Letter

Multiphoton fluorescence excitation and detection with a single negative curvature hollow core fibre

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Abstract

In this letter, we propose a single fiber-based sensor setup allowing for simultaneous excitation and detection of multiphoton fluorescence. Presented sensor’s key element is the negative curvature hollow core fiber (NCHCF) with three transmission bands in the visible spectral range (414–423 nm, 510–552 nm and 680–784 nm), allowing for nearly dispersion-free guidance of 160 fs-long laser pulses at 730 nm photon wavelength. Total temporal broadening of a laser pulse propagating in the proposed sensor setup is only (7 ± 1) fs. The NCHCF output beam was additionally focused with a microlensed, multimode fiber tip, increasing the efficiency of multiphoton absorption. The usefulness of the sensor for the multiphoton spectroscopy experiments is tested on the solutions of fluorescein and flavin adenine dinucleotide. This optical fiber sensor combines simplicity, minimal size, and good optical properties, and can be found an interesting solution for the non-linear optical methods used in chemistry, biology and medicine.

Keywords: microstructured optical fibers, hollow core antiresonant fibers, photonic fiber sensors, multiphoton fluorescence, non-linear optics

(Some figures may appear in colour only in the online journal)
1. Introduction

Ever since the implementation of the hollow core photonic bandgap fiber (HC-PBF) idea nearly 20 years ago [1], the interest in optical fiber-based photonic structures is constantly growing. Indeed, the appearance of the HC-PBF which combined the purity of light guiding medium (air) with the control over transmitted light beam’s trajectory was a true milestone for development of the optical fiber technology. Numerous publications have emerged, proving that HC-PBFs are useful for delivery of high power laser beams and ultrashort laser pulses [2–5], as well as for different types of sensing and imaging applications [6–8]. Further development of the microstructured optical fibers has resulted in the appearance of a negative curvature hollow core fiber (NCHCF) [9, 10]. The NCHCF’s main advantages over a standard HC-PBF’s are less complicated cladding structure and, usually, better optical performance in terms of the transmission bandwidths and dispersion properties. Although lots of work has already been done in terms of guiding light in the NCHCF from the UV [11, 12] up to mid-IR spectral range [13], the topic of optical sensing utilizing these particular fibers is still slightly underdeveloped, with researchers focusing mainly on the topic of filling such fibers with gases [14] or liquids [15, 16]. Nevertheless, excellent power handling capabilities [17], combined with almost negligible dispersion across wide optical bands [18] seem to make these fibers perfectly suited for the non-linear optical (NLO) methods, such as multiphoton excited fluorescence (MPEF) or second harmonic generation. In 2016, Sherlock et al [19] have proven that NCHCF is useful for multiphoton microscopy, while in our previous work [20] its application in a multiphoton fluorescence sensor has been shown. However, in both these cases NCHCF’s were used only for the transmission of ultrashort laser pulses.

In this letter, we present a proof-of-concept of the MPEF sensor setup based on a single NCHCF. By utilizing two out of three transmission bands in the visible spectral range of the fiber, we have successfully excited and collected multiphoton fluorescence from aqueous solutions of fluorescein and flavin adenine dinucleotide (FAD), out of which the latter is an important endogenous fluorophore, playing a significant role in the cellular metabolism and being one of the sources of emission in the multiphoton microscopy [21]. To increase the efficiency of MPEF, the NCHCF output beam was additionally focused by a microlensed, multimode fiber (MMF) tip. Obtained results pave the way towards the idea of photonic fiber sensors reduced to a diameter of a single fiber. Such sensor combines both the minimal size, and excellent optical properties, and can be tailored for the NLO-based measurement methods, reducing the problem of diagnostic invasiveness in biological and medical applications.

