Photonic crystal fiber based evanescent-wave sensor for detection of biomolecules in aqueous solutions

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We demonstrate highly efficient evanescent-wave detection of fluorophore-labeled biomolecules in aqueous solutions positioned in the air holes of the microstructured part of a photonic crystal fiber. The air-suspended silica structures located between three neighboring air holes in the cladding crystal guide light with a large fraction of the optical field penetrating into the sample even at wavelengths in the visible range. An effective interaction length of several centimeters is obtained when a sample volume of less than 1 μL is used.

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Photonic crystal fibers[1] (PCFs) are characterized by a pattern of air holes running along the entire length of the fiber. With careful choice of fiber parameters, such as lattice pitch Λ and air-hole diameter d, a large fraction of the optical field propagates through the fiber as an evanescent field,[2] penetrating into samples positioned in the air holes. Previously, solid-core PCFs were used for evanescent-wave sensing of gases.[3] In conventional fibers one must remove the coating and cladding to obtain an overlap between the optical field and the sample. The air holes of the PCF provide access for the sample to regions with a strong optical field and PCF-based evanescent-wave sensors can keep both the cladding and the coating on, thereby ensuring a robust device. For wavelengths in the visible range, a strong evanescent field from modes guided in the solid core requires a lattice pitch well below 2 μm and a large relative air-hole diameter, $D = d/\Lambda$. Such fibers are difficult to fabricate and furthermore probe only the sample volume placed in the first ring of air holes surrounding the core. Here we demonstrate highly efficient evanescent-wave sensing in the photonic crystal of a PCF without a solid core,[4] henceforth referred to as a strong-penetration photonic crystal fiber (SP-PCF). In this fiber, visible light propagates in the cladding crystal, where it interacts strongly with sample volumes in all the air holes of the fiber. The functionality of the SP-PCF-based evanescent-wave sensor is illustrated by detecting Cy5-labeled DNA molecules in submicroliter volumes of aqueous solutions positioned in the air holes of a SP-PCF with a lattice pitch of 4 μm.

A micrograph of a SP-PCF is depicted in Fig. 1a. Air holes with an average diameter of 3.4 μm are arranged in a triangular lattice with a 4-μm pitch. For evanescent-wave sensing applications, the crystal should ideally cover the entire microstructured part of the fiber. A hollow fiber center is difficult to realize, and in our experiments it represents only a dead volume with little interaction between light and sample. As illustrated in Fig. 1b, the cladding crystal resembles a large number of air-suspended index-guiding corelike structures connected by thin silica bridges. Given the width of these structures, there is a significant penetration of the evanescent field into the sample, as is also the case for wavelengths in the visible range. We used a freely available full-vectorial plane-wave method,[5] to determine the fraction γ of the optical field in the fundamental space-filling cladding mode that propagates in the aqueous sample. For light with a free-space wavelength $\lambda^* = 650$ nm guided through a water-filled SP-PCF we find that $\gamma = 5.2\%$. In addition to having a strong evanescent field, the SP-PCF has the advantage that sample volumes positioned in all the air holes are probed. Figure 1c shows a micrograph of the distribution of the optical field when the entire microstructured part of the opposite fiber end is illuminated by a white-light

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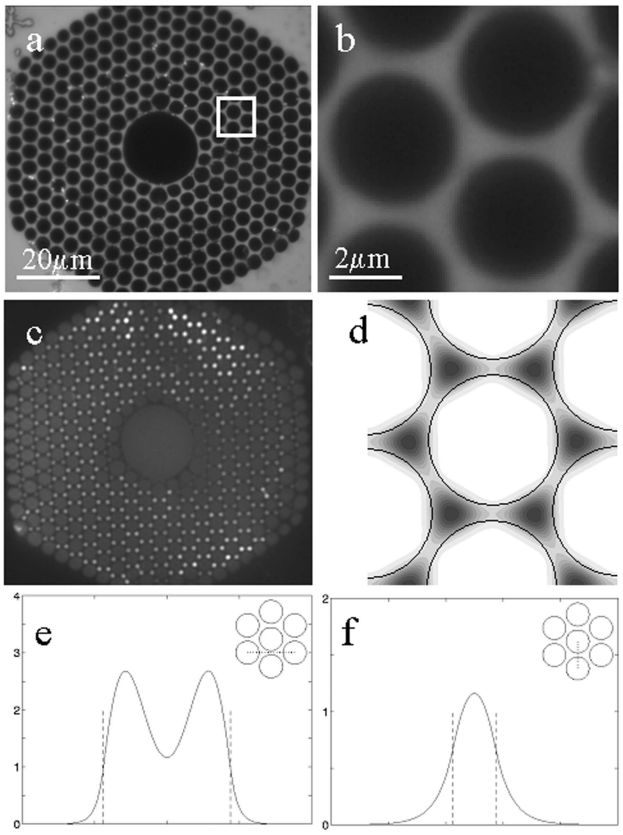

Fig. 1. a, Micrograph of the microstructured part of the SP-PCF. b, Close-up of the white-outlined section in a, showing the air-suspended corelike structures in the cladding crystal. c, Micrograph of the fiber with the entire cladding crystal illuminated from the opposite fiber end. d, Field distribution in the sample-filled fiber calculated with the plane-wave method.[5] e, f, Mode field profiles calculated along the dotted lines shown in the respective insets.

source. Light is evidently guided through the silica in the entire cladding, with the intensity peaking in the corelike structures. A calculation of the field distribution for the cladding crystal assuming that $D = 0.9$ supports this observation, as can be seen from Fig. 1d. To estimate the penetration of the optical field into the sample along the perimeter of the air holes, field intensity profiles were calculated for a crystal with $D = 0.85$, along the dotted lines indicated in the insets of Figs. 1e and 1f. The depicted intensity curves also show that the guided mode has a significant intensity in the thin silica bridges and most importantly that the strength of the evanescent field just outside the bridges is comparable with that outside the corelike structures. Thus the light propagating through the fiber probes the sample volume along the entire perimeter of all the air holes of the SP-PCF.

