ACS Combinatorial Science

Supporting Information

Polymer Microarrays for the Discovery and Optimization of Robust Optical Fibre-based pH Sensors

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Abbreviations

MMA	Methyl methacrylate
DMAEA	dimethylaminoethyl acrylate
DEAA	N, N-diethylacetoacetamide
PAA	polyacrylic acid
MEMA	2-methoxyethyl methacrylate
DMAA	dimethylacrylamide
HEMA	2-hydroxyethyl methacrylate
HPMA	N-(2-Hydroxypropyl) methacrylamide
HBMA	4-hydroxybutyl methacrylate
DEAEMA	2-(diethylamino)ethyl methacrylate
DEAEA	2-(diethylamino)ethyl acrylate
MTEMA	(methylthio) ethyl methacrylate
BAEMA	t-(butylamino)ethyl methacrylate
DMAPMAA	N-[3-(dimethylaminopropyl)] methacrylamide
VAA	vinylacetic acid
VI	1-vinylimidazol
VPNO	1-vinyl-2-pyrrolidinone
VP-4	4-Vinyl pyridine
VP-2	2-Vinyl pyridine
DAAA	diacetone acrylamide
BACOEA	2-[[(butylamino)carbonyl]oxy]ethyl acrylate
A-H	acrylic acid
MA-H	methacrylic acid

EGMP	ethylene glycol methacrylate phosphate
EMA	ethyl methacrylate
BMA	N-butyl methacrylate
DMAEMA	2-(dimethylamino)ethyl methacrylate
GMA	glycidyl methacrylate

PA101 synthesis and characterization

The hit polymer **PA101** was resynthesized by free-radical polymerisation, using 2,2'-azobis(2-methylpropionitrile) (AIBN) as an initiator. Reaction conditions are given in Table S1. The monomers, AIBN and the solvent were mixed in a glass vessel, and polymerisation carried out for 48 h under a N_2 atm. The polymers were precipitated by the dropwise addition of the reaction mixture into hexane, collected by filtration, washed hexane, and dried overnight *in vacuo* at 40 ºC. The polymer **PA101** was characterised by GPC (Table S2), NMR (Figure S1) and FT-IR (Figure S2).

Table S1. Synthesis of **PA101**.

Polymer	**Monomers**		**AIBN**	**Solvent**	**T**	**Yield**
PA101	MMA 45 mmoL	DMAEA 5 mmoL	0.0625 mmol	Toluene (6mL)	60 ºC	89%

Table S2. Molecular weight (Mw and Mn) and polydispersity index (PDI) of **PA101**.

