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Controlled Core-to-Core Photo-polymerisation – Fabrication of an Optical Fibre-Based pH Sensor

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The fabrication of fluorescence-based pH sensors, embedded into etched pits of an optical fibre via highly controllable, and spatially selective photo-polymerisation is described and the sensors validated.

Optical sensors, utilising the many advantageous properties of visible light, have been developed to measure a variety of analytes, such as O2, CO2, pH and glucose.1-5 Optical fibres have been widely used in this area, driven by the increasing demand for small, compact, and simple robust sensors that can be delivered to a location remote from the optical measurement apparatus. Many optical fibre-based sensors have been constructed by immobilising indicators at the distal end of an optical fibre, where the indicators interact with the analytes, resulting in measurable change in the optical properties.6-7 This is often achieved by removing the cladding from the optical fibre and it is replaced by a coating containing the specific indicator of choice.8,9 In some cases, the tips of the fibres have been chemically etched, and the indicator and/or a solid matrix deposited into the etched cavities.10,11

Several fluorescence-based optical sensors have been fabricated using photo-initiation methods, employing the discrete optical pathways found in optical fibres.12,13 The advantages of photo-induced polymerisation in controllable polymerisation are myriad, thus the polymer can be made in situ at room temperature, and patterned in defined locations.7,14 Importantly, using an etched fibre means that the polymers generated are firmly anchored into the fibre, whereas placing indicators within a polymer matrix, and dip-coating onto the fibre tips, while widely reported,13,15,16 can result in sensor leaching from the polymer matrices, poor attachment to the fibre itself, and photo-bleaching.6,16

Multicore optical fibres, due to their physical nature, offer a natural multiplexing potential. Walt has reported fibre-based photo-polymerisation by coupling light at the proximal end of an imaging fibre, thereby inducing photo-polymerisation of acryloyl fluorescein and 2-hydroxyethyl methacrylate, broadly on the illuminated area (ø = 125 μm) at the distal end of the imaging fibre, thereby creating sensing regions for pH analysis.7 This technique was expanded to the fabrication of a multi-analyte probe for pH, O2, and CO2, on a single imaging fibre (3000 individual elements), with discrete sensing areas (ø = 27 – 47 μm).14

To date, no work has described the fabrication of an optical fibre-based sensor with discrete sensing cores within a multi-mode multi-core fibre. Herein, we report the fabrication of a novel, sensitive, and robust (physically and optically) fluorescence-based pH sensor by photo-polymerising fluorescein acrylate co-polymers specifically at individually illuminated cores (with 405 nm illumination), which had been chemically etched into pits by hydrofluoric acid. The use of polymerisable fluorescent monomers overcomes the dye leaching problem,17-21 improves sensor photo-stability, while allowing photo-polymerisation directly into an etched core, thereby securing the location of the sensor, resulting in enhanced fluorescence, and robust attachment. The performance of the pH sensors was shown to be robust.

The general approach used for photo-polymerisation at the tip of the optical fibre is shown in Figure 1. The optical fibres used in this work were multi-mode multi-core, consisting of 19 cores (diameter = 20 μm), made up from germanium-doped silica, and surrounded by a pure silica cladding. Each optical fibre was cut to 1 m length, with a 5 cm length of coating at each side removed, and followed by cleavage of each end (~1cm) to give a smooth flat surface. One end of the optical fibres (distal end) was chemically etched in hydrofluoric (HF) acid, which removed the Ge-doped silica that formed the cores to form a cavity (or a pit) on the distal surface (Figure 1a-b).22 The etched fibres were then silanized with 3-(trimethoxysilyl)propyl methacrylate (TMSPMA) (20 v/v% in acetone), thereby introducing acrylate groups into the etched pits on the fibres. A 405 nm laser was used for polymerisation as it was compatible with the absorbance spectrum of the photo-initiator, diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO), a common dental photo-initiator that absorbs in the UV region up to about 425 nm (Figure 1c).23-25 To ensure uniform and controlled photo-polymerisation, the laser source was used at low power levels so that only a single core of the 19 cores was illuminated. Once coupled, the distal end of the fibre was placed into the polymerisation solution with the irradiation time and the laser power optimized for efficient photo-polymerisation. The polymerisation solution consisted of pH-sensitive fluorescein O-methacrylate (10 mol%), poly(ethylene glycol) diacrylate (PEGDA) (87 mol%) as a cross-linker, and the photo-initiator TPO (3 mol%) in N,N-dimethylacetamide (DMA) solvent. The head-on and side view of the optical set-up used for photo-polymerisation and fluorescence measurement are shown in Figure S1.

