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DNA Probe Detection within 3D Hydrogel Matrix in a Hollow Core Photonic Crystal Fibre

Monika Rutowska, Jiadi Lu, Fatima C. Garcia Gunning and Andrew D. Ellis
Photonic Systems Group
Tyndall National Institute and Department of Physics, University College Cork, Ireland

ABSTRACT

In this paper, we report for the first time the detection of a Cy5-labelled DNA probe immobilised within a 3D hydrogel matrix, formed inside a hollow core Photonic Crystal Fibre (HC-PCF). We show both the sensitivity of fluorescence detection inside the HC-PCF using a supercontinuum light source and of the variation of the luminescence intensity with the different concentrations of DNA probe within the hydrogel. The 3D hydrogel matrix is a network of polymer chains, which is expected to provide highly sensitive detection and selection of bio-molecules, in comparison with 2D coverage. The biocompatibility of hydrogel in the HC-PCF suggests numerous applications associated with immobilised DNA probe detection for point-of-care or remote systems.

Keywords: Photonic crystal fibres, hollow core, biosensing, silanisation, hydrogel, DNA, fluorescence

1. INTRODUCTION

The use of microstructure fibres for numerous sensing applications is rapidly expanding. The detection of molecules in gas or liquids, as well as the detection of bio-molecules anchored inside the fibres have established MOFs as sensitive and reliable candidates for sensors[1-4]. Such fibres are unique, due to the light guiding mechanism inside their structure[5] which enables to shift the guided frequencies by changing the refractive index within their holes[6] and improves the light interaction within sample.

In a recent paper, we propose to combine the optical benefits of such fibres, especially the HC-PCF, and use them for molecule immobilisation, tagging, and measurement based on hydrogel[7]. Hydrogel is a competing technology which offers three dimensional matrix for the immobilisation of molecules and it is considered to offer higher sensitivity than two dimensional immobilisation, common to a surface coverage devices[8] for the detection and selection of attached bio-molecules inside its structure. It consists a network of polymer chains cross-linked via chemical bonds. Hydrogel has been widely used for biomedical applications[9] due to its hydrophilic character and biocompatibility[10], which are mainly due to the well controlled growing process of hydrogel, swelling, molecular diffusion and permeability[10-12].

Individually, both of these approaches offer advantages over conventional micro-array assays, and would offer significant benefits for DNA based research for a variety of health applications. In this paper, we combine the 3D immobilisation properties of hydrogel with the improved ligand-light interaction of HC-PCFs, reporting for the first time, the detection of the immobilised DNA probe inside a 3D hydrogel (25% PEG-DA w/v) structure formed within a HC-PCF, and present the sensitivity of the system for fluorescence measurements, using a supercontinuum source. In this system, fluorescence tagged DNA is bound to pre-polymer molecules, which are injected into the HC-PCF. Hydrogel formation is then photo-initiated enabling subsequent fluorescence analysis by illuminating the fibre at the absorption wavelength of the flourochrome. The HC-PCF enables an interaction length of several cm and provides an efficient collection mechanism for the emitted fluorescence.

2. HC-PCFs PREPARATION

To form hydrogel within the HC-PCF, that will enable the DNA probe detection, chemical surface preparation, treatment and photo-polymerisation process are required inside the fibre[7]. The photo-polymerisation process occurs in the frequency range of the shifted bandgap, which is 510-680 nm. In a HC-PCF 1060[6] with a core diameter of 9.8 μm, this is achieved by changing the refractive index to 1.33. Therefore, the first batch of HC-PCFs 1060 was silanised following a specific chemical protocol as described in[7]. Briefly, the internal surface of each fibre was covered with silane agents

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(solution of AMPTS 3%) and photo-initiator molecules (solution of 0.5 mM Eosin Y and 25 mM EDAC) resulting in fibre cross sections under white light illumination, as shown in Fig. 1.

