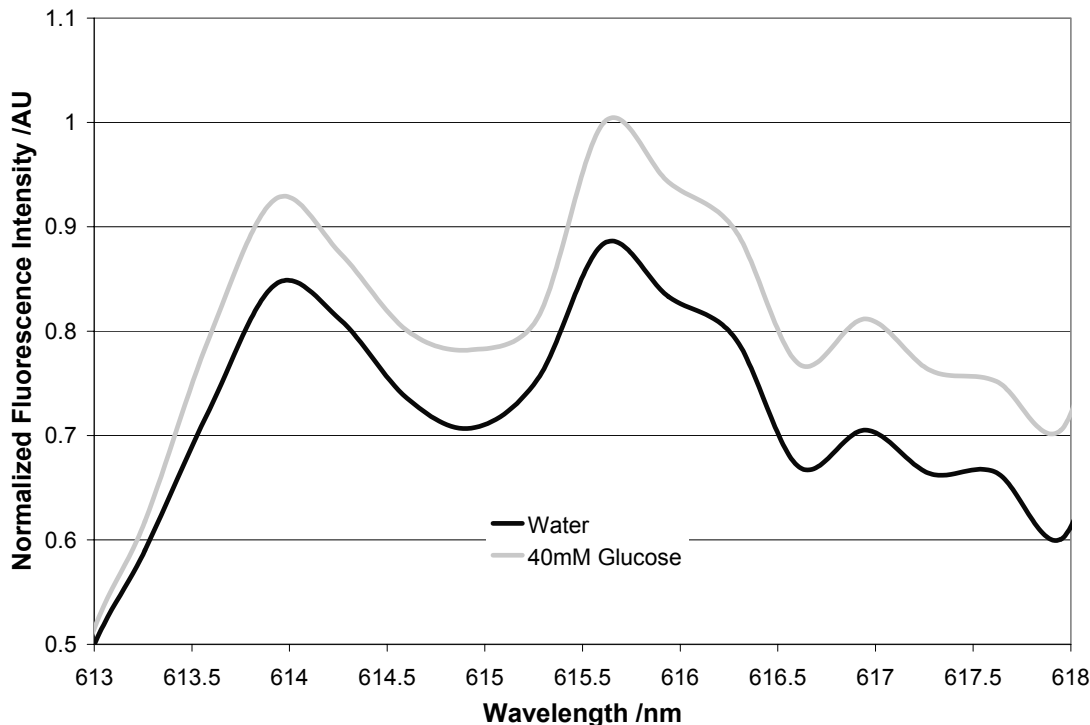


Figure7. Normalized spectra for glucose sensitivity response

5. CONCLUSIONS

An optically-based PDMS waveguide glucose sensor was successfully produced and tested, demonstrating that the methodology of thermally-defined PDMS waveguides on a PDMS substrate for integrated optical sensors is viable. Although usable readings were produced by the sensor for the purpose of this study, the overall sensitivity was generally too low for effective use. The low fluorescence intensity is thought to be caused by both the failure of the vane demolding and a general lack of sensitivity of the spectrometer. Both of these issues can be resolved, the former by using a higher quality SU-8 exposure light source, and the latter by substituting a high-sensitivity detector such as a photomultiplier tube or an avalanche photodiode for the spectrometer. Further work is currently being performed to investigate variations of this sensor system in an effort to improve the sensitivity.