Figure 2 shows the top view of the optical fibres after photo-polymerisation with fluorescein O-methacrylate. Too higher power, and/or long irradiation times caused large bulbous polymers to form that extended beyond a single pit, covering, in extreme cases, the whole distal face of the fibre (Figure S2). Using an illumination power of 100 μW (405nm laser) and a 5 seconds illumination time, the polymerisation could be controlled, with filling of the pit, of a single core (Figure 2a-b). By coupling the laser to only one core at a time during the photo-polymerisation process, individual polymers could be grown at different cores, producing a sensor with multiple sensing elements. Figure 2c shows the top view of an optical fibre, containing three fluorescein O-methacrylate-based polymers.
Figure 1. (a) - (b) The side view images of an etched optical fibre. The pit’s depth was approximately 30 \( \mu \text{m} \). The distal end of the etched optical fibre was HF-etched and silanized in 3-(trimethoxysilyl)propyl methacrylate (TMSPMA) (20 % in acetone) to introduce acrylate groups onto the distal surface of the pit. (c) The absorbance spectrum of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) (2.5 mM in ethanol) (black line), and the 405 nm laser spectrum (blue line). TPO absorbs in the UV range up to about 425 nm, and although not optimal, it allowed sufficient excitation and radical generation by TPO (it is worth noting that the light source used by dentist with this initiator are typically in the wavelength range of circa 380 to 520 nm (dual peak LED). (d) The method used for photo-polymerisation at the distal end of the optical fibre. Only seven (of the 19) cores of the optical fibre are shown for clarity. The laser was coupled to a specific core of the fibre via an XYZ translational stage. Once coupled, the distal end was dipped into the polymerisation solution containing fluorescein O-methacrylate (10 mol%), PEG-diacylate (87 mol%), and TPO (3 mol%).
A 485nm laser was coupled to the “polymer cores” using a low excitation power (1.0 µW) to minimize the effects of photo-bleaching, with the photo-polymerised polymers showing a strong and robust pH dependent fluorescence, when pH > 6. The pH response of the sensor between pH 3 and pH 10 is shown in Figure S3. Sensor analysis between pH 6.6 and 7.6 was measured in 0.1 increments (in random order) with three calibration points (pH 6, 7, and 8) (Figure 2d). Analysis of the integrated fluorescence spectra showed little sign of photo-bleaching when the sensor was continuously illuminated in air or PBS for three minutes with the 485nm laser operating at 1.0 µW (Figure 2e). Randomised measurements in the 6.6 to 7.6 range were generally within error of the trend from the calibration data – although with some variation between the three replicates (Figure S4), presumably from power variations in the laser. Analysis of the sensors response time (measured by measuring changes in fluorescent intensity following movement of the sensor from pH 6 to pH 8 and back to pH 6 gave a response time of 31±3 seconds (Figure S5).

In conclusion, an optical fibre-based pH sensor with discrete sensing cores was prepared. By combining photo-polymerisation and the discrete optical pathways of optical fibres, fluorescein-based polymers could be fabricated at individually irradiated cores. This allowed easy identification of the specific sensing cores for measurement and analysis, while the use of etched fibres allowed the fabrication of robust optical sensors. Furthermore, this technique allowed three polymers to be grown individually at three distinct cores, showing the potential of fabricating of a multi-analyte sensor.

Fluorescein O-methacrylate-based polymers at the irradiated

Figure 2. Photo-polymerisation of fluorescein O-methacrylate and PEG diacrylate at the end of the optical fibre. (a) Image of the distal surface of an optical fibre, containing one fluorescein polymer core in the middle core (white circle) (b) The polymer fluoresced green under 488 nm excitation (white circle). (c) The distal surface of an optical fibre showing three fluorescein polymers (labelled 1, 2, and 3) at different cores. The polymers were grown individually, with defined core-to-core photo-polymerisation, with fresh polymerisation solution used for every polymerisation. The polymer labelled 1 distorted during photo-polymerisation thus extending to the nearest core. The polymers generated filled the depth of the pits, thus giving polymer features of approximately 30 µm in depth (d) The integrated fluorescence intensity of the fluorescein O-methacrylate-based sensor at 0.1 pH increments from pH 6.6 to 7.6 (Figure S4), with the three calibration points (pH 6, 7, and 8) (n = 2) (R² = 0.99). The fluorescence measurements of 0.1 pH increments were in random order, while the calibration points were in ascending order. (e) The integrated fluorescence intensity of the fluorescein polymer with continuous illumination for 180 seconds in PBS (pH 7.4) (blue circles) and in air (red squares), showing the photo-stability of the sensor. Very little evidence of photo-bleaching was observed. In a realistic usage environment, discreet measurements would be performed, rather than the continuous illumination demonstrated here (180 seconds continuous exposure). The small variation of the intensity may also be attributed to the intensity fluctuations in the excitation source. All measurements were made in a dark room with excitation wavelength = 485 nm, with the laser operating at 1.0 µW.
cores showed robust pH dependence under physiological conditions with good repeatability. The use of an etched core enabled good attachment of the polymer, making the sensor package more robust in comparison to non-etched (flat-faced) optical fibres, where there is a risk that polymer detaches from the distal end. Future work will focus on the developing of a dual emission pH optical sensor to allow an internal reference to be added to the system, to produce more reproducible optical pH sensors.