Polymer	**Mw**	**Mn**	**PDI**
PA101	226KDa	140KDa	1.6

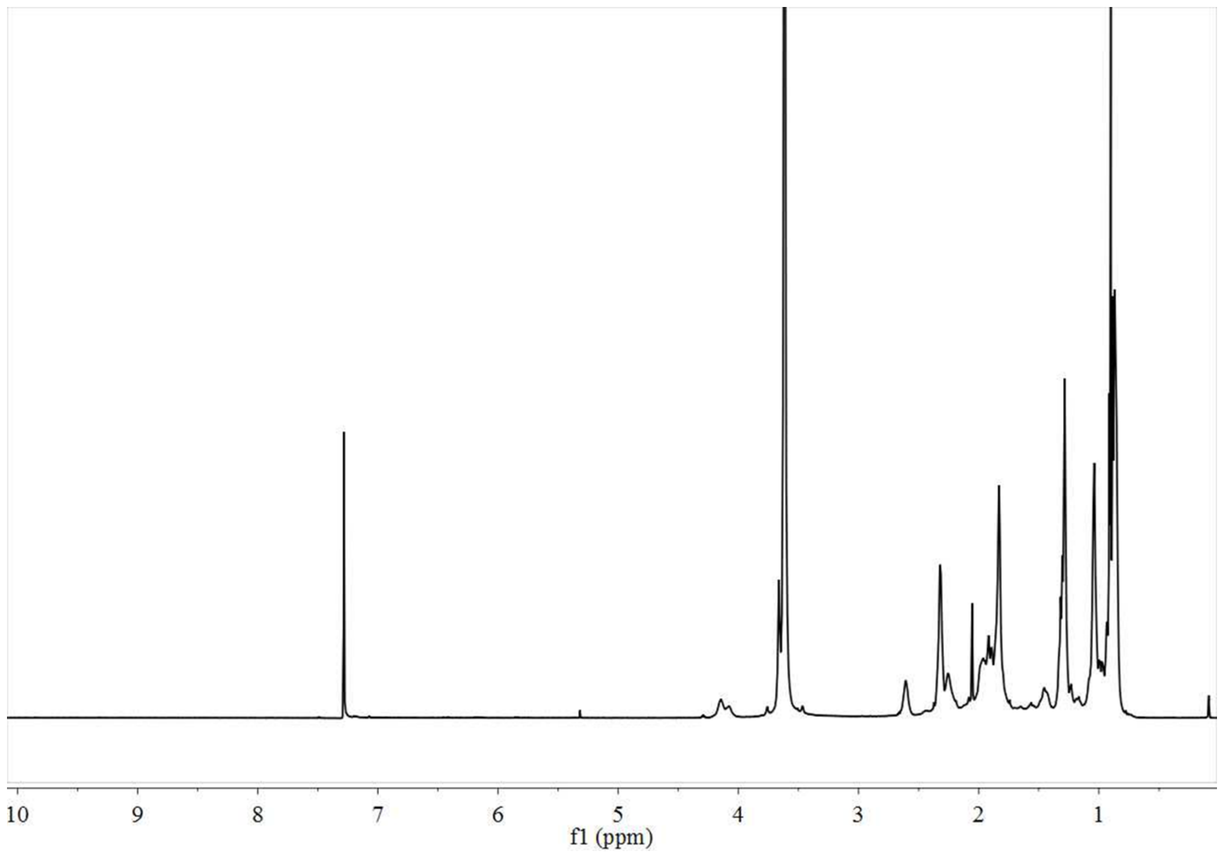

Figure S1. NMR of **PA101**

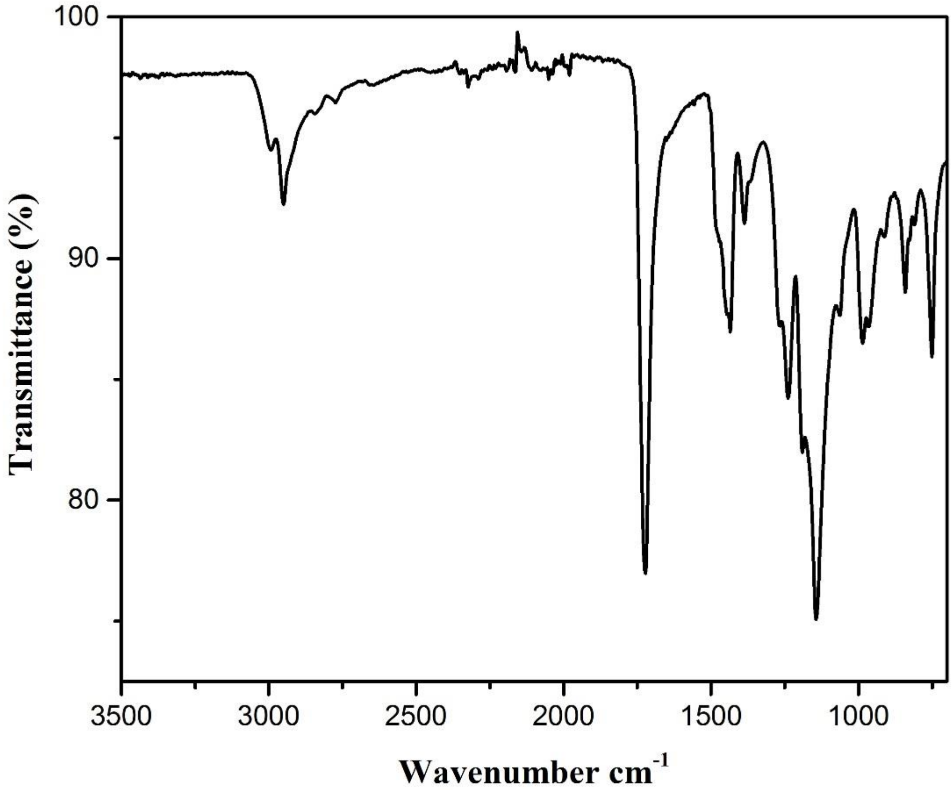

Figure S2. FT-IR of **PA101**

Table. S3. Fold increase in fluorescence for the polymer spots mixed with 5(6)-carboxyfluorescein when changing pH buffer from pH 4.0 to 10.0. The fold increase was calculated from the average of 4 microarray slides. A=0.025% 5(6)-carboxyfluorescein, B= 0.01% 5(6)-carboxyfluorescein, C = 0.04% 5(6)-carboxyfluorescein.

Polymer	Monomer 1	Monomer 2	Monomer 3	Ratio of Monomers	Fold increase (A)	Fold increase (B)	Fold increase (C)
PA1	St	DEAA		90:10	1.24	1.82	2.50
PA2	St	DEAA		70:30	1.15	1.74	2.22
PA3	St	DEAA		50:50	1.16	1.60	1.80
PA4	St	PAA		90:10	1.18	1.65	2.66
PA5	St	PAA		70:30	1.30	1.09	2.77
PA6	MMA	DEAA		90:10	1.25	1.47	1.85
PA7	MMA	DEAA		70:30	1.23	1.60	2.30
PA8	MMA	DMAA		90:10	1.30	1.71	3.02
PA9	MMA	DMAA		70:30	1.20	1.73	3.06
PA10	MMA	DEAA		50:50	1.21	1.79	2.67
PA11	MMA	PAA		90:10	1.24	1.67	2.55
PA12	MMA	PAA		70:30	1.36	1.81	2.83
PA13	MMA	PAA		50:50	1.16	1.92	3.54
PA14	MEMA	DEAA		90:10	1.25	1.52	2.12
PA15	MEMA	DEAA		70:30	1.20	1.65	2.16
PA16	MEMA	DEAA		50:50	1.18	1.62	2.95
PA17	MEMA	DMAA		90:10	1.33	1.85	2.82
PA18	MEMA	DMAA		70:30	1.21	1.37	1.78
