Introduction

Recently, a surge of research activities has focused on development of novel cost-effective and field-portable biosensors for environmental monitoring and bioprocess monitoring. Among them, enzymatic biosensors are widely implemented and many enzymatic biosensor designs based on conductometric transducers, potentiometric transducers, amperometric transducers, thermal transducers and fiber optics have been reported. Among these, photoluminescent fiber-optic biosensors possess attractive qualities such as flexibility, reversibility and resistance to electrical noise. However, incorporating a bio-analytical component and indicator dye at the end of an optical fiber is mechanically complicated by the fiber’s small diameter and non-planarity. Moreover, end-of-fiber sensors exhibit substantial reflection of excitation light requiring the use of expensive optical filters, increasing the design cost. Hence, there exists a need for mass-producible, economical, low excitation interference enzymatic biosensor devices that also exhibit the advantages of photoluminescence-based fiber optic sensors.

A plastic total internal reflection photoluminescence device for enzymatic biosensing

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A total internal reflection photoluminescence (TIRPh) device employing an easily fabricated PMMA/PDMS waveguide system provides a detection limit comparable to the best reported results but without using an excitation filter. The optical mechanism is similar to total-internal-reflection-fluorescence (TIRF) but uses a ruthenium-based phosphorescent dye (Ru(dpp))3 deposited on the PMMA core, motivating the generalized term of photoluminescence to include both fluorescence and phosphorescence. An enzymatic hydrogen peroxide (H2O2) biosensor incorporating catalase was fabricated on the TIRPh platform without photolithography or etching. The O2-sensitive phosphorescence of Ru(dpp)3 was used as a transduction mechanism and catalase was used as a biocomponent for sensing. The H2O2 sensor exhibits a phosphorescence to scattered excitation light ratio of 76 ± 10 without filtering. The unfiltered device demonstrates a detection limit of (2.2 ± 0.6) μM with a linear range of 0.1 mM to 20 mM. The device is the first total internal reflection photoluminescence based enzymatic biosensor platform, and is promising for cost-effective, low excitation interference, field-portable sensing.

Total internal reflection (TIR) configured photoluminescence biosensors reduce excitation interference when photoluminescence is collected perpendicular to the waveguide. Planar waveguide-based TIR biosensors have been reported and typically incorporate optical components such as a lens or prism to properly couple the excitation light as well as fluidics for supplying reagents/analytes for sensing. The alignment requirements of optical components and fluidics increase the complexity of system integration. Conventional TIR-based biosensors used glass materials for optical waveguides. However, waveguides made of glass materials may be more costly and fragile than polymer materials. Recent designs of TIR-based biosensors were made using microfabrication techniques including photolithography and etching with elastomer-based fluidics and semiconductor, or polymer-based optics. Fabrication techniques such as photolithography and etching require expensive microfabrication equipment. Chronis et al. reported detection of fluorescent beads using their device, while the device of Huang and Tseng incorporated an immunoassay. Chang-Yen et al. did not employ photolithography or etching in spin coating a SU-8 slab waveguide core on a glass slide to realize a fluorescence based oxygen sensor. However, excitation and fluorescence collection shared the same in-plane optical path, resulting in collecting residual excitation that was many times the fluorescence power. This paper reports the first plastic total internal reflection photoluminescence (TIRPh) enzymatic biosensor fabricated without photolithography or etching. The device comprises a
PMMA optical waveguide core and PDMS cladding and molded fluid cell. The use of directly coupled fiber optics simplifies excitation and collection optics and does not require lenses. The fluid cell and fiber openings are created with macroscopic molds, and the slab geometry requires no lithographic patterning. The dye and enzyme layers are deposited on the PMMA core using common chemistry lab tools and techniques. Using these simplified approaches, an enzymatic hydrogen peroxide sensor is demonstrated.

Experimental

Design of the TIRPh device

The TIRPh enzymatic biosensor uses waveguided excitation light to excite a photoluminescent dye. The propagation axis of the excitation light is orthogonal to the emission collection direction, which reduces excitation interference with the photoluminescence signal compared to coaxial fiber optic and coplanar slab waveguide configurations.