2. Experimental setup

2.1. Measurements of the NCHCF transmission in the visible spectrum region

The NCHCF utilized in the setup is presented in figure 1. Its structure has the well-known ‘revolver’ shape, consisting of eight glass capillaries surrounding the region of the hollow core, very similar to the one presented in [18]. Its core diameter and wall thickness of the capillaries are 21 and 1.5 μm, respectively. To determine spectral properties of this fiber in the visible region, its transmission spectrum was measured by coupling a broadband light source (AQ 4305, Yokogawa) into the NCHCF. The light source was coupled into the NCHCF with a ~50 cm long, single-mode (SM) fiber (SMF-28, Corning) pigtail, in a similar way as in [12], ensuring that no cladding/glass modes of the NCHCF were excited. To fully confirm the latter, the NCHCF’s output end, with the white light source coupled into it, was additionally imaged under a light microscope.

The output end of the NCHCF was coupled into a standard, 50 μm core diameter, ~1 m long, multimode fiber (OM3 type) pigtail, connected to an USB spectrometer (USB2000, OceanOptics). Since the NCHCF was ~1.5 m-long, it was not possible to measure its attenuation with the cut-back method. However, in the presented setup, by simply removing the NCHCF and coupling the SM pigtail output into the MM pigtail, it was possible to determine the insertion loss (IL) introduced by the NCHCF.

2.2. Microlensed fiber tip (MFT) fabrication

Proper focusing of an excitation beam is an important factor for the efficiency of MPEF [22]. However, the standard approach, employing microscope objectives or bulk lenses, was unacceptable, as it would significantly increase the size of the proposed sensor. Thus, the NCHCF has been equipped with a custom-made MFT, allowing for both the excitation beam focusing and keeping the sensor’s diameter as small as a single fiber. Although the idea of such lensed fiber tips was presented many years ago [23], combining it with photonic crystal fibers is still not very well established. Few concepts of such microlensed photonic crystal fibers have already been shown [24–26], with the most interesting one presented recently by Lombardini et al [27], where a 30 μm microsphere was inserted into the core of a double-clad, Kagomé-lattice hollow core fiber, and spliced with a CO2 laser. The GRIN lens approach, presented by Kasztelan et al [28], although not applied directly to a microstructured fiber, is also promising. Nevertheless, successful arc fusion splicing of the NCHCF has not been reported so far. The photonic structure collapse has already been stated a problem [29–31] in the case of the HC-PBFs. The same problem occurred for the NCHCF, and due to its limited length, a decision has been made to omit the splicing and perform a butt-coupling procedure between the NCHCF and MFT instead. This approach has additionally ensured better control over the MFT output beam profile, which was an important factor in the experiment, and is further described in the results section.

The microlens concept is based on the idea presented by Kim et al [32]. However, the combination of a coreless silica fiber (CSF) and a polymer microlens tip was found to be relatively sophisticated in terms of fabrication, as it required very short lengths of the CSF (≤700 μm) and precisely deposited droplets of the UV curable polymer. Instead, a MM fiber...
As stated earlier, the efficiency of MPEF depends on the focusing properties of the excitation optics. However, it is also influenced by the excitation laser pulse width (\(\tau\)). Thus, in addition to the measurements of the MPEF itself, focusing properties of the fabricated MFT and temporal broadening of the excitation laser pulse in the proposed fiber sensor setup were also determined. Optical setup for all the aforementioned measurements is presented in figure 2. An ultrafast Ti:Sapphire oscillator (Mira-HP, Coherent) was used as a light source, providing laser pulses of width \(\tau_{\text{laser}} = 160\) fs at \(\lambda = 730\) nm wavelength and repetition frequency \(f_{\text{rep}} = 80\) MHz. The laser beam, reflected with a dichroic mirror (650dcspxr, Chroma), was coupled into the NCHCF with a 10× microscope objective. The NCHCF’s output beam was then coupled into the MFT with a XYZ translation stage (MBT616D/M, Thorlabs). The average power at the MFT output was \(P_{\text{avg}} = 60\) mW for all the aforementioned measurements, unless stated otherwise. The element at the output of the fiber sensor was exchanged according to the measurement needs, i.e. to determine the fabricated MFT’s output beam spatial properties, such as the output beam profile in the farfield, lens working distance, and beam diameter at the focal point, a beam profiler (BP-209VIS, Thorlabs) and CMOS camera (HDCE-X5, Carton Optical Instruments) were used. Autocorrelator (pulseCheck, A.P.E.) was used to measure excitation laser pulse widths in the setup with and without the optical fiber sensor. Finally, MPEF was excited in the fluorescein and FAD solutions (Sigma-Aldrich), placed in a custom-made sample cuvette. The details of all the measurements mentioned above, will be further described in the following sections.