PA19	MEMA	DMAA		50:50	1.18	1.36	1.65
PA20	MEMA	PAA		90:10	1.18	1.42	1.73
PA21	MEMA	PAA		70:30	1.23	1.56	1.85
PA22	MEMA	PAA		50:50	1.17	1.64	2.63
PA23	MEA	PAA		50:50	1.10	1.53	2.49

PA24	HEMA	DEAA		70:30	1.13	1.51	2.08
PA25	HEMA	DMAA		90:10	1.17	1.40	2.00
PA26	HEMA	DMAA		70:30	1.21	1.62	2.50
PA27	HPMA	DEAA		50:50	1.08	1.51	2.82
PA28	HPMA	DMAA		50:50	1.18	1.40	1.84
PA29	HBMA	DMAA		70:30	1.23	1.50	1.87
PA30	HBMA	DMAA		50:50	1.21	1.49	1.56
PA31	HBMA	PAA		90:10	1.31	1.46	3.24
PA32	HBMA	PAA		70:30	1.23	1.84	2.62
PA33	HBMA	PAA		50:50	1.38	1.83	2.69
PA34	MEMA	DEAEMA		50:50	1.33	1.46	2.60
PA35	MEMA	DMAEMA		90:10	1.50	1.81	2.31
PA36	MEMA	DMAEMA		70:30	2.36	2.82	4.23
PA37	MEMA	DMAEMA		50:50	2.33	2.23	3.95
PA38	MEMA	DEAEA		70:30	1.43	1.88	3.58
PA39	MEMA	MTEMA		70:30	1.36	1.60	2.53
PA40	MEMA	MTEMA		50:50	1.34	1.65	2.01
PA41	MEMA	BAEMA		90:10	1.60	2.05	2.93
PA42	MEMA	BAEMA		70:30	1.35	1.89	2.89
PA43	MEMA	BAEMA		50:50	1.58	2.45	4.77
PA44	MEMA	DMAPMAA		90:10	1.41	2.43	3.50
PA45	MEMA	VAA		90:10	1.24	1.44	2.42
PA46	MEMA	VAA		70:30	1.18	1.52	2.07
PA47	MEMA	VAA		50:50	1.24	1.79	2.17
PA48	MEMA	VI		70:30	1.20	1.46	2.29
PA49	MEMA	VPNO		90:10	1.26	1.53	2.81
PA50	MEMA	VPNO		70:30	1.24	1.50	2.86
PA51	MEMA	VPNO		50:50	1.15	1.27	2.34
PA52	MEMA	VP-4		90:10	1.19	1.75	2.45
PA53	MEMA	VP-4		70:30	1.18	1.90	2.23

