

Integration of a 3D Hydrogel Matrix with a Hollow Core Photonic Crystal Fibre for DNA Probe Immobilisation

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Abstract. In this paper, we demonstrate the integration of a 3D hydrogel matrix within a hollow core Photonic Crystal Fibre (HC-PCF). In addition we also show the fluorescence of Cy5-labelled DNA molecules immobilised within the hydrogel formed in two different types of HC-PCF. The 3D hydrogel matrix is designed to bind with the amino-groups of biomolecules using an appropriate cross-linker, providing higher sensitivity and selectivity than the standard 2D coverage, enabling a greater number of probe molecules to be available per unit area. The HC-PCFs, on the other hand, can be designed to maximise the capture of fluorescence to improve sensitivity and provide longer interaction lengths. This could enable the development of fibre-based point-of-care and remote systems, where the enhanced sensitivity would relax the constraints placed on sources and detectors. In this paper, we will discuss the formation of such polyethylene glycol diacrylate (PEGDA) hydrogels within a HC-PCF, including their optical properties such as light-propagation and auto-fluorescence.

KEYWORDS:

Photonic crystal fibres, hollow core, bio sensing, hydrogel, fluorescence, DNA immobilisation, silanisation

1. Introduction

Optical fibre sensors have been investigated over many decades and recently sensors using Photonic Crystal Fibres (PCF) and microstructure optical fibres (MOF) have shown great potential for a number of applications. Unlike standard optical fibres, PCFs and certain MOFs have a photonic crystal-like structure surrounding the core, which enables light to be propagated through the core without the assistance of co-dopants [1,2]. Hollow core photonic crystal fibre (HC-PCF) which operate through the photonic bandgap effect (PBG) [3,4,5,6,7] offer the prospect of common confinement of the sample and the optical field. In a PBG fibre only a restricted band of wavelengths is confined in the core by the photonic bandgap effect induced by a microstructure fabricated within the cladding. Samples which are typically confined to the hollow core of the PBG fibre have improved the interaction between light within the guided wavelength band and the sample under test [8].

Recently, bio sensing using glass PCFs [9], glass MOFs [10], and polymer MOFs [11] were verified, and the possibility of antibody immobilisation within the microstructure has been demonstrated [9,12]. The use of such fibres enables biosensors with high sensitivity, because the interaction length can be extended to several centimetres if not meters, and fibres can also be designed to maximise fluorescence guidance [13]. They also enable selectivity, with the possibility for multiple antigens to be attached and analysed with a multi-wavelength optical source. HC-PCFs have been used for immobilisation, capture and detection of DNA probe molecules [14]. However, such immobilisation techniques are based on standard techniques for surface coating on a two dimensional (2D) scale.

Lately, three dimensional (3D) hydrogels have been demonstrated as alternative substrates for microarray applications [15] with higher sensitivity [16,17] than the 2D matrices when grown on glass substrates. Hydrogels (also called aquagels) are mostly liquid, yet behave like solids due to a three-dimensional crosslinked network within the lattice structure. They consist of a group of polymer chains that are water-insoluble. Hydrogels contain binding sites for the covalent attachment of biological compounds within their porous structure via the use of an appropriate cross-linking agent. For the immobilisation of probe DNA molecules, they are first modified with an amino group in order to allow for successful attachment to the polymer backbone. The immobilisation occurs simultaneously during the formation of the hydrogel, in a photo-polymerisation process [18].

The advantages of using a hydrogel include the increased capacity for the immobilisation of the biomolecules, due to a greater number of probe molecules to available per unit area. The hydrogel's high affinity for water can provide a homogeneous environment with the surrounding aqueous solution, which allows for the free diffusion or mass transfer of the biomolecules into the hydrogel matrix. This increases the probability of target DNA molecules of binding to and interacting with complementary probes immobilised within the hydrogel matrix. They also offer the possibility of reducing steric hindrance which is a factor contributing to sensitivity and reproducibility limits in the most of 2D systems [16,17].

In this paper we demonstrate the immobilisation and fluorescent detection of a Cy5-labelled DNA probe in a polyethylene glycol diacrylate (PEGDA) hydrogel platform formed within a glass-based HC-PCF. We show, for the first time, the studies of light propagating through the hydrogel structure, indicating the refractive index of the hydrogel within the fibre.