A schematic of the TIRPh enzymatic biosensor is presented in Fig. 1. The periphery of the device, noted as Region 2 in the figure, consists of a symmetric slab waveguide with a high index core (polymethyl methacrylate – PMMA) and lower index cladding (polydimethylsiloxane – PDMS). The upper PDMS piece surrounds a fluid chamber in the interior where a photoluminescence dye layer is deposited on the top surface of the core. The sample fluid serves as the upper cladding in the interior. The dye layer behaves as either a conventional cladding or anti-guiding cladding depending on its precise refractive index, which is close to that of PMMA. Excitation light coupled to the edge of the waveguide through a multimode fiber creates a long decay length evanescent field or a shallow angle propagating wave in the interior dye layer. Upon excitation, the dye layer isotropically emits photoluminescence, a portion of which is directed to an optical detector without an intervening filter. A biocomponent enzyme layer immobilized on top of the dye layer catalyzes a reaction of analyte molecules in solution to produce (or consume) particular molecules in a manner that is proportional to the analyte concentration. These “reporter” molecules reduce the photoluminescence of the dye layer in relation to their concentration, generating the optical signal for sensing. The photoluminescence strength is measured by a photodetector to indirectly measure the analyte concentration.

Material selection, low cost methods of waveguide and fluidics fabrication, and efficient deposition of dye and enzyme layers are key features of the plastic TIRPh biosensor design.

Material selection for the TIRPh device

The TIRPh device is made of two materials with high optical transmittance, low autofluorescence and different refractive indices; PMMA for the waveguide core and PDMS for cladding as shown in Fig. 1.

Thermoplastic materials possess the advantageous properties of ease of fabrication, low cost, and shock resistance over glass materials with sufficiently comparable optical properties.11,12 Also, thermoplastic materials are easily polished to obtain optically smooth surfaces and edges. These properties make the thermoplastic materials suitable for the waveguide core if they have large enough refractive index, \( n_1 \), compared to the cladding material.

A second material with refractive index \( n_2 \) is used as a cladding material as well as defining the fluidic channels. It should be easily shaped and possess optically smooth surfaces at the core-cladding interface. Silicon-based elastomers, such as PDMS, possess excellent mold-ability13 and chemical resistance.14 Fluidic channels of various sizes and shapes can be molded in PDMS more easily than any thermoplastic. These properties make PDMS the most suitable material for cladding and making fluidics. The refractive index of PDMS is 1.47 to 1.43 over the visible spectrum. Choosing PDMS as the material for the cladding makes it imperative to choose the waveguide core material with refractive index larger than that of PDMS.

Refractive index and transmittance values for some candidate thermoplastic materials such as styrene acrylonitrile (SAN), polymethyl methacrylate (PMMA), polycarbonate (PC), polystyrene(PS), cyclic olefin copolymer (COC-5013LS) from Topas and cyclic olefin polymer (COP-480R)-Zeonex and PDMS appear in Table 1 for three wavelengths in the visible spectrum. All the materials listed in Table 1 have refractive

| Table 1 Refractive index values (at wavelengths of 481.6 nm, 587.6 nm, 656.3 nm) and optical transmittance (for 2-mm thick sheets) of several thermoplastics and PDMS |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Refractive index | Transmittance (2 mm) |
| 486.1 nm | 587.6 nm | 656.3 nm |
| PMMA16,17 | 1.4973 | 1.4914 | 1.4880 | 92% |
| COC16,17 | 1.5399 | 1.5332 | 1.5304 | 91.2% |
| PC16,17 | 1.5994 | 1.5849 | 1.5782 | 88% |
| SAN16,17 | 1.578 | 1.567 | 1.5616 | — |
| PS16,20 | 1.6056 | 1.5917 | 1.5853 | 90% |
| COP18 | 1.5317 | 1.5251 | 1.5224 | — |
| PET19 | ≈1.57 to 1.54 | — | — |
| PDMS15 | ≈1.47 to 1.43 | — | — |
index values greater than that of PDMS. PMMA and COC have the highest known transmittance of the plastics listed.