PA54	MEMA	VP-4		50:50	1.13	1.60	2.55
PA55	MEMA	VP-2		90:10	1.27	1.76	2.32
PA56	MEMA	VP-2		70:30	1.15	1.70	2.67
PA57	MEMA	VP-2		50:50	1.45	2.19	2.97
PA58	MEMA	DAAA		90:10	1.25	1.74	2.64
PA59	MEMA	DAAA		70:30	1.30	1.64	2.28
PA60	MEMA	DAAA		50:50	1.32	1.76	2.68
PA61	HEMA	DEAEMA		50:50	2.08	1.88	2.36
PA62	HEMA	DMAEMA		90:10	1.39	2.06	3.44
PA63	HEMA	DMAEMA		50:50	2.54	2.49	5.95
PA64	HEMA	MTEMA		90:10	1.26	1.62	2.28
PA65	HEMA	BAEMA		90:10	1.23	1.79	3.05
PA66	HEMA	BAEMA		70:30	1.37	1.56	2.24
PA67	HEMA	DMAPMAA		90:10	1.32	1.88	2.86
PA68	HEMA	DMAPMAA		70:30	1.41	2.24	4.30
PA69	HEMA	DMAPMAA		50:50	1.73	2.50	3.51
PA70	HEMA	BACOEA		90:10	1.26	1.54	2.58
PA71	HEMA	VAA		90:10	1.26	1.78	2.66
PA72	HEMA	VAA		70:30	1.18	1.59	2.04
PA73	MMA	A-H		70:30	1.14	1.30	1.55
PA74	MMA	MA-H		90:10	1.15	1.47	2.28
PA75	MMA	MA-H		50:50	1.20	1.47	1.74
PA76	MEMA	MA-H		90:10	1.10	1.44	1.72
PA77	MEMA	MA-H		70:30	1.18	1.40	2.03
PA78	MEMA	MA-H		50:50	1.20	1.48	2.15
PA79	MEMA	EGMP		90:10	1.16	1.26	1.45
PA80	MEMA	EGMP		70:30	1.14	1.21	1.51
PA81	MMA	A-H	DEAEMA	70:20:10	1.16	1.36	1.66
PA82	MMA	A-H	DEAEA	70:20:10	1.16	1.29	1.76
PA83	MMA	A-H	DEAEA	70:15:15	1.13	1.31	1.48