2. HC-PCF preparation

2.1 HC-PCF samples

Short pieces (10 to 30 cm) of commercially available HC-PCFs (NKT Photonics A/S) were used, which were originally designed for 1060 and 1550 nm propagation (HC-PCF 1060 and HC-PCF 1550), with core diameters of approximately 9.8 and 11.0 μm respectively (figure 1).

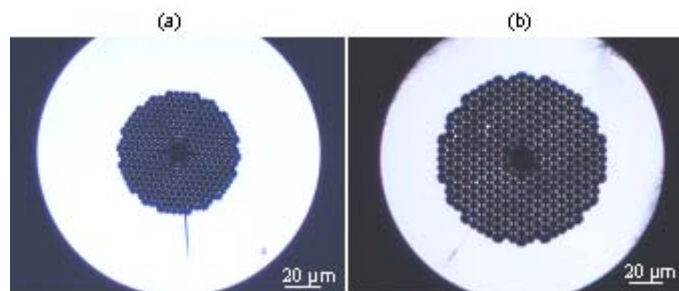


Figure 1: Cross section of fibres (a) HC-PCF 1060 and (b) HC-PCF 1550.

The chemical preparation of the hole surfaces and the photo-initiation of the 3D hydrogel took place within the hollow core and the cladding holes, although selective filling could also have been used [19]. Instead of relying on a lengthy filling process based on capillary action, a syringe pump (KD Scientific 410) was used to assist both the chemical filling and rinsing. Each chemical solution described below was pumped for about 5 minutes, even though the hollow core of the PCF can be filled within seconds and were kept incubated within the microstructure for a certain period of time for each step. This protocol was followed in order to ensure a good surface coverage. All chemicals and reagents used were purchased from Sigma-Aldrich Ltd, unless stated otherwise.

2.2 Silanisation process and immobilisation of Eosin Y

The holes of the HC-PCFs were chemically treated as per [20]. The holes were pumped with a 3% (v/v) solution of (3-Aminopropyl) trimethoxysilane (APTMS), which was prepared in 19:1 solution of methanol and deionised water. The filling process was monitored via a microscope (Nikon Eclipse ME600), where one

of the fibre ends was held vertically and its cross-section observed; and the other end was attached to the syringe pump. This solution of silanising agent was kept in the fibre and incubated for 1 hour at room temperature. Afterwards, the silanising solution was removed by several rinses of methanol and cured in an oven for 2 hours, at 60°C. A 10 mM NaH₂PO₄ solution was prepared in order to make up a solution containing 0.5 mM Eosin Y and 25 mM N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) and the resultant solution was pumped into the fibre (figure 2(b)). After 90 minutes, the sample was rinsed with deionised water and dried in an oven at 35°C for 15 hours. After this process, a green reflection from the fibre was observed using microscope, indicating successful Eosin Y immobilisation on the silanised surface (figure 2 (c)).

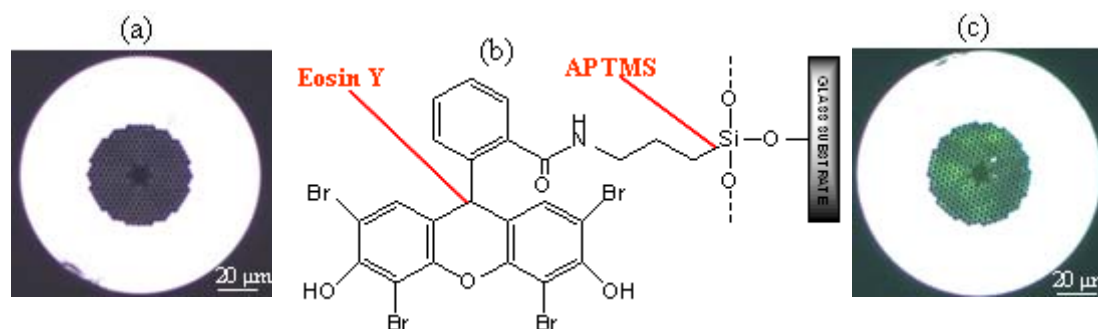


Figure 2: Cross section of HC-PCF 1060 (a) before a silanisation process, (b) the silanised glass substrate with attached Eosin Y, (c) HC-PCF 1060 after a silanisation process.

