# Fibre Platform for Multiplexed Physiological Sensing in the Distal Lung with Fluorescent Probes on Multicore Fibres

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**Abstract:**
A flexible miniaturised multiplexed sensing optrode based on fluorescent microspheres attached to multi-core fibre is reported. Photonic measurements of pH and oxygen concentration in the distal alveolar space of a perfused *ex vivo* lung are performed.


## 1. Introduction

Physiological sensing deep in tissue remains a clinical challenge. Here a flexible miniaturised sensing optrode providing a platform to perform minimally invasive *in situ* measurements is reported[1]. Silica microspheres covalently coupled with a high density of ratiometrically configured fluorophores were deposited into etched pits on the distal end of a 150 µm diameter multicore optical fibre. With this platform, photonic measurements of pH and dissolved oxygen concentration in the distal alveolar space of the lung are reported. We demonstrate and investigate the phenomenon that high-density deposition of carboxyfluorescein covalently coupled to silica microspheres shows an inverse shift in fluorescence in response to varying pH. This platform delivered fast and accurate measurements (± 0.02 pH units and ± 0.6 mg/L of oxygen), near instantaneous response time and a flexible architecture for addition of multiple sensors.

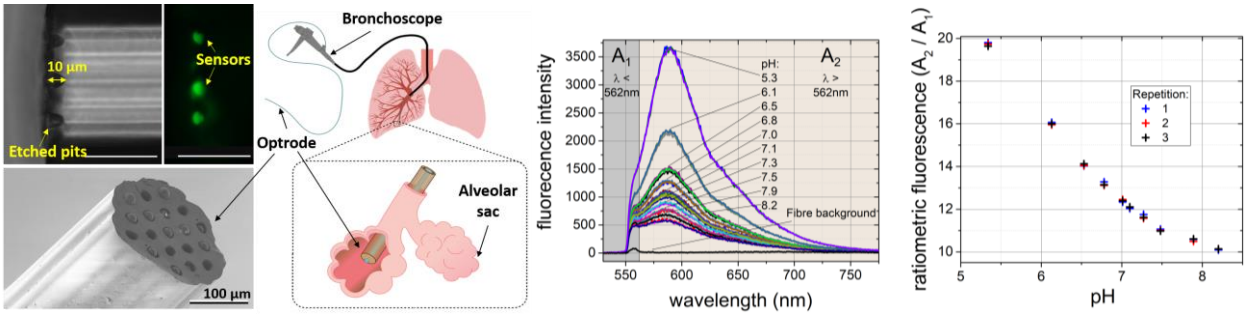


Fig. 1. Left: Optical and electron microscopy images of the fibre optrode with multiple fluorescent microspheres embedded in the etched cores of a multi-core optical fibre. Cartoon shows usage, as experimentally demonstrated in [1]. Right: fluorescence response of pH sensor, showing spectral variation in fluorescence and ratiometric response to pH changes over 3 randomly ordered repetitions. Figures adapted from [1].

## 2. Optrode architecture

Numerous optical fibre probes exist based upon sensors attached to the fibre tip, including Raman and fluorescent based systems. In some architectures multiplexing of distinct sensors can be achieved spectrally if responses are well defined (e.g. surface enhanced Raman spectroscopy probes). However, Raman based fibre probes suffer from weak signals and accordingly the challenge of overcoming backgrounds from the optic fibre requiring novel probes or systems[2,3].

In comparison fluorescence based sensors have strong signals but are hard to multiplex spectrally due to relatively broad emission bands. If two fluorophores are able to be illuminated by a single pump wavelength, fluorescent emission will likely have significantly overlapping spectra requiring spectral unmixing[4]. Fluorophores with widely differing absorption bands can be used in combination with multiple illumination sources (e.g. blue and red regions)[5], however bandwidth quickly limits the number of sensors which can be multiplexed. Here we instead

address the challenge of spatially multiplexing fluorescent probes on individual measurement channels while maintaining a truly miniaturized architecture through the use of multi-core optical fibres.

The multiplexed probe consists of fluorescent pH sensors (fluorescein and rhodamine based) and oxygen sensors (palladium porphyrin complex based) covalently bonded to silica microspheres (10 µm) loaded on the distal facet of a 19 core (10 µm core diameter) multicore fibre (total diameter of ~150 µm excluding coating). Pits are formed by selectively etching the cores using hydrofluoric acid, multiplexing is achieved through the self-location of individual probes on differing cores. While we demonstrate the combination of two sensors, the architecture is appropriate for notable expansion of the number of unique sensor types to simultaneously investigate further tissue parameters.

## 3. Fluorescent sensors

Fluorescence based sensors are often limited by measurement instabilities and particularly photobleaching. This challenge is especially acute for miniaturized fibre probes[6,7], where the fluorophore must endure relatively high illumination flux in order to achieve sufficient measurement signal from a small volume of reporter. To address this, we have developed sensors based on high fluorophore loading on silica microspheres. High loading enables additional relaxation mechanisms through energy transfer (e.g. FRET), and sensors are therefore more robust to photo-damage. We study the effect of varying fluorescein loading, observing the effects of energy transfer spectrally and through single photon counting time resolved measurements. Observing changes in fluorescent lifetimes provides direct insight into the increased degeneracy of the coupled energy level system.

In response to pH changes, the highly loaded sensor exhibits a reverse amplitude dependence as compared to that normally expected and observed with lower fluorophore loading. However, the highly loaded sensor demonstrates much improved photostability which we directly compare between sensors of varying loading.

For robust pH measurement we include rhodamine co-loaded with the fluorescein, forming a well know FRET pair. Observing distinct spectral contributions from each component, the rhodamine contribution varies in response to pH due to energy transfer from the fluorescein. The result is an extremely repeatable and stable response to pH for the fibre optrode as seen in Fig 1.

Similar loading of an established palladium porphyrin complex offers oxygen sensing on a separate channel. Both the pH and oxygen channels have a combination of spectral features that vary and remain constant in response to stimuli, allowing for a ratiometric measurement scheme robust to system fluctuations.

## 4. Experimental demonstration

Illumination at 520 nm is performed sequentially with an automated scanning system across the cores of the fibre. Short (100ms) measurement times at low (~10µW) illumination powers are triggered electronically synchronized with the laser illumination on demand at each fibre core. As such we demonstrate independent parameter measurements with an optrode of ~200 µm size, although in this case increased to approximately 1 mm size when packaged in available tubing for bronchoscopic deployment.

We performed on bench calibration and tests of *in vitro* tissue models and an ovine whole lung model for sensor validation. The pH sensor is demonstrated in the physiologically relevant range of pH 5 to pH 8.5 and with an accuracy of ± 0.02 pH units. The oxygen sensor is demonstrated in gas mixtures downwards from 20% oxygen and in liquid saturated with 20% oxygen mixtures (~8mg/L) down to full depletion (0mg/L) with ~0.5mg/L accuracy. In the ventilated and perfused whole ovine lung model, we observe the expected function of the sensors in response to changing conditions[1].

## 5. References


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