Some plastics exhibit high background fluorescence which can confound measurements of dye fluorescence. Piruska et al.\textsuperscript{21} studied the autofluorescence magnitude and dynamics of Borofloat glass, several plastic materials and associated plastic chips at four different laser excitation wavelengths; 403 nm, 488 nm, 532 nm and 633 nm. They found the autofluorescence of the plastic materials higher than the Borofloat. Significantly higher fluorescence magnitudes were found for 403 nm and 488 nm excitation. The auto-fluorescence magnitude was found to be reduced for longer excitation wavelengths. PDMS and PMMA exhibited the lowest autofluorescence magnitudes. Due to their high optical transmittance and low autofluorescence, PMMA ($n_1 = 1.49$) and PDMS ($n_2 = 1.44$) were selected as the most appropriate materials for the TIRPh biosensor device. The predominantly polyurethane elastomer dye layer has an estimated refractive index of 1.46 to 1.52.\textsuperscript{22–24}

Materials

PMMA slabs were purchased from a local plastics vendor, which cut them to desired dimensions using a CO\textsubscript{2} laser. A silicone elastomer (PDMS) kit Sylgard 184 was purchased from Dow Corning, and base and curing agent were used in 10:1 proportions. Hydrophilic thermoplastic polyurethane elastomer pellets with 15% water absorption capacity were purchased from AdvanSource Biomaterials. Professional grade acrylic adhesive (IPS Weld-On 3, liquid) was purchased from a retail vendor. Phosphorescent indicator dye Ru(dpp)\textsubscript{3} (tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride), 3-APTES (3-aminopropyl triethoxysilane), aqueous suspension of catalase from bovine liver, 99.5% glycerol, 25% aqueous glutaraldehyde, phosphate buffer saline powder (pH 7.4), albumin from bovine serum in lyophilized powder form and 30% (w/w) H\textsubscript{2}O\textsubscript{2} solution were purchased from Sigma Aldrich.

Fabrication of the TIRPh device

The TIRPh device (Fig. 2(c)) is fabricated by irreversibly bonding molded PDMS upper and lower cladding pieces to a surface modified PMMA core slab. The assembly of the PDMS molds and bonding protocols are described in detail below.

Acrylic molds and molding of PDMS cladding pieces.

A method for making novel acrylic molds was developed to fabricate relatively thick (few mm) features. Molds were created by joining different sized and shaped acrylic blocks cut to size from a 0.090 ’’ (\approx 2.2 mm) thick PMMA sheet by a commercial plastic supplier using a CO\textsubscript{2} laser. Two acrylic blocks with dimensions of 5 mm $\times$ 15 mm $\times$ 2.2 mm (base) and 25 mm $\times$ 35 mm $\times$ 2.2 mm (chamber) served as a mold for the PDMS upper cladding piece, and three acrylic blocks of 1 mm $\times$ 5 mm $\times$ 2.2 mm (excitation fiber-groove), 15 mm $\times$ 25 mm $\times$ 2.2 mm (waveguide-core socket) and 25 mm $\times$ 35 mm $\times$ 2.2 mm (base) were assembled to mold the PDMS lower cladding piece. A semi-transparent tape was applied on the bottom surface of each of the 25 mm $\times$ 35 mm $\times$ 2.2 mm base blocks and marked with a pattern to indicate the position for gluing the corresponding blocks on the top surface. After placing blocks on the top surface of each base block, a commercial acrylic adhesive (IPS Weld-On 3, liquid) was applied at the edges of the smaller block using a pipette so as to be drawn into the entire interface between the blocks by capillary action. The adhesive was cured for 30 minutes at room temperature before the alignment tapes were peeled off the base blocks, and the molds were ready to use, as shown in Fig. 2(a)(i) and Fig. 2(b)(i).