2.3 Photochemical initiation and hydrogel growth

A pre-polymer solution was prepared in phosphate buffer solution (PBS), consisting of 25% (v/v) poly(ethylene glycol) diacrylate (PEGDA), 225 mM triethanolamine, and 37 mM 1-vinylpyrrolidinone. Solutions were stored in the fridge at ~ 4°C. The pre-polymer solution was deposited into the HC-PCF fibres which were previously functionalised with silane anchor molecules and Eosin Y photo-initiator, and then irradiated with visible light at a wavelength of 530 nm for about 6 minutes; and the optical setup for the hydrogel growth is shown in figure 3.

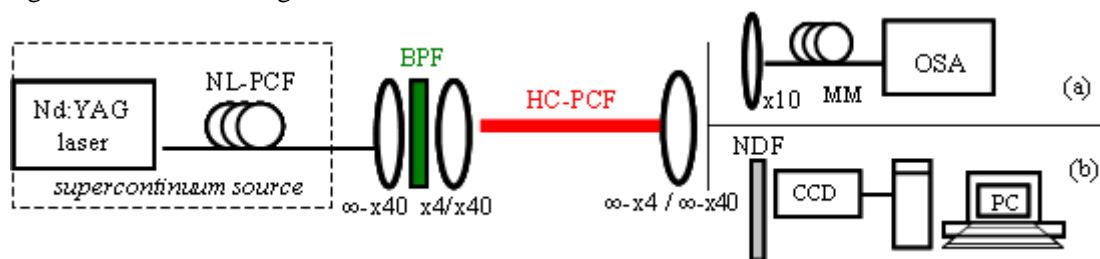


Figure 3: Optical experimental setup used for photochemical initiation and spectral analysis. BPF = bandpass filters, MM = multimode fibre, OSA = optical spectrum analyser, NDFs – neutral density filters, CCD = camera, PC=desktop (a) spectrum analysis and (b) near-field images.

A Q-Switched semiconductor pumped Nd:YAG laser (JDS Uniphase), centred at 1064 nm (6.85 kHz repetition rate, 0.55 ns pulse width, and a ~10.7 μJ/pulse) together with a 20m highly nonlinear PCF (NL-PCF) were used as a supercontinuum source [21]. The light was coupled to the fibre by a combination of collimating and focusing lenses. Photochemical initiation of the polymerization process was achieved by filtering the supercontinuum spectrum using a broad bandpass filter (10 nm FWHM) centred at 530 nm. Typically 5 to 10 minutes was sufficient for hydrogel formation through the entire length of the fibre, with average input powers of about 1μW. Exposure to the light results in the excitation of Eosin Y to a triplet state and in turn, activates the triethanolamine co-initiator to release a neutral Eosin Y radical. These radicals

initiate free-radical polymerisation reactions which convert the PEGDA pre-polymer into a hydrogel which is covalently bound to the inner surface of the HC-PCF.

3. Experiments:

3.1 Hydrogel filled HC-PCF

Following the photo-initiation process, the ends of filled HC-PCFs were analysed with assistance of a standard optical microscope, as per figure 4 (a) and (b) shown as examples of the fibres HC-PCF 1060 and HC-PCF 1550 with hydrogel. Although it is known that hydrogels can be porous [15,22] it is difficult to predict its uniformity within the HC-PCF, even if the same rule-of-thumb applies. Figure 4c, for example, shows a scanning electron microscope (SEM) image of the HC-PCF 1550 fibre with hydrogel partly formed in the core. We would anticipate that the impact of the porosity would affect the light guidance in the HC-PCF, which is discussed in section 3.1.2.