2.3. Optical setup for measurements of the MPEF, autocorrelation function of ultrashort laser pulses and focusing properties of the MFT

(FG025LJA, Thorlabs) was used, with its tip directly shaped with a commercial fiber fusion splicer (FSU975, Ericsson). We used the splicer’s fiber tapering programme, consisting of the following parameters: 3 pulling steps with 20 mA arc current (\(I_{\text{arc}}\)) at each, pulling times (\(t_{\text{pull}}\)): 2 s, 1 s and 1 s for steps 1, 2 and 3, respectively. The fiber tip was additionally moved \(\approx 250\) \(\mu\)m away from the centre of the arc, allowing to obtain smaller tip curvatures. Approximately 2 cm long MM fiber piece with the lensed tip was then cleaved with a fiber cleaver (FC-6S, Sumitomo Electric), eventually making the MFT, used later for the multiphoton spectroscopy experiments. The choice of the aforementioned MM fiber as a MFT material was based upon it meeting the criteria of matching the core size and numerical apertures of the NCHCF (\(d_{\text{core,NCHCF}} = 21\) \(\mu\)m, \(N_{\text{NCHCF}} \approx 0.04\)), allowing for efficient coupling between both, without the risk of exciting cladding modes in the MM fiber tip. The fact that core diameters of the NCHCF and MFT are similar has also positively influenced the coupling of the collected fluorescence signal between both fibers.

2.3.1. Focusing properties of the MFT. Coupling the NCHCF’s output into the MFT is one of the factors to be concerned during the experiment. For such short fiber lengths, different excitation geometries of the fiber core can result in very similar output powers, but carried by different modes, which, in turn, can influence the beam divergence at the fiber output. Therefore, to correctly determine focusing properties of the MFT and justify its fabrication, we have measured the output beam profiles in the far field, using the previously mentioned beam profiler with its dedicated software (ThorlabsBeam, Thorlabs). This measurement was performed for two MM fiber tips—without and with the microlens at its end. The distance between the output of the fiber tips and the beam profiler was 10 mm. The beam profiler was placed on a single-axis translation stage, allowing to scan the output beam along its main optical axis, and, in turn, determine the output beam divergence for both fiber tips. The goal was to ensure similar mode excitation conditions of the latter, which resulted in similar output powers and beam profiles at the far field. The final step was the measurement of the MFT’s working
distance and beam spot diameter. In this case, the beam profiler was replaced with the previously mentioned CMOS camera, equipped with a 20× microscope objective. The camera was placed on a XYZ translation stage, and the output beam of the MFT was scanned along its optical axis. Scan spanned from the sensors output plane to the point of minimal beam diameter, with 10 µm steps.

2.3.2. Autocorrelation function measurements of the ultra-short laser pulses transmitted in the fiber sensor setup. Dispersion in optical fibers is a well-known problem, causing a substantial temporal elongation of transmitted laser pulses, and making it difficult to obtain high power densities required by the MPEF. The sensor consisted of 2 different types of optical fibers—a hollow-core fiber (NCHCF) and a classic, solid core one (MM fiber used for the fabrication of MFT), out of which the latter was expected to have a significant influence on the fs long laser pulses. Thus, the previously mentioned autocorrelator was used to determine the temporal shape of the pulses and their widths for 3 different cases—without any optics (further referred to as ‘base pulse’), the base pulse coupled into the NCHCF and the NCHCF output pulse coupled into the MFT.