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Notes and references
† The fluorescence set-up used a laser emitting at 485 nm (operated at 1.0 µW with a >510 nm band-pass filter). Measurements were performed by manually opening a shutter and recording the fluorescence spectrum with an integration time of 1 second. The manual shutter was closed between measurements to avoid any unnecessary photo-bleaching.
§ pH 6 - 8 solutions were prepared using potassium dihydrogen phosphate (KH₂PO₄, 0.1 M), sodium hydroxide (NaOH, 0.1 M), and distilled water. The pH values were measured using a glass-electrode pH meter (Mettler Toledo), and where necessary adjusted using the appropriate acid or base.

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Controlled Core-to-Core Photo-polymerisation – Fabrication of an Optical Fibre-Based pH Sensor

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Content:

Figure S-1 – The optical set up for photo-polymerisation and fluorescence measurement

Figure S-2 – Photo-polymerisation optimisation of fluorescein O-methacrylate and PEG diacrylate at the distal end of etched-silanized optical fibres

Figure S-3 – The pH response from pH 3 to pH 10.

Figure S-4 – The three replicates of integrated fluorescence spectra of the fluorescein O-methacrylate-based sensor at 0.1 pH increments from pH 6.6 to 7.6

Figure S-5 – The time response study between pH 6 and pH 8.

Experimental

- Chemicals
- Optical Fibre
- Optical Instruments
- Sensor Fabrication
- Absorbance and Fluorescence.
Figure S-1. The top view (a) and side view (b) of the optical set-up for photo-polymerisation and fluorescence measurement. Table S1 shows the list of the components used to assemble the set ups.

Table S-1. List of the components used to assemble the optical set-ups.

| No. | Component                                                                 |
|-----|---------------------------------------------------------------------------|
| 1   | Single mode fibre patch cable (1m, 405 – 532nm)                           |
| 2   | 5 mm travel XY translation mounts, connected to Z-axis translation mount |
| 3   | Molded glass aspheric lenses (350 – 700nm broadband antireflective (AR) coating) |
| 4   | 30 mm cage system – includes rods, and adaptors for fibres and lenses    |
| 5   | Beam-splitter with dichroic mirror and bandpass filters                   |
|     | For 485 nm laser, the bandpass filter has the cut-on wavelength > 510 nm  |
| 6   | Bare fibre terminator                                                    |
| 7   | Compact USB 2.0 CMOS cameras (resolution of 1280 X 1024 pixels, color sensor), connected to a display |
| 8   | Spectrometer collection (Ocean Optics USB2000+VIS-NIR-ES).               |
Figure S-2. (a) – (c) Photo-polymerisation of fluorescein O-methacrylate and PEG diacrylate into the etched-silanized optical fibres with variation of laser power, time of irradiation, and polymerisation solution composition: (a) 2.1 mW and irradiated for 1 minutes – large global polymer deposition (polymerisation solution consisted of fluorescein O-methacrylate (1 mol%), TPO (1 mol%) and PEG-diacrylate (98 mol%)). (b) 3.0 mW and irradiated for 5 seconds. (c) 1.1 mW and irradiated for 5 seconds. (d) 100 μW and irradiated for 5 seconds. Fresh polymerisation solution used for every polymerisation (the polymerisation solution (for b – d) consisted of fluorescein O-methacrylate (10 mol%), TPO (3 mol%) and PEG-diacrylate (87 mol%)).
**Figure s-3.** The pH response of the fluorescein-based sensor from pH 3 to pH 10 (n = 3). The sensor was excited at 485 nm, operating at 1.0 µW, with 500 ms pulses. The black dotted line showing the trend for the whole pH titration, and the red dotted line showing the linear trend from pH 6 to pH 8. The linear trend from pH 6 and pH was plotted by considering only the data with their standard deviations at those pH’s only (pH 6 – pH 8).
Figure S-4. The three replicates of integrated fluorescence spectra of the fluorescein o-methacrylate-based sensor at 0.1 pH increments from pH 6.6 to 7.6. For pH 6.6 to 7.5, all three points (black, red, and blue) were used to calculate the average total fluorescence intensity at each pH. Due to the anomaly, black point (Replicate 1) of pH 7.6 was not used to calculate the average fluorescence intensity at pH 7.6.
Figure s5. The time response of the sensor was found to be 31 + 3 s. Excitation 485 nm, 1.0 µW, 500 ms pulse triggered system.
Experimental.