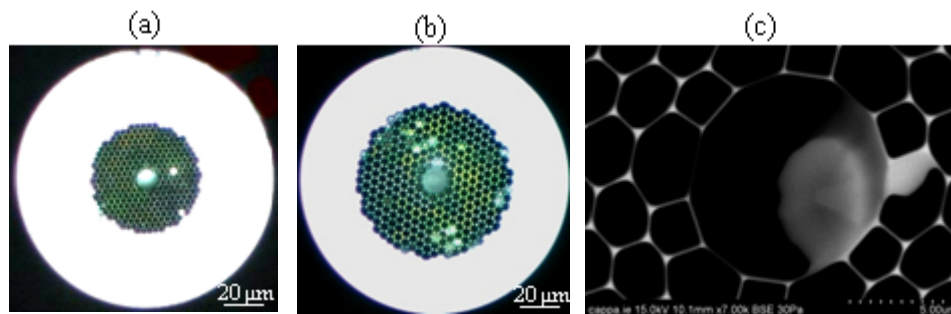


Figure 4: Cross section of fibres filled with hydrogel (a) HC-PCF 1060 (b) HC-PCF 1550 (c) SEM image of hydrogel inside the core of fibre HC-PCF 1550.

3.1.1 Spectral and near-field analyses. In order to analyse the transmission spectrum of the filled HC-PCFs after the hydrogel formation, the 530 nm bandpass filter was removed from the set-up and the entire supercontinuum was launched through the fibre via a x4 lens and its output was coupled into a multimode (MM) optical fibre for spectral analysis. For comparison, the supercontinuum spectrum (black line - Figure 5a) was obtained in the absence of the HC-PCF, but using the same lens configuration, albeit with a lateral shift of the output lens assembly. The spectral results for a 20 cm HC-PCF 1060 before and after filling with hydrogel are also shown in figure 5a. In this case the overall signal power was maximised. Strong attenuation was observed at all wavelengths for the empty fibre (red filled circles). In the presence of hydrogel (blue open squares), however, there was a substantial improvement in transmission for all wavelengths, especially in the infrared region (figure 5(c)), probably due to the change in refractive index. Similar effects were recently observed for aerogel filled HC-PCFs [23], where a shift in the bandgap was observed accompanied by the appearance of region of broadband guidance.

Such results are intriguing, especially for the empty fibre, as one would expect propagation for the allowed bandgap only. Therefore the setup in figure 3 was modified in order to analyse the near-field images after light propagation through the fibre samples. This was achieved using a CCD camera (LTC 0335 Series Dinion Monochrome), positioned as figure 3b. In this case, it was necessary to reduce light intensity using neutral intensity filters. It is clear from figure 5b that light guidance occurred not only through the core, but also through the cladding microstructure and the solid cladding even for the empty fibre.

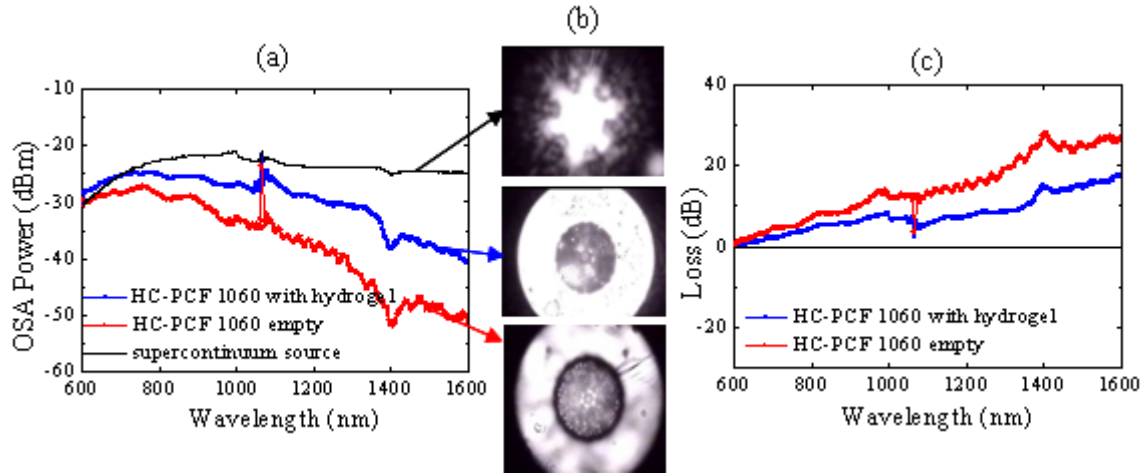


Figure 5: (a) Spectra for supercontinuum source without any fibre (black line), after empty fibre (red) and after hydrogel filled fibre (blue). (b) CCD images before HC-PCF (top), after hydrogel filled (middle) and empty (bottom) fibres. (c) Inferred insertion loss for empty (red) and hydrogel filled (blue) fibres.