The molds were used to form matching upper and lower cladding pieces using conventional methods for 5 mm think PDMS. Uncured PDMS mixture (Sylgard 184 in 10:1 ratio) was poured over the molds in a petri dish and was cured at room temperature for 24 hours. The cured PDMS claddings were peeled off the molds. Fig. 2(a)(ii) and Fig. 2(b)(ii) show the resulting PDMS upper and lower cladding pieces.

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Three 1-mm diameter holes were punched in the ceiling of the fluid cell of the PDMS upper cladding.

The width of 5 mm and the length of 15 mm for the fluid cell were chosen to keep the holes at least 1 mm away from one another and the walls, allowing for 1 mm alignment tolerances and for tolerances in the positions of the deposited dye-enzyme spot and holes. The height of the fluid cell was chosen to be approximately 2 mm to allow enough space between the core surface and the fluid cell ceiling to easily deposit the dye-enzyme layer using a pipette tip inserted through the collection fiber hole.

**PDMS-PMMa and PDMS-PDMS bonding.**

Following PDMS molding, a PMMA core slab of size 15 mm × 25 mm × 2.2 mm was cut and coated with a self-assembled layer to facilitate PDMS-PMMA bonding. After CO2 laser cutting of the PMMA, which left smooth edges so that polishing was not required, the PMMA slab was treated with O2 plasma using a micro RIE plasma chamber at 200 W RF power, 50 sccm O2 flow rate and 50 mTorr pressure for 60 seconds. Immediately after plasma chamber venting, the slab was immersed in activated 5% 3-APTES (3-aminopropyl triethoxysilane) solution for 1 minute to create a self-assembled layer. The solution was activated by heating it at 85 °C for 5 minutes. The slab was kept at 65 °C for 10 minutes after immersion in order to stabilize the self-assembled layer on all sides of the PMMA slab.

Next, the PMMA core slab with the self-assembled layer was first bonded to the PDMS upper cladding, and that assembly was subsequently bonded to the lower PDMS cladding. Just prior to bonding, the slabs were treated by air corona discharge using a Plasmaline chamber at 50 W RF power and 0.9 Torr air pressure for 60 seconds. Immediately after venting, the treated surfaces of the slabs were brought together in firm contact for 30 seconds to obtain irreversible bonding between them. Corona discharge is believed to produce active sites on the surfaces temporarily to provide very strong means for irreversible bonding. The bonded PDMS-PMMa-PDMS configuration featured a groove to hold the excitation fiber firmly at the desired position aligned against the PMMA core slab, which was fixed in a PDMS socket.

**Indicator dye deposition**

After assembly of the PMMA and PDMS pieces, a phosphorescent dye, Ru(dpp)$_3$, was deposited on the core surface. A hydrophilic thermoplastic polyurethane elastomer (AdvanSource Biomaterials) was used to attach the Ru(dpp)$_3$. The elastomer has the ability to rapidly absorb water while maintaining high tensile strength and high elongation, resulting in a permanently lubricious polymer, which makes it biodurable and biocompatible, and hence appropriate to facilitate depositing dye in biosensor applications. The elastomer pellets were dissolved in chloroform to produce 5% (w/v) solution (Solution A). Ru(dpp)$_3$ dye was dissolved in chloroform to produce 1.5% w/v solution (Solution B). Solutions A and B were mixed in a 1:1 proportion to make the deposition solution. A 3-μL volume of the deposition solution was pipetted onto the core surface through the collection fiber hole of the assembled TIRPh device. The chloroform evaporated in approximately 10 minutes after deposition, leaving a film of the elastomer firmly attaching the dye to the PMMA core surface.