2.3.3. MPEF measurements. Two different commercially available fluorophores were used: fluorescein and FAD. While fluorescein can be treated as a control compound in our experiment, with relatively high two-photon absorption cross...
sections within the 700–800 nm range, the choice of FAD is due to its important role in multiphoton microscopy. Both compounds were dissolved as suggested by their specification sheets [33, 34], while their concentrations (C_{FAD} = 10^{-5} M, C_{Fluo} = 10^{-4} M) were chosen according to [21, 35], ensuring the correctness of their emission spectra. Solutions were placed in a 0.5 mm-deep V-groove channel, milled in a small aluminium block, with a 0.1 mm-thick cover-glass placed between the MFT and the sample. Fluorescence signal was collected back by the MFT, transmitted through the NCHCF, and subsequently collimated by the 10× objective and directed to the MM fiber bundle (BF13LSMA01, Thorlabs) ended with the USB spectrometer (5 s integration time).

3. Results and discussion

3.1. NCHCF transmission in the visible spectral range

Results presented in figure 3 confirm both the high confinement of the coupled halogen lamp’s VIS spectrum within the NCHCF core (figure 3(a)), and the presence of VIS transmission bands (figures 3(b) and (c)). To determine the spectral widths of the aforementioned transmission windows, the corresponding spectral intensities for the recorded spectra from figure 3(b) were substituted into the IL formula:

\[ \text{IL}(\lambda) = 10 \log_{10} \left( \frac{I_{\text{Lamp}}(\lambda)}{I_{\text{NCHCF}}(\lambda)} \right), \]

where \( I_{\text{Lamp}}(\lambda) \) and \( I_{\text{NCHCF}}(\lambda) \) are the measured halogen lamp spectral intensity in a setup with and without the NCHCF, respectively. Obtained IL values are plotted in figure 3(c). Three windows of reduced losses (IL \( \leq 10 \) dB) of the NCHCF are revealed: blue (414–423 nm), green (510–552 nm) and red (680–784 nm), with corresponding loss minima: IL_{BLUE} = 8.93 dB at 420.5 nm, IL_{GREEN} = 6.29 dB at 537.2 nm and IL_{RED} = 3.81 dB at 741.2 nm. The last two windows are particularly interesting for the biochemical applications—the green one covers the emission spectrum of many important endogenous fluorophores, like melanin, lipofuscin or flavins [36], while the red one covers the wavelengths well suited for the multiphoton excitation of the aforementioned compounds.

3.2. Light focusing properties of the MFT

Table 1. Output beam (\( \lambda = 730 \) nm) parameters for different fiber setups used in the experiment. The sine of divergence (\( \sin(\alpha) \)) values are included for the sake of easier comparison with the numerical aperture values of the fibers. The placement of the output plane is presented in figure 4(a). Working distances and focal point beam diameters were not observed for the fibers without a microlens (NCHCF and NCHCF + lensless MMF tip).

| Fiber type                      | Divergence angle \( \alpha \) \( ^{\circ} \) (\( \sin(\alpha) \) value in the parenthesis) | Working distance WD [\( \mu \)m] | Output plane beam diameter [\( \mu \)m] | Focal point beam diameter [\( \mu \)m] |
|--------------------------------|-------------------------------------------------|-------------------------|--------------------------------------|--------------------------------------|
| NCHCF                          | 2.34 (0.04)                                      | 21                      |                                     |                                     |
| NCHCF + lensless MMF tip        | 2.27 (0.04)                                      | 22                      |                                     |                                     |
| NCHCF + MFT                     | 4.96 (0.09)                                      | 42                      |                                     | 15                                   |