In order to investigate the propagation of the broadband supercontinuum source through the filled hydrogel fibre, wavelength dependent images were taken by adding filters just before light was launched through the fibre samples. The same setup as described in section 2.3 was used, including the x4 focusing-lens. Figure 6 below illustrates the preliminary results obtained with hydrogel filled fibre HC-PCF 1060 of approximately 46 mm in length. In figure 6(a), an un-filtered supercontinuum signal was transmitted, which shows that both the core and the cladding were inevitably excited. Filtering of the source signal, using standard bandpass filters (full width at half maximum ~ 10 nm), with the centre wavelengths at 530, 650, 800 and 1050 nm respectively, enabled the analysis of the transmission of specific wavelengths. In figure 6(e), where the centre wavelength is close to original bandgap (1060 nm) marginal light confinement was observed with no light confined in the core. At 650 nm and below, however, light was observed in the core of the fibre, but with substantial propagation within the cladding area due to the over illumination of the fibre outside the core. Note that at 650 nm the light intensity within the core was maximised, confirming the capture and guidance of light at this wavelength, and suggesting the bandgap might have shifted to this value. This guidance wavelength corresponds closely to the absorption and emissions wavelengths of the common fluorophores suitable for use of detection of DNA in a hydrogel matrix.

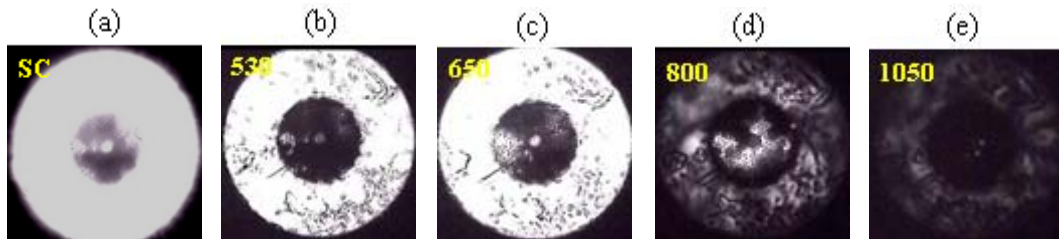


Figure 6: CCD images of an HC-PCF 1060 filled with hydrogel after transmission of a broadband supercontinuum source un-filtered (a) and filtered (b, c, d, and e). Captions indicate the centre wavelengths.

We believe that, by replacing the focusing x4 lens with a x40 lens for the optical setup shown in figure 3, it would be possible to reduce the solid cladding propagation and ensure that primarily the fibre core of a filled hydrogel HC-PCF would be illuminated. This improvement will be shown in section 3.1.2.

3.1.2 Refractive index characterisation. It is possible to estimate if there is a bandgap shift of the HC-PCF used, if the refractive index of the filling material is known [8]. In order to determine the wavelength dependence of mode propagation within the hydrogel filled fibre, the refractive index of hydrogel was estimated. For an initial estimate, hydrogel spots (diameter ~ 7 mm) were formed on a microscope slide, which

was first modified as described in the protocol in section 2.1. Pre-polymer solution was deposited onto the silanised glass surface, and irradiated with light from an epi-fluorescence microscope filtered with a dichroic Cy3-filter (wavelength range 550/570 nm). The photo-polymerisation was initiated upon light exposure of the pre-polymer solution, using illumination times in the range from 5 to 120 seconds. These hydrogel spots were analysed using conventional ellipsometer, where changes in light polarisation can be used to determine certain properties of the material, including optical constants [24]. In this case a M-2000 Spectroscopic Ellipsometer from J.A.Woolen Co. Inc. with CompleteEASE data analysis software was used. The measurements (figure 7(a)) were significantly degraded, which we believe is due to the rough, porous surface of the hydrogel, which could have presented multiple reflecting surfaces, affecting the observed overall polarisation rotation. The estimation for n and k values (figure 7(b)), assuming the fitting Cauchy model from figure 7(a), and it gives a refractive index of approximately 1.46 at 600 nm.