**TIRPh based enzymatic hydrogen peroxide (H$_2$O$_2$) sensor using catalase**

The utility of the TIRPh device as an enzymatic biosensor was investigated by combining it with catalase to create an enzymatic H$_2$O$_2$ sensor. Hydrogen peroxide was chosen as a benchmark analyte for purposes of comparison because many papers have reported enzymatic and non-enzymatic H$_2$O$_2$ sensors. The H$_2$O$_2$ sensor was made by depositing a layer of catalase enzyme on top of the Ru(dpp)$_3$ layer. The catalase causes catalytic decomposition of H$_2$O$_2$ molecules into H$_2$O and O$_2$ molecules. In this scheme, the quenched phosphorescence intensity due to the increase in catalyzed O$_2$ concentration correlates with the increase in H$_2$O$_2$ concentration.

Albumin from bovine serum (A9418, Sigma Aldrich) was used to immobilize the catalase enzyme on the deposited dye layer. In detail, a mixture of aqueous solutions of enzyme and albumin was crosslinked using an appropriate amount and concentration of glutaraldehyde and glycerol. The catalase-albumin solution was made by mixing 5 μL of catalase, 45 μL of BSA solution (56 mg ml$^{-1}$ of bovine serum albumin in water), 1.19 μL of glycerol, and 25 μL of a 2.5% aqueous glutaraldehyde. Immediately after mixing, 1.5 μL of the solution was pipetted onto the dye layer and was cured for 30 minutes at room temperature before it crosslinked to provide an attached enzyme layer.

**Experimental apparatus**

Spectral measurements of the TIRPh device and H$_2$O$_2$ biosensor were performed using a large numerical aperture, multi-mode optical fiber with large core diameter that has a higher light-gathering capacity than a single mode fiber. Moreover, it facilitates light coupling by decreasing sensitivity to alignment. Hence, a N.A. = 0.48 multi-mode fiber with a large core diameter (SH4001-1.3, 980 μm core diameter, Industrial Fiber Optics) was used as the collection fiber to collect the phosphorescence emitted from the immobilized dye. The proximal end of the collection fiber was held in the collection hole of the TIRPh device while the collected phosphorescence was directly delivered to a fluorescence spectrometer (USB4000-FL, Ocean Optics) through the ST-connected distal end of the collection fiber without any intervening optical filter. The same type of optical fiber was used to couple blue excitation light (470 nm) from an LED (Industrial fiber optics, IF92B) into the waveguide. The spectrometer was connected to a PC running data acquisition software SpectraSuite (OceanOptics). While a single detector would likely be employed in low-cost versions of the device, the spectrometer allows analysis of both excitation and emission.
light. Inlet and outlet tubes held in the upper PDMS piece were connected to a syringe containing PBS and a rinse collection beaker, respectively.

Method for measuring calibration curves and detection limit of the TIRPh enzymatic H$_2$O$_2$ sensor

Using the aforementioned apparatus with the TIRPh enzymatic H$_2$O$_2$ sensor, the spectrometer was set to record one spectrum every second. Each spectrum spanned the wavelengths from 400 nm to 850 nm to encompass both the desired phosphorescent red emission as well as the interfering blue excitation. In steady state, 60 spectra acquired during one minute were averaged and subsequently integrated from 400 to 850 nm to obtain a single data point for total optical power that consists of phosphorescence (integrated from 550 to 850 nm) and interfering scattered excitation (integrated from 400 to 550 nm).

The biosensor’s calibration curve was obtained by measuring and plotting the data points of total optical power for different concentrations of H$_2$O$_2$. The experimental procedure was designed to extract the device performance parameters including limit of detection, linear range, sensitivity, and response time from a single experiment. Phosphate-buffered saline (PBS) was used as a solvent to make analyte solutions for the blank measurements (to measure detection limit) and for the measurements of the calibration curve. Since the quenching of the Ru(dpp)$_3$ phosphorescence is O$_2$-sensitive, the PBS solution was made anoxic (devoid of dissolved O$_2$) before it was used to make analyte (H$_2$O$_2$) solutions. The catalyzed oxidation reaction of sodium sulphite (Na$_2$SO$_3$) in the presence of cobalt chloride (CoCl$_2$) was used to consume all dissolved O$_2$ in the PBS solution. CoCl$_2$ and Na$_2$SO$_3$ were added in PBS solution in 1:10000 and 1:1000 proportions, respectively. The dissolved O$_2$ content of the anoxic PBS solution was measured to be 1.0 ± 0.2 nM over 15 minutes using an O$_2$ probe from In-Situ Inc. The resulting anoxic PBS solution was used to make analyte (H$_2$O$_2$) solutions with different concentrations of 1.5 mM, 15 mM, 37.5 mM, and 150 mM. These solutions were sealed in air-tight vials immediately after they were made.