In both cases the profiles are Gaussian-like, with the MFT output profile being more uniform at its transverse (\( Y \)) plane and significantly larger in terms of the beam diameter than its lensless counterpart. Divergence angle (\( \alpha \)) of the output beam increased from \( \sim 2.7^{\circ} \) for the lensless MMF tip, to \( \sim 4.96^{\circ} \) for the MFT, suggesting that the signal collection capabilities of the latter are significantly better. In the case of the lensless tip, the fact that its divergence value is well below the numerical aperture of the fiber specified in its datasheet (\( NA_{\text{MMF}} = 0.1 \), which corresponds to \( \alpha = 5.74^{\circ} \)) can be explained by the short length of the tip, which makes it impossible to properly excite all the core modes, hence reducing the divergence of the output beam. Output average powers for both fiber tips were 60 \( \pm 1 \) mW under similar beam profiles. Therefore, it was assumed that excitation conditions of the cores of the lensless tip and the MFT were nearly identical. Focusing properties of the MFT are confirmed by imaging its output beam cross-sections on two planes: (i) an output plane (figure 4(d)), placed at the MFT’s face, and (ii) the focal plane (figure 4(e)), found to be 200 \( \mu \)m away from the output one. That distance is also the working distance of the MFT (\( WD_{\text{MFT}} \)). The beam diameters at the MFT’s output (\( d_{\text{MFT, output}} \)) and focal planes (\( d_{\text{MFT, focal}} \)) were 42 and 15 \( \mu \)m, respectively. Although the obtained value of \( d_{\text{MFT, focal}} \) is not enough for high resolution imaging applications, it is enough for the purpose of multiphoton spectroscopy experiments.

3.3. Ultrafast laser pulse width measurements in the fiber sensor setup

Obtained autocorrelation traces of the laser pulses are presented in figure 5. It is assumed that pulses have a sech\(^2\) temporal shape, and their widths were calculated as \( \tau_{\text{pulse}} = \tau_{\text{ACF}}/1.54 \). The results are rounded up to the value of autocorrelator’s resolution, which is \( \pm 1 \) fs. Temporal broadening of the base laser pulse, caused by the NCHCF’s dispersion, was as low as 2 fs (from 162 to 164 fs), similar to the previously reported results [20]. After coupling the NCHCF output beam into the MFT the pulse was broadened by another 5 fs (169 fs). This result shows that the MFT had a major contribution to the total increase in the transmitted pulse’s width, despite its short (2 cm) length. However, the total base laser pulse broadening is 7 fs, and can be considered negligible in this experimental setup.
working distance, spot diameter, divergence) of the additional work: the output beam parameters (beam profile, divergence) of the NCHF are shown, explaining the distortions in the spectral shape of fluorescein and FAD emission. Of course, the presented optical fiber sensor concept requires being high enough to exceed a 10 dB-loss limit.

The resultant shape of the fluorescein emission spectrum is similar to its free-space collected counter-part [35], as it fits well within the W2. Compared to fluorescein, the FAD fluorescence spectrum collected in free space is slightly red-shifted and spectrally broader. Thus, the observed change in the FAD emission spectrum shape, collected via the fiber sensor, is larger. The W2 window covers only the strongest part of the FAD fluorescence [21], making the collected spectrum much more symmetrical. Although in case of this experiment the spectral width of the W2 is rather a drawback, it has a potential of being considered an advantage for other applications, i.e. when filtering different emission spectra would be required.

4. Conclusions

To summarize, a multiphoton fluorescence excitation-emission sensor based on a single NCHCF was presented. Three transmission bands in the VIS region of this fiber were reported: blue (414–423 nm), green (512–528 nm) for the fluorescein and FAD, respectively, and are in good agreement with the results previously reported in [21], [35]. Both emission spectra exceed the boundaries of the NCHCF transmission window W2 due to their intensities being high enough to exceed a 10 dB-loss limit.

Figure 6. Collected MPEF spectra of FAD and fluorescein. The boundaries of W2 transmission window (green dashed line) of the NCHF are shown, explaining the distortions in the spectral shape of fluorescein and FAD emission.

3.4. Multiphoton fluorescence measurements

Fluorescence emission spectra of tested chemical compounds are presented in figure 6. Their emission maxima are 520.2 and 528.6 nm for the fluorescein and FAD, respectively, and are in good agreement with the results previously reported in [21], [35]. Both emission spectra exceed the boundaries of the NCHCF transmission window W2 due to their intensities being high enough to exceed a 10 dB-loss limit.

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