REFERENCES

1. P.-A. Auroux, D. Iossifidis, D. R. Reyes and A. Manz, *Anal. Chem.*, **74**, 2637-2652, 2002
2. S. Camou, H. Fujita and T. Fuji, *Lab on Chip*, **3**, 40-45, 2003
3. S. Aubonnet, H. F. Barry, C. von Bültzingslöwen, J.-M. Sabattié and B. D. MacCraith, *Electron. Lett.*, **39**, 913-914, 2003
4. J. M. Ruano, A. Glidle, A. Cleary, A. Walmsley, J. S. Aitchison and J. M. Cooper, *Biosens. and Bioelec.*, **18**, 175-184, 2003
5. B. G. Splawn and F. E. Lytle, *Anal. Bioanal. Chem.*, **373**, 519-525, 2002
6. D. Neuschäfer, W. Budach, C. Wanke and S.-D. Chibout, *Biosens. Bioelec.*, **18**, 489-497, 2003
7. T. Bachinger, U. Reise, R. K. Eriksson and C.-F. Mandenius, *Biosens. Bioelec.*, **17**, 395-403, 2002
8. P. M. Schmidt, C. Lehmann, E. Mathes and F. F. Bier, *Biosens. Bioelec.*, **17**, 1081-1087, 2002
9. I. Moser, G. Jobst and G. A. Urban, *Biosens. Bioelec.*, **17**, 297-302, 2002

10. E. Brynda, M. Houska, A. Brandenburg and A. Wikerstål, *Biosens. Bioelec.*, **17**, 665-675, 2002
11. P. S. Petrou, I. Moser and G. Jobst, *Biosens. Bioelec.*, **17**, 859-865, 2002
12. L. D. Williams and S. Blair, *Proceedings of the SPIE*, **4982**, 156-161, 2003
13. D. Chang-Yen and B. Gale, *Lab on a Chip*, 2003, DOI:10.1039/B305358J
14. R. Horváth, L. R. Lindvold and N. B. Larsen, *J. Micromech. Microeng.*, **13**, 419-424, 2003
15. O. J. A. Schueller, X.-M. Zhao, G. M. Whitesides, S. P. Smith and M. Prentiss, *Adv. Mater.*, **11**, 37-41, 1999
16. J. W. Grate, S. L. Rose-Pehrsson and D. L. Venezky, *Anal. Chem.*, **65**, 1868-1881, 1993
17. A. L. Jenkins, O. M. Uy, and G. M. Murray, *Anal. Chem.*, **71**, 373-378, 1999
18. J. Wang, M. Pumera, M. P. Chatrathi, A. Escarpa and M. Musameh, *Anal. Chem.*, **74**, 1187-1191, 2002
19. M. Alstein, G. Segev, N. Aharonson, O. Ben-Aziz, A. Turniansky and D. Avnir, *J. Agric. Food Chem.*, **46**, 3318-3324, 1998
20. A. Mulchandani, P. Mulchandani, W. Chen, J. Wang and L. Chen, *Anal. Chem.*, **71**, 2246-2249, 1999
21. T. Inoue, J. R. Kirchhoff and R. A. Hudson, *Anal. Chem.*, **74**, 5321-5326, 2002
22. D. Chang-Yen, Y. Lvov, M. McShane and B. Gale, *Sens. Actuators, B*, **87**, 336-345, 2002
23. O. S. Wolfbeis, *Anal. Chem.*, **74**, 2663-2678, 2002
24. T. E. Plowman, J. D. Durstchi, H. K. Wang, D. A. Christensen, J. N. Herron and W. M. Reichert, *Anal. Chem.*, **71**, 4344-4352, 1999
25. P. Thiébaud, L. Lauer, W. Knoll and A. Offenhäusser, *Biosens. Bioelec.*, **17**, 87-93, 2002
26. J. R. Anderson, D. T. Chui, R. J. Jackman, O. Cherniavskaya, J. C. McDonald, H. Wu, S. H. Whitesides and G. M. Whitesides, *Anal. Chem.*, **72**, 3158-3164, 2000
27. M. Fang, P. S. Grant, M. J. McShane, G. B. Sukhorukov, V. O. Golub and Y. M. Lvov, *Langmuir*, **18**, 6338-6344, 2002
28. T. Wu, K. Efimenko and J. Genzer, *Macromol.*, **34**, 684-686, 2001
29. M. Colic, A. Chien and D. Morse, *Croatica Chem. Acta*, **71**, 905-916, 1998