A 165-μL aliquot of the anoxic PBS solution was used to obtain blank measurements because the fluid cell has a volume of approximately 165 μL. A total of seven such measurements were performed, each time by flushing the fluid cell with 160 μL of anoxic PBS solution. For a blank measurement, 7 values of total optical power, each obtained from one minute of spectra acquisition, were averaged.

The concentration of H$_2$O$_2$ in the fluid cell was then increased in 5-μM steps by adding 0.5 μL of 1.5 mM anoxic H$_2$O$_2$ solution using a pipette for addition and mixing with the fluid in the chamber at each step. For each new step in concentration, the spectrometer trace was monitored to see if a perceivable (≥0.05%) decrement in the total optical power was found. A decrease of less than 0.05% was seen for 5 μM H$_2$O$_2$, which was not distinguishable from the blank. However, a decrease of greater than 0.05% was observed at 10 μM H$_2$O$_2$. Therefore, the H$_2$O$_2$ concentration corresponding to the first such perceivable decrement was 7.5 ± 2.5 μM, which is the detection limit based on the empirical method defined and used in literature.$^{34,35}$

After finding the empirical LOD, the H$_2$O$_2$ concentration was increased in approximately 20-μM steps by adding and mixing 2 μL of 1.5 mM H$_2$O$_2$ solution until the total concentration in the fluid cell was 200 μM. Then, the concentration was increased in approximately 200-μM steps by adding and mixing 2 μL of 15 mM H$_2$O$_2$ solution until the total concentration was 1 mM. Finally, the concentration was increased in 3-mM steps until no further change (decrease) in the total optical power with increase in concentration was found. For each step, 10 minutes was allowed for the total optical power to reach a steady state of a new decreased level. Normalized data points (I$_0$/I) for the total optical power were obtained by dividing the blank measurement (I$_0$) by the total optical power data points (I) and were plotted against the corresponding H$_2$O$_2$ concentrations to obtain calibration curves. The standard deviations in the normalized data points on the calibration curves were obtained from standard deviations in the corresponding blank measurement and optical power data points. Three iterations of the experiment were performed to obtain three calibration curves for one such H$_2$O$_2$ sensor.

Results and discussion

Excitation light coupling and scattering

As depicted earlier in Fig. 1, the PMMA core slab is conceptually divided into three region types, denoted as Region 2, Region 3 and Region 4. Region 2 has PDMS as cladding both on top and bottom of the core, Region 3 has the fluid in the fluid cell as the upper cladding and PDMS as the lower cladding, and Region 4 has the dye layer as the upper cladding and PDMS as the lower cladding. For all three regions, the lower cladding is PDMS. Thus, Region 2 is a symmetric planar waveguide, whereas Region 3 and Region 4 have asymmetric refractive index profiles. The symmetric waveguide of Region 2 serves as the initial coupling region. Given the PMMA core and PDMS cladding refractive indices of 1.49 and 1.44, the numerical aperture of the waveguide in the vertical direction is 0.38. Since the waveguide’s numerical aperture is smaller than that of the optical fiber (0.48), the acceptance cone of the waveguide is completely filled by the launch cone of the excitation fiber. This overfilled launch condition guarantees some light is coupled to higher order modes that have longer evanescent field decay lengths. However, the mismatched vertical N.A. of the multimode waveguide and multimode fiber still allow for a calculated 88% coupling efficiency, assuming uniform brightness within the fiber’s emission pattern, while providing a ±6° tilt tolerance. The 2.2 mm thickness of the PMMA core provides a ±0.6 mm vertical alignment tolerance for the 1 mm core diameter fiber. The core
thickness is restricted to the multimode regime by mechanical requirements.