PA84	MMA	MA-H	DEAEA	70:20:10	1.37	2.35	3.58
PA85	MMA	MA-H	DEAEA	70:15:15	1.12	1.34	2.29
PA86	MMA	MA-H	DEAEA	70:10:20	1.28	1.60	2.10
PA87	MEMA	A-H	DEAEMA	70:20:10	1.13	1.81	2.20
PA88	MEMA	A-H	DEAEA	70:20:10	1.33	1.93	3.08
PA89	MEMA	A-H	DEAEA	70:15:15	1.25	1.72	2.72
PA90	MEMA	A-H	DEAEA	70:10:20	1.19	1.77	2.36
PA91	MEMA	MA-H	DEAEMA	70:20:10	1.18	1.80	2.53
PA92	MEMA	MA-H	DEAEMA	70:15:15	1.09	1.27	1.89
PA93	MEMA	MA-H	DEAEA	70:20:10	1.35	2.02	2.94
PA94	MEMA	MA-H	DEAEA	70:15:15	1.26	1.78	2.67
PA95	MEMA	MA-H	DEAEA	70:10:20	1.23	1.71	2.62
PA96	MMA	DEAEMA		90:10	1.20	2.30	2.94
PA97	MMA	DEAEMA		70:30	1.48	2.08	3.07
PA98	MMA	DEAEMA		50:50	2.45	3.12	3.80
PA99	MMA	DEAEA		90:10	1.86	5.32	11.76
PA100	MMA	DEAEA		70:30	1.22	1.62	2.01
PA101	MMA	DMAEA		90:10	2.23	5.63	14.24
PA102	MMA	DMAEA		70:30	2.03	2.67	4.14
PA103	HPMA	DEAEA		70:30	1.29	1.69	2.21
PA104	HBMA	DEAEMA		70:30	1.23	1.52	2.35
PA105	EMA	DEAEMA		90:10	1.70	1.53	2.77
PA106	EMA	DEAEMA		50:50	1.34	1.63	2.77
PA107	EMA	DMAEMA		70:30	1.78	2.48	2.09
PA108	EMA	DEAEA		90:10	1.21	2.39	1.91
PA109	EMA	DMAEA		90:10	1.14	1.74	2.67
PA110	BMA	DEAEMA		90:10	1.33	2.04	2.25
PA111	BMA	DMAEMA		90:10	1.39	2.07	2.86
PA112	BMA	DMAEA		90:10	1.49	2.20	2.84
PA113	MMA	GMA		90:10	1.30	1.82	2.78

PA114	MMA	GMA		50:50	1.96	3.20	4.06
PA115	MEMA			100%	1.24	2.58	2.15
PA116	MMA			100%	1.22	1.53	2.13
PA117	HBMA			100%	1.20	2.16	3.86
PA118	HPMA			100%	1.28	1.46	2.03
PA119	BMA			100%	1.52	1.59	3.97
PA120	HEMA			100%	1.26	1.95	3.23
PA121	EMA			100%	1.26	1.81	3.62