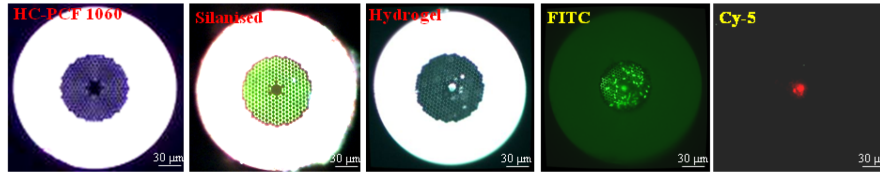

Fig. 1. The images of HC-PCFs 1060, where from the left side: (1st) HC-PCF 1060, (2nd) after the silanisation process, (3rd) the hydrogel formed in the core, (4th) the FITC image for a hydrogel, (5th) the Cy-5 DNA immobilisation in the fibre core.

As detailed in[7], a 25% PEG-DA pre-polymer was prepared also here, which was a phosphate buffer (PBS), consisting of 25% (v/v) poly(ethylene glycol) diacrylate (PEG-DA), 225 mM triethanolamine (TEA), and 37 mM 1-vinylpyrollidinone (VP) which could be polymerised into hydrogel by photo-initiation. A 100 µM concentration of amino-modified oligonucleotides (Metabion International AG), with attached Cy-5 Fluor, were added to pre-polymer with a NHS-PEG-acrylate cross-linker. The pre-polymer solutions were injected into fibre samples using a syringe pump setup and uniform hydrogel growth in the core was initiated by focussing a 50 mW 532 nm laser into the fibres samples using a x4 lens. The light was transmitted through the fibre samples for approximately 15 minutes.

To investigate the sensitivity of the optical system for use in the latter phase of experiments, another fibre batch of HC-PCFs 1060 was also prepared. After changing the refractive index of the fibre core to 1.33, we enabled guiding the wavelength range of 650-750 nm and not of the original wavelength at 1060 nm. Since the majority of commercially available fluorochromes, suitable for DNA tagging, interact in or near the visible wavelength range and are water soluble compounds, we decided to apply selective filling, with the help of the fusion splicing method[13]. Hence we cleaved HC-PCFs 1060 into pieces of approximately 30 cm in length and modified the front-end of each one of them up to a structure depth of 100 µm. Then we were able to fill only the core of the prepared fibre pieces with fluorochrome sample, diluted in water.

3. EXPERIMENTS AND RESULTS

The optical setup for the DNA probe detection is shown in Fig. 2. The source is a supercontinuum[14] generated by a Q-Switched semiconductor pumped Nd:YAG laser, centred at 1064 nm (6.85 kHz repetition rate, 0.55 ns pulse width, ~10.7 µJ pulse energy) together with a 20 m highly nonlinear PCF (NL-PCF), covering the range of wavelengths from 350 nm to 1700 nm. The light was collimated with an infinity corrected objective, and passed through a series of filters. To obtain the optimum excitation of the fluorochrome and detection of the resulting a back-fluorescence, selected filters were placed before and after the sample, as detailed in Table 1. The fluorescence was collected through a focusing lens (x10), coupled into a multimode fibre which was connected to a photomultiplier tube with a sensitivity peak at 630 nm. A 16 bit 400ks/s analogue to digital converter was used to record data, which was then processed to give the total number of fluorescence counts in a 0.1 second interval.

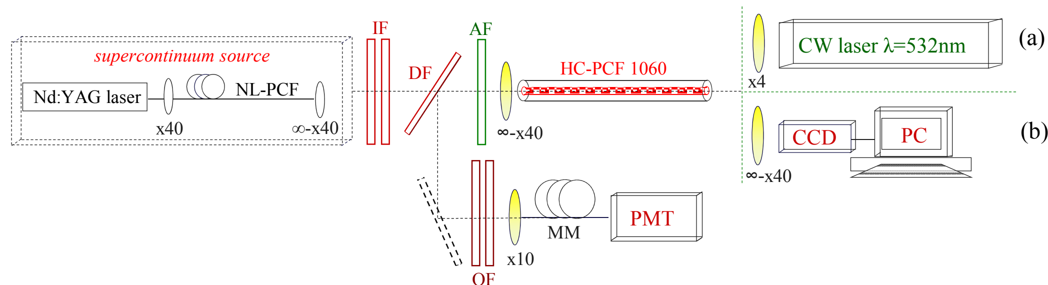

Fig. 2: Optical experimental setup where: NL-PCF=highly nonlinear PCF, IF =input filters, DF=dichroic filter, AF=alignment filter, OF= output filters, PMT = Photomultiplier tube, MM= multi-mode fibre. The HC-PCF output was either connected to (a) a CW laser for the photo-initiation, or (b) a CCD for monitoring purposes.