To determine which regions of the waveguide might contribute to scattering of excitation light that could interfere with unfiltered detection of photoluminescence, a color picture of the TIRPh device with deposited Ru(dpp)₃ was taken using a digital camera. Then, the red Ru(dpp)₃ phosphorescence was digitally filtered from the picture using the "rgb2ind(6)" command in MATLAB. Fig. 3 is the resulting filtered picture of blue excitation light scattered from the device. It is evident that once the light is coupled into the waveguide, the strongest scattering of excitation light occurs at the Region 2 to Region 3 interface and the Region 3 to Region 4 interface. The scatter at the Region 3 to Region 4 interface is more critical, as it is closer to the collection fiber and thus more likely to interfere with the phosphorescence signal.

Interfacial scattering is caused by differences in the upper cladding refractive index in different regions, which leads to mismatches in evanescent field decay lengths. The index difference from an aqueous sample (n = 1.33) to either PDMS (n = 1.43 to 1.47) or the polyurethane based dye layer (n = 1.46 to 1.52) is greater than the index difference would be between PDMS and a dye layer, suggesting scattering could be further reduced by abutting the PDMS and dye layer.

Phosphorescence (P) to scattered excitation (S) ratio

To measure the relative amount of excitation light scattered into the collection fiber, spectra of the phosphorescence and the scattered excitation light carried by the collecting fiber were measured for three nominally identical TIRPh devices with Ru(dpp)₃ dye spots using the aforementioned apparatus. Fig. 4(a) shows the resulting spectra. The peaks at 630 nm in all the spectra correspond to the phosphorescence, while the peaks at 470 nm correspond to the scattered excitation light. The ratio (P/S) of the phosphorescence integrated from 550 through 850 nm to the scattered excitation light integrated over 400 to 550 nm was computed for each device and tabulated in Fig. 4(a).

Zhong et al. previously made oxygen optodes for toluene biosensors using the same dye material on the tips of optical fibers, but using a different deposition protocol. Three such optodes were reproduced and their spectra were measured using the same bifurcated fiber-based instrument used in that study but without using any filter. The resultant spectra and P/S ratios for all three optodes are shown in Fig. 4(b). The optodes exhibited an average P/S ratio of 0.2 ± 0.01. In contrast, the TIRPh device exhibited an average P/S ratio of 76 ± 10. This also demonstrates that the TIRPh configuration suppresses the scattered excitation light more than 350 times better than the optodes, which greatly relaxes the need for costly optical rejection filters in the TIRPh device.

Large P/S ratios both increase maximum allowed phosphorescence signal power and reduce optical noise yielding larger dynamic range and lower limits of metrology. An unfiltered photodetector must be able to provide a linear or at least calibrated response to a combined optical power of up to \( P_0 + S \), where \( P_0 \) is the maximum phosphorescence. This maximum power in the presence of scatter is \( (P_0 + S)/P_0 = 1 + (S/P_0) \) times larger than the phosphorescence alone. Hence, scatter reduces the allowed maximum optical power by a factor \( P_{\text{max}} = 1 + (S/P_0) \), which will reduce dynamic range by the same factor.

Shot noise serves as an optimistic model of the impact of scatter on optical noise. The mean-square shot noise current for a photodetector of responsivity \( R \) and bandwidth \( \Delta f \) is
Calibration curves and detection limit of the TIRPh enzymatic H$_2$O$_2$ sensor