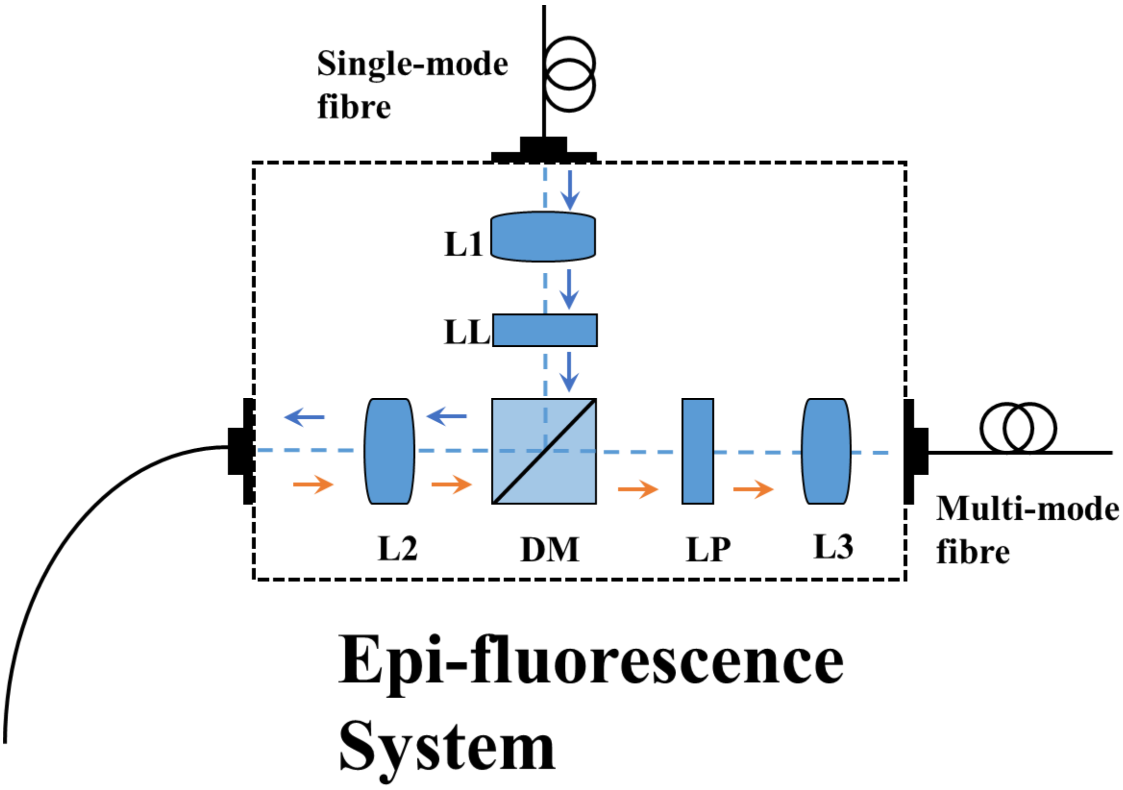

Figure S3. An epi-fluorescence system was used to couple the excitation light into the optical fibre probe with coupling of the excitation fluorescence signal light into the spectrometer. The illuminating light from the laser (485 nm) was launched from a single mode fibre (MFD ~ 5µm), with alignment of the fibre controlled relative to a compact aspheric lens (L1) to provide a collimated on axis beam into the fibre coupling system. After passing through a laser line filter (LL) to ensure a clean spectrum of the desired wavelength, the excitation light was then reflected by a dichroic mirror and focused onto the core of the optical fibre probe using lens L2. The position of the core was noted through imaging of the distal end of the fibre, with the XY and Z control of the optical fibre using a translational stage. The fluorescent light emitted from the probe was roughly collimated by L2, and passed straight through the dichroic mirror, with a long-pass filter (LP, cut-on of wavelengths >510 nm). The fluorescent light was coupled into a spectrometer collection multi-mode fibre (50 µm collection fibre) using lens L3. In all cases, compact aspheric lenses (350-700 nm broadband with an antireflective (AR) coating) were used, with identical lenses were used in all locations to give unity magnification of the laser source.

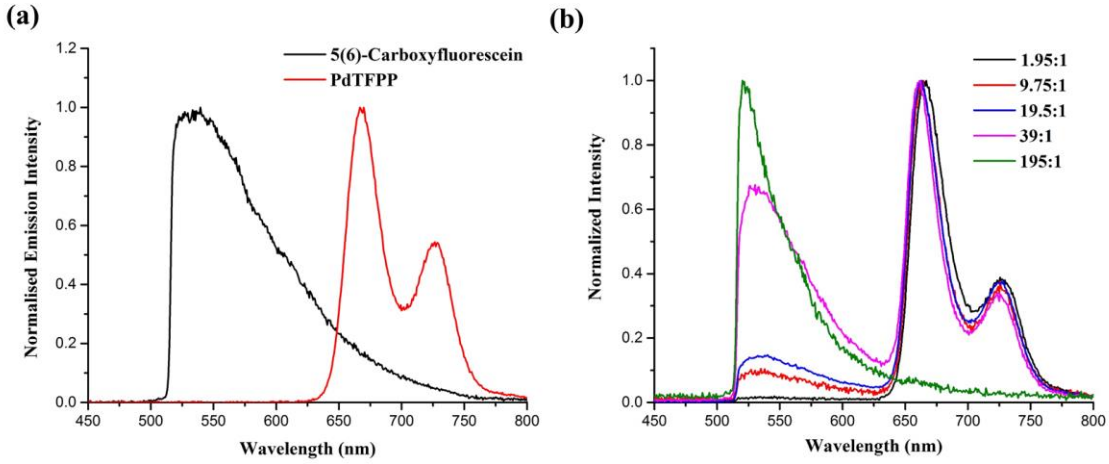

Figure S4. (a) Emission spectra of 5(6)-carboxyfluorescein and PdTFPP and (b) emission spectra of the optical fibre 5(6)-carboxyfluorescein/PdTFPP sensors fabricated with different ratios of 5(6)-carboxyfluorescein/PdTFPP. Excitation at 485 nm, 1 µW. The spectra were recorded with an integration time of 100 ms. The sharp leading-edge is due to the excitation filter that cuts off wavelengths < 510 nm.