For comparison, we repeated the calculations of the evanescent-field strength for a solid-core PCF with a triangular lattice of air holes and a relative hole size of $D = 0.9$. The calculations show that the penetration of the optical field into the air holes surrounding the solid core is 0.2%, 2.4%, and 5.2% for fibers with a lattice pitch Λ of 4.0, 1.6, and 1.2 μm, respectively. Solid-core PCFs with the same strength of the evanescent field as that achieved in the SP-PCF evidently requires a much smaller lattice pitch. Because of the small lattice pitch, the fiber will be significantly more difficult to manufacture than the SP-PCF, and furthermore the light guided in the solid core probes only the sample positioned in the first ring of air holes surrounding the core.

The labeled DNA was detected by obtaining a Cy5 absorbance spectrum derived from the transmission spectrum of the sample-filled PCF. In the presence of an absorbing molecule in the aqueous solution, there is a frequency-dependent drop in the intensity of the transmitted signal as described by the Lambert–Beers law[6]: $I(v) = I_0(v) \times 10^{-A(v)}$, where $I_0(v)$ and $I(v)$ are the intensities before and after the sample, respectively, and v is the frequency of the light. The absorbance, A, is proportional to the interaction length, L, and the concentration of the biomolecules, c, through $A(v) = \epsilon(v)cL$, where $\epsilon(v)$ is the frequency-dependent molar extinction coefficient of the absorbing molecule. The frequency dependence of the background is compensated for by subtracting a reference spectrum taken on a PCF containing pure water, thereby replacing $I_0(v)$ with a constant I_0 in the Lambert–Beers law. The frequency-dependent absorbance introduces a dip in the transmission spectrum centered at the wavelength where the Cy5 absorbance spectrum peaks, $\lambda^* = 650$ nm. The air holes of the PCFs were filled with the aqueous samples, either by utilizing capillary forces or by applying pressure to the sample. Calculations based on the Navier–Stokes equations for the flow of liquids assuming circular air holes show that the time required to fill a 20-cm section of the crystal cladding of a SP-PCF with an aqueous solution is of the order of 10 min. This time is reduced significantly when pressure or heat is applied to the liquid. When, e.g., a pressure difference of 200 kPa is maintained, the filling time for the 20-cm section of the SP-PCF drops below 4 min. This was confirmed experimentally, although variations of several centimeters in the filled lengths were observed. The increase in the flow velocity with temperature is the most likely explanation for the variations observed when filling with a fixed pressure difference. Evidently, these fluctuations make quantitative detection difficult unless a reference molecule with a known concentration is added to the solution or the length of the filled section is measured. A quantitative detection, measuring the concentration of the labeled DNA, is also influenced by any gradients in the concentration along the fiber. Such gradients have been observed especially in fibers filled by use of capillary forces. When pressure is applied to the liquid, the full length of the fiber is filled. Samples were tested by measurement of the transmission spectrum of the liquid-filled PCFs. White light from a tungsten halogen lamp was launched into the microstructured part of the SP-PCF by butt-coupling to a conventional multimode step-index fiber with a core diameter of 100 μm. This ensured efficient excitation of the guided modes in all the air-suspended cores in the fiber cladding. The transmission spectrum was measured with an ANDO AQ6315 optical spectrum

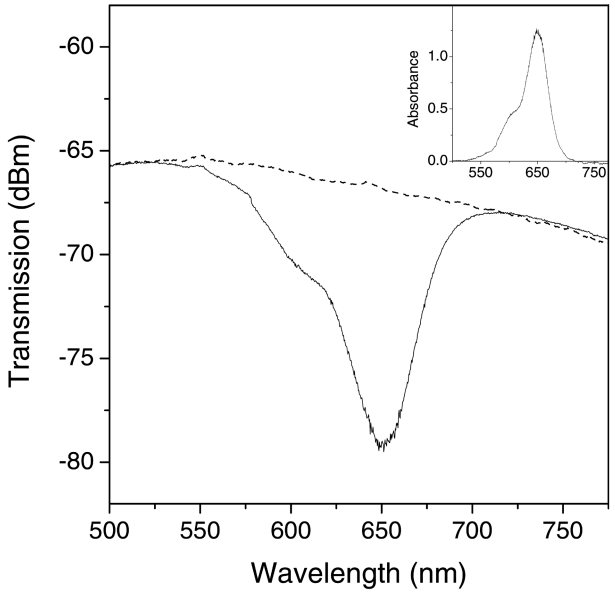

Fig. 2. Transmission spectra of SP-PCFs with a 5-μM Cy5-labeled DNA solution (solid curve) and pure water for reference (dashed curve), respectively. Inset, derived absorbance in the sample containing the Cy5-labeled DNA solution.

analyzer with a resolution of 1 nm. When the intensity of the transmitted signal is measured on the decibel scale, the maximum absorbance is proportional to the