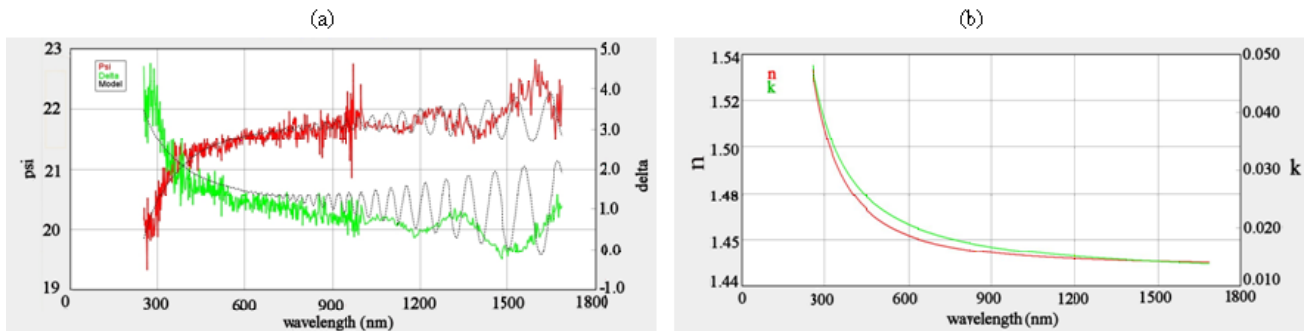


Figure 7: (a) spectroscopic ellipsometric (SE) data measurements, (b) the fitting Cauchy model for n and k .

Furthermore, as seen above, the natural porosity of the hydrogel gives rise to noise in the ellipsometric results, which, in turn, could give rise to a non-uniform hydrogel distribution along the fibre length (figure 4c). The non-uniformity may be enhanced by the end illumination technique used here, due to the interaction between the partly formed hydrogel and the 530 nm photo-initiation signal. This may have two significant effects. Firstly, the non-uniformity will result in a significant increase in scattering, increasing the background loss of the system. Secondly, the non-uniformity will result in large variations in the bandgap shift. However, we could anticipate that the diffraction length of a signal outside the bandgap is significantly lower than the porous size, which will result in a spatially averaged bandgap shift, with a degree of residual broadening. We believe that techniques to minimise porosity [22] and improve volumetric uniformity throughout the core and microstructure cladding should minimise scattering and cladding propagation and a well-defined bandgap shift should be observed.

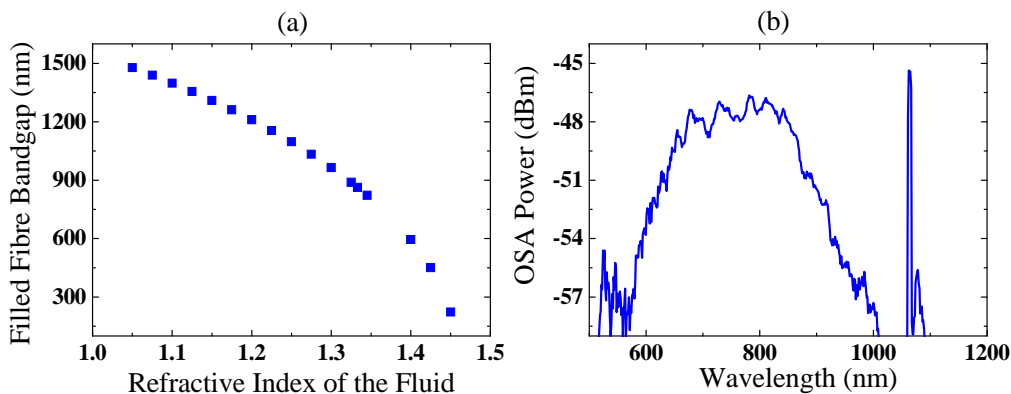


Figure 8:(a) dependence of bandgap shift of a HC-PCF 1550 on refractive index; (b) transmission spectrum of a 21 cm HC-PCF 1550 filled with hydrogel.

Assuming the analysis presented in [8] which is represented in figure 8a, it seems that for the spectral dependence of the refractive index shown in figure 7b (~1.46), the bandgap would shift to wavelength below 200 nm for an HC-PCF 1060 and HC-PCF 1550, contrary to the observed wavelength shift in figure 8b. Note that, for this spectrum, a x40 launching-lens was used in order to confine the light at the filled core, unlike figure 4a. This result reveals a bandgap shift to around 700-800 nm, which would indicate a refractive index of about 1.33. The composition of the 25% pre-polymer solution contains 75% water which gives an estimated refractive index for the hydrogel of 1.33, which is close to that of water, for wavelength ~863 nm, as expected [8].