**Chemicals.** All chemical were purchased from Sigma Aldrich Co. and were used without further purification, unless otherwise stated. Di-styryl fluorescein was previously synthesized by members of Bradley Group.1

**Optical Fibre.** The multicore fibre was fabricated using the “stack and draw” process commonly used to fabricate photonic crystal fibres.2 Initially, a Ge-doped optical fibre preform (ø = 32 mm, numerical aperture = 0.3, Draka-Prysmian) with a parabolic refractive index profile and a thin pure silica jacket was drawn down to rods (ø = 5.75 mm). To increase the core-to-core separation in the final fibre, each of the rods was then further jacketed with a pure silica tube (outer ø = 10 mm), and drawn down again to 19 rods (ø = 2.4 mm). The rods were then stacked in a hexagonal close-packed array, placed into a jacket tube, and drawn down under a vacuum to form the final fibre. The final diameter of the cores was 20 μm, with a center-to-center separation of 46 μm.

**Optical Instruments.** The 405nm laser was purchased from CVI Melles Griot, and the 485nm from PicoQuant. All other optical instrumentation was purchased from Thorlabs. The input and output powers of all light sources were measured using a power meter (Thorlabs).

**Sensor Fabrication.** A 5 cm length of coating at each end of a 1 m length of fibre optic (19 cores, ø = 20 μm) was removed using a clean razor blade, followed by cleavage of each end (~1cm) to give a smooth surface. The cleaved fibres were cleaned using deionized water in an ultrasonic bath for 3 minutes. The fibre facets were etched in 40% hydrofluoric acid (HF) for 60 seconds before sonication in deionized water for 5 minutes. For surface activation, the etched fibres were dipped in methanol for 30 minutes, which was followed by dipping the fibres into a 20% (v/v) solution of 3-(trimethoxysilyl)propyl methacrylate in acetone overnight. The fibre was then washed in deionized water, and dried prior to polymerization.

The head-on and side view of the optical set-up used for photo-polymerisation and fluorescence measurement are shown in the Figure S1. The illuminating laser light was launched from a single mode fibre with alignment of the fibre controller relative to the first (launch) lens to provide a collimated on axis beam into the fibre coupling system. The light was passed through a laser line filter (to ensure a clean laser spectrum of the desired wavelength) and the light reflected by a dichroic mirror onto the desired core of the optical fibre. The collimated beam was focused onto a single core of the optical fibre with XY and Z control of the optical fibre using a translational stage. The position of the core was noted through imaging the distal end of the fibre.

For fluorescence measurements, light emitted from the optical fibre (sensor) was roughly collimated by the coupling lens, and passed straight through the dichroic mirror, with an additional filter to remove any remaining laser excitation light. The fluorescent light was coupled into a spectrometer collection fibre with the same lens arrangement.

In all cases, compact aspheric lenses were used, where identical lenses were used in all locations to give unity magnification of the laser source (single mode fibre, MFD ~ 5 μm) onto the test optical fibre (19 cores, ø = 20 μm), and imaging of the test fibre core onto the spectrometer collection optics (50 μm collection fibre) for efficient coupling.

Dichroic and filters: A beam-splitter cube was used to mount the dichroic mirror and both the excitation and emission filters. During polymerization, the set up was used optimal for 405nm pump light, while for fluorescence measurements, the cube was replaced with a one containing a filter set for 485nm laser illumination and collection of wavelengths > 510nm into the spectrometer.

All stock solutions were prepared in N,N-dimethylacetamide (DMA). For the optimized polymerization, the polymerization solution comprising of fluorescein O-methacrylate (10 mol%), poly(ethylene glycol) diacrylate (PEG diacrylate, Mw 250) (87 mol%) and diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) (3 mol%). After successful core-laser coupling, the distal end of the fibre was placed into the polymerization solution. The power of the laser was optimized to be 100 μW from the 405nm laser.

**pH solutions.** pH 6 - 8 solutions were prepared using potassium dihydrogen phosphate (KH2PO4, 0.1 M), sodium hydroxide (NaOH, 0.1 M), and distilled water. The pH values were measured using a glass-electrode pH meter (Mettler Toledo), and where necessary adjusted using the appropriate acid or base.

**Absorbance and Fluorescence.** The absorbance spectrum of TPO was recorded using a UV-Vis spectrophotometer (UV-1800 Shimadzu). The fluorescence set-up was the same as the polymerization set-up but using a laser emitting at 485 nm (operated at 1.0 μW with a >510nm band-pass filter). Fluorescence spectra were captured on a spectrometer (Ocean Optics USB2000+VIS-NIR-ES). Measurements were performed by manually opening a shutter and recording the fluorescence spectrum with an integration time of 1 second. The manual shutter was closed between measurements to
avoid any unnecessary photo-bleaching. Fluorescence imaging on the distal tip was captured through a fluorescent microscope (EVOS Fl, Bothell, WA).

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