Fig. 5(a) shows the calibration curves obtained for three iterations of a hydrogen-peroxide sensor as a function of H$_2$O$_2$ concentration. The vertical error bar for each data point on the calibration curves represents two times the standard deviation. The calibration curves exhibited nonlinearity for very small H$_2$O$_2$ concentrations as well as for relatively large values of H$_2$O$_2$ concentrations, as shown in the figure. The nonlinearity at higher concentrations is due to the maximum quenching of all the dye molecules. The nonlinearity at low H$_2$O$_2$ concentrations is associated with the nonlinear relationship between Ru(dpp)$_3$ phosphorescence and O$_2$ concentration. This relation is modeled using the two-site model of the Stern–Volmer equation.$^{37,38}$ Eqn (1) represents the two-site model:

$$I_0 = \frac{1}{I} = \frac{f_1}{1 + f_1 K_{SV1}[Q]} = \frac{f_2}{1 + f_2 K_{SV2}[Q]}$$  \hspace{1cm} (1)$$

where, $[Q]$ = quencher (O$_2$) concentration, $I_0$ = phosphorescence in absence of quenchers, $I$ = phosphorescence in presence of quenchers, $f_1$, $f_2$ = fractional contribution of dye layer site-1, site-2 to the total phosphorescence, and $K_{SV1}$, $K_{SV2}$ = Stern–Volmer sensitivities of site-1, site-2.

Fig. 5(b) shows the linear and non-linear (at small [H$_2$O$_2$]) portions of the calibration curves. Fitting parameters ($f_i$, $K_{SVi}$) and $R^2$ values of the trendlines fit to the curves are given in Table 2. These parameters are used to determine the analytical value of limit of detection. Accordingly, the limit of detection (LoD) for three iterations was found to be 1.7 μM, 2.9 μM and 2.1 μM. Therefore, the average value of limit of detection is 2.2 ± 0.6 μM. Also, the sensor exhibits a linear detection range of 0.1 mM to 20 mM.

The linear range and limit of detection values of the plastic TIRPh enzymatic hydrogen peroxide sensor were compared to those of enzymatic hydrogen peroxide sensors reported previously (Table 3).$^{37-31}$ The present device exhibits a statistically equivalent or better linear detectable range and limit of detection than these earlier published reports, even without an excitation filter. The TIRPh-based biosensor is expected to have sensitivities to the measurement environment that are similar to those of other enzymatic biosensors,
including to temperature, pH, salinity, competing substrates (usually with similar molecular structures), and specific inhibitory molecules. Catalase is a relatively specific enzyme with the only other reported substrate being methyl hydrogen peroxide. 39

Fluorophore stability can impact the long-term performance of photoluminescence-based sensors, and the temperature dependence of phosphorescence should also be considered. While the TIRPh device is not constrained to be used with any particular chemically-sensitive dye, both photobleaching and temperature affect the phosphorescence efficiency of the oxygen-sensitive Ru(dpp)3 used for this demonstration. Ru(dpp)3 emission power decreases with time due to photobleaching, 40 and its emission power has a notable and linearly decreasing relation with temperature. 41 However, other reports show that Ru-based luminescence sensors 42,43 give acceptable stability and photobleaching rates at nominal excitation power levels. Also, Ru-based luminescence sensors have measurement accuracy which is comparable to other reports 42,43 at nominal temperatures. For the measurements reported in this paper, the phosphorescence was observed to decrease by less than 2% over time.

Conclusions

The TIRPh device described here is the first published demonstration of an enzymatic biosensor based on a total internal reflection platform. The construction of the device is particularly simple and does not require photolithography or etching. Macroscopically molded PDMS serves as the waveguide cladding, defines fluid channels, and offers fiber alignment features. Lensless, direct coupling of a plastic optical fiber to the PMMA waveguide core simplifies optical source integration. Guided wave excitation along an axis well outside the photoluminescence collection cone greatly reduces interference from excitation light, which minimizes or eliminates the need for an excitation filter and in the future permits a cost effective on-chip or above-chip photodetector. The performance of the device can be further improved by reducing the differences in the upper cladding refractive index in different regions, which would further reduce the residual excitation scatter from cladding interfaces. The planar format lends itself to printing of multiple dye spots on a single PMMA core for a multiple analyte array sensor. Thus, the TIRPh device is very promising as a simple, cost-effective, low interference and miniaturized enzymatic biosensor.

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References