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Filters	Cy-5 Fluor		Alexa 750 Fluor	
Input Filters	band pass filter at 650 nm, 10 nm bandwidth	low pass filter, at 650 nm	band pass filter at 740 nm, 10 nm bandwidth	low pass filter, at 750 nm
Output Filters	band pass filter at 670 nm, 10 nm bandwidth	high pass filter, at 665 nm	band pass filter at 780 nm, 10 nm bandwidth	high pass filter, at 775 nm
Dichroic Filter	transmission at 650 nm, reflection a 670 nm		transmission at 750 nm, reflection a 780 nm	
Alingment Filter	band pass filter at 530 nm, 10 nm bandwidth		band pass filter at 530 nm, 10 nm bandwidth	

Table 1. The description of set filters for a Cy-5 Fluor and Alexa 750.

To verify the sensitivity of the system at the predicted loss minima of water filled HC-PCF, the prepared fibre samples were filled with the various concentrations of Alexa Fluor 750 (diluted in distilled water), from 500 nM to 0.5 nM, with excitation and emission wavelengths of 750 nm and 780 nm, respectively. The average launched power into the fibre was around 62 µW. The fluorescence bleaching curves, for the different concentrations, are shown in Fig. 3(a), where the lowest detectable concentration of flourochrome was 1 nM. Given a fibre length of 33 cm this corresponded to a 24 nL volume of sample (~14×10^6 flourochrome molecules). To enable the detection of lower concentrations, the fusion splicing parameters were set to modify the selective filling process and the sample was allowed to fill the core and 18 additional capillaries in the cladding. As a result, a lower concentration of 0.5 nM was detectable with the fibre length of 31 cm, corresponding to the sample volume of 53 nL (~16×10^6 flourochrome molecules) as shown in Fig. 3(b).

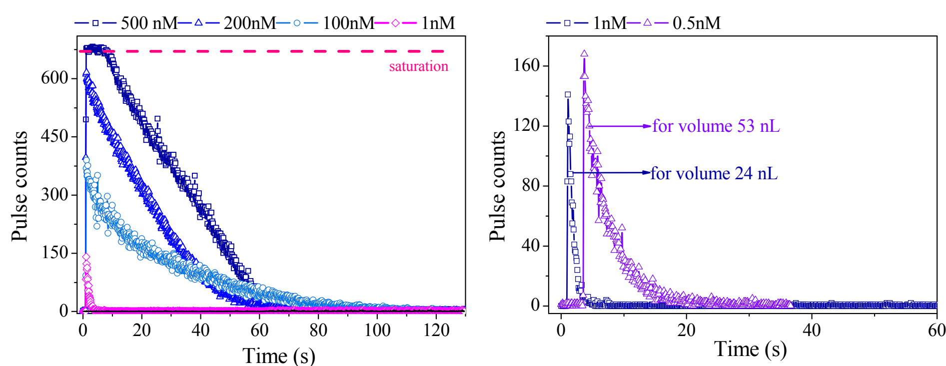

Fig. 3: (a) Fluorescence decay curves as a function of concentration of Alexa-Fluor 750, (b) Flourescence decay curves for 1 nM filled core (square symbols) and 0.5 nM filled core and surrounding 18 holes (triangular symbols).