3.2 DNA probe immobilisation

The hydrogel is a 3D matrix specifically designed for detection of DNA microarrays. In this paper we report the detection of Cy5-labelled probe DNA strands immobilised with the hydrogel matrix within HC-PCFs. In order to immobilise probe DNA within the hydrogel, amino-modified oligonucleotides (Metabion International AG) were added to a volume of the pre-polymer solution to give a concentration of 260 nM. Roughly 0.1 mg of NHS-PEG-acrylate cross-linker in powdered form (Nektar Therapeutics, Huntsville, AL) was added to this mixture, in order to provide an anchor or binding site for the probe sequence. The formation of the hydrogel and the immobilisation of probe DNA occur simultaneously during the photo-polymerisation process (as per section 2.3), as the NHS-PEG-acrylate cross-linker allows for the covalent attachment of the probe to the polymer backbone.

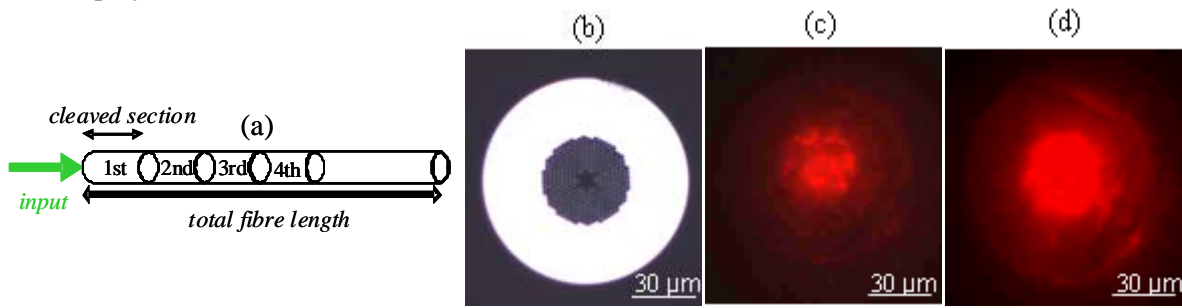


Figure 9: (a) Fibre cleaving schematic. Also showing cross-section images (x20 magnification) of a HC-PCF 1060 for (b) an empty fibre as a visual guide; and Cy5-fluorescence for hydrogel and DNA filled fibres with (c) 20 ms and (d) 50 ms integration times.

A standard fluorescence microscope (Olympus BX51 with Cell[^]F software) was used to analyse the fluorescence effectively and reproducibly. The microscope contained the appropriate dichroic filters to excite the Cy5-fluorophore (~650 nm) and detect only the fluorescence (~670 nm). However, it was not possible to analyse the entire length of fibre, and therefore, for this preliminary experiment, short pieces of the prepared fibres (0.3 to 1 cm) were cleaved (figure 9(a)) and mounted vertically on a microscope slide. Precise cleaving and dust-free surfaces were paramount. Figure 9 (c) and (d) shows the cross-section of the 4th centimetre cut from input of a HC-PCF 1060 and the Cy5 fluorescence analysis for two different integration times.

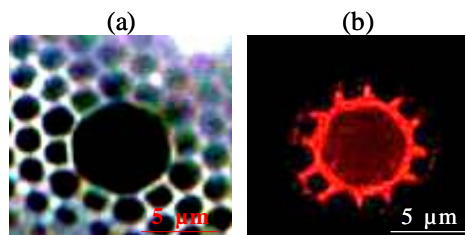


Figure 10: Cross-section images (x100 magnification) of a HC-PCF 1550 for (a) an empty fibre, and (b) for hydrogel and Cy5 labelled DNA filled fibre with 100 ms integration time.

The same analysis was performed with a HC-PCF 1550 sample. Figure 10 shows the results obtained with a 0.3 cm length sample, x100 magnification and 100 ms integration time. Although the fibre sample was extremely short, it was still possible to detect the Cy5-fluorescence. Note that, in the case of the HC-PCF 1550 nm fibre, the shifted bandgap did not correspond to the fluorescence wavelength, and the detected fluorescence appears to be guided by the solid elements of the microstructure. However, it is also possible that the launch conditions (x100 lens) lead to increased coupling to the cladding modes. Re-optimisation of the launch, taking into account any changes in fibre N.A. due to the presence of the hydrogel, may improve these results.