Experimental procedures for pH measurement on the ovine tissue sample

The fabricated optical fibre probe was calibrated using a range of phosphate buffers (pH 6.0 to 8.0) before tissue measurements. The fibre tip of the probe was placed in contact with the surface of the tissue and left in place for approximately 1 min, ensuring that the fluorescence spectrum was stable before measurements were taken. Four sample locations were used and at each location three measurements were recorded, with the probe washed between each measurement.

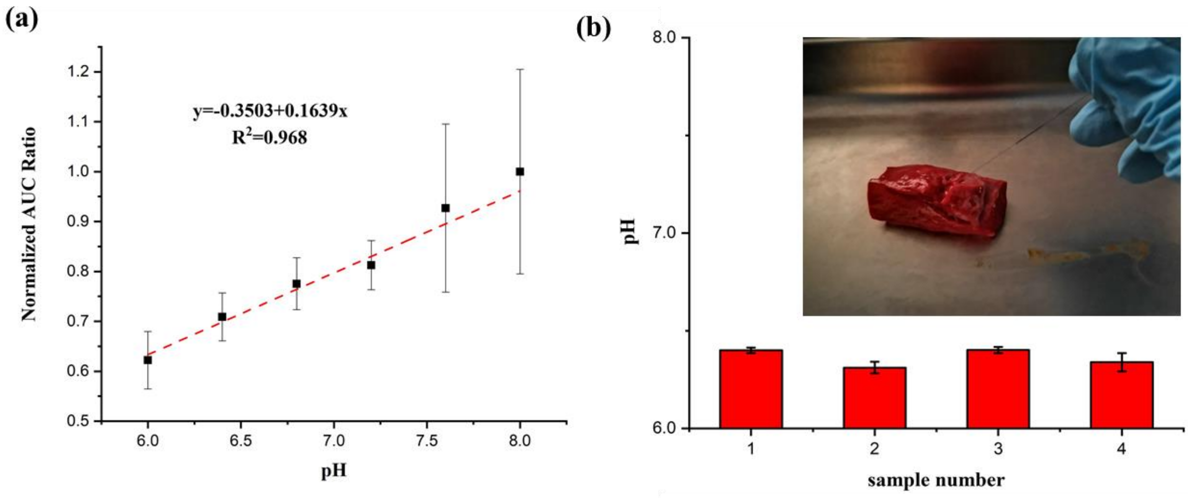

Figure S5. (a) Calibration of the fibre pH probe prepared for tissue measurement and (b) The tissue pH variation measured using the fabricated probe at each sample locations. Inset: Example photograph of tissue pH measurement using the fabricated probe.

Matlab script for fluorescent sensor distribution analysis on the end of the fibre.

```
%%
% This script is created originally on 27/11/2018;
% version='1.0';
%
% Author: Jingjing Gong, (s1547470@ed.ac.uk)
% University: University of Edinburgh
% All rights reserved

%Algorithm description:
%   this is used to process the fluorescent image and plot the
%   radialaverage intensity
%   radialavg function can be found at:
%   https://uk.mathworks.com/matlabcentral/fileexchange/46468-radialavg-zip

%% main body of algorithm
function []=main(img_dir)
% close all figures and clear all variables
clc, clear, close all;

% Parameters setting
M = 255;
Xo = 0  ;
Yo = 0  ;

if nargin==0
    % default img_dir
    img_dir='./put/your/image/directory';
end

% Read fluorescent image
raw_img = imread(img_dir);

% Process the image
[Zr, R] = radialavg(raw_img,M, Xo, Yo);

% Plotting results
figure(1)
plot(R, Zr, '-')
xlabel('Radial distance')
ylabel('Fluorescent intensity')
end
```