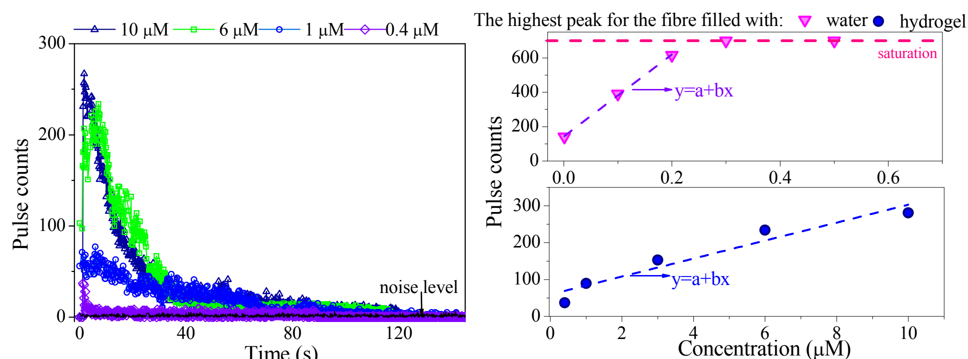

Fig. 4: (a) Fluorescence decay curves as a function of concentration for a Cy-5 DNA probe incorporated in a fibre filled with hydrogel, (b) comparison of fluorescence peak intensity for Alexa-Fluor 750 in water (top), and hydrogel immobilised oligos (bottom).

Although the refractive index of hydrogel is close to that of water, it was found that fibre filled with hydrogel guided wavelengths closer to 650 nm than to 750 nm[15]. In order to work at this shorter wavelength Cy-5 Flour was ordered to be attached to the oligos array. Cy-5 has excitation and emission wavelengths at 650 nm and 670 nm, respectively. Before the fluorescence measurements, a hydrogel filled fibre was observed using the microscope to confirm confinement of the hydrogel to the fibre core, as shown in Fig. 1 (3rd from the left). Fig. 1 also shows the auto-fluorescence of the hydrogel and the surface coverage due to photo-sensitive Eosin Y (4th from the left), detected with a 490/520 nm dichroic filter. The Fig. 1 (5th image from the left) was taken using 650/670 nm dichroic filter shows that the DNA was immobilised within hydrogel, at the core of the HC-PCF. Quantitative fluorescence measurements were then arranged using the setup of Fig. 2, with Cy-5 filters set, as detailed in Table 1. However, the average illumination, using the full set input filters,

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for wavelength of 650 nm was only 14 μW, due to the supercontinuum intensity profile. From measurements taken using the HC-PCF 1060 filled with hydrogel, we observed a higher transmission loss than for pure water measurements. Hence, we applied the average illumination of 260 μW, which was achieved by removing the band pass filter at 650 nm. The output of fibre was monitored with CCD camera confirm for all measurements that the light was correctly focused into the filled core and a strong interaction between the light and the sample was achieved, at last the fluorescence decay curves were detected. Fig. 4(a) shows the corresponding fluorescence decay curves for concentrations varying from 0.4 μM to 10 μM, using HC-PCF 1060 lengths of approximately 10 cm. Measurable responses were observed even for the lowest oligos concentration of 0.4 μM ($\sim 1.7\times10^9$ oligos) immobilised in the HC-PCF. Fig. 4(b) shows the variation in the fluorescence amplitude, measured at the peak of the fluorescence decay curve with sample concentration for both water with Alexa-Fluor 750 (upper curve) and hydrogel with Cy-5 DNA oligos. The data clearly demonstrates PMT saturation for the highest concentrations, however, a linear fit is observed for concentrations up to 0.2 μM for the Alexa-Fluor 750 reference, and around 10 μM for the Cy-5 DNA oligos fibres. A larger dynamic range would clearly be available using a set of neutral density filters.

Note that these results were obtained using a standard 25% PEG-DA (w/v) solution. This concentration has been shown previously, as being the optimum for allowing variations in the PEG-DA, TEA, and VP chemical compounds to alter optimise the permeability of hydrogel, as well as, the crosslink density, and thickness coverage[10-12]. Re-optimisation of the pre-polymer solution and the optical properties of the HC-PCF might optimise guidance at the fluorescence wavelength when filled with hydrogel, and is expected to result in higher sensitivity of the immobilised probes within hydrogel in the fibre.

4. CONCLUSION

We have demonstrated the Cy5-labelled DNA oligonucleotides detection within a 3D hydrogel matrix formed inside the holes of a hollow-core PCF. A 0.4 μM oligonucleotides sensitivity was observed via fluorescence measurements using a 260 μW illumination. The potential sensitivity of the scheme is demonstrated, using water based solutions in the HC-PCF 1060, to be in the range of 0.5 nM.

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