It is important, however, to distinguish between the results obtained above and auto-fluorescence, as hydrogels are known to be highly fluorescent in the visible range, due to the use of Eosin Y as a photo-initiator for the silanisation process [16]. The hydrogel auto-fluorescence was detected for Cy3 dichroic filters, instead of Cy5, where the excitation wavelength is ~ 550 nm and the emission at ~ 570 nm. The hydrogel filled fibre with and without labelled DNA microarrays inside its structure were analysed. Figure 11 shows an example of the detected auto-fluorescence of the hydrogel in the fibre in comparison to Cy-5 labelled DNA-probe immobilisation detection. Hydrogel auto-fluorescence is observed in figure 11b at the locations suggested by the optical image of the fibre (figure 11(a)). Although fluorescence is only observed at locations corresponding to hydrogel auto-fluorescence, not all hydrogel sites exhibited Cy-5 fluorescence, consistent with the randomness of the DNA immobilisation.

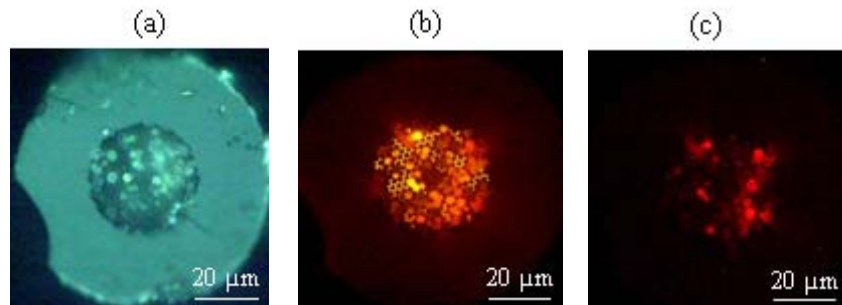


Figure 11: Cross section images (x100) magnification of a HC-PCF 1060, for (a) bright light, (b) for Cy3 dichroic filter, with integration time 2ms (c) for Cy5 dichroic filter, with the integration time 2s.

In figure 12 the hydrogel is formed within the core and cladding holes containing no probe DNA molecules. The hydrogel auto-fluorescence was observed in the range ~ 570 nm as expected, while there was no light detection in the range ~ 670 nm due to absence of Cy5 tagged DNA.

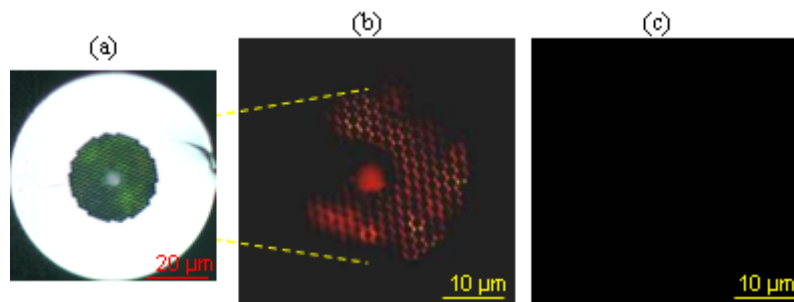


Figure 12: Cross section images of a HC-PCF 1060, for (a) bright light, with (x50) magnification, and (b) (x100) magnification for Cy3 dichroic filter, with integration time 2ms (c) (x100) magnification for Cy5 dichroic filter, with the integration time 2s.

4. Conclusion

We have demonstrated the growth of a 3D hydrogel matrix within the holes of a Hollow-Core Photonic Crystal Fibre. The light behaviour of these hydrogel filled fibres and the estimated refractive index of the hydrogel is in good agreement with theoretical discussions. Moreover Cy5-labelled DNA immobilisation and

hydrogel auto-fluorescence inside HC-PCF structure were detected. The integration of the hydrogel with PCF is a promising technique for highly sensitive bio-detection devices. Such a scheme would provide a compact solution as a point-of-care device for rapid detection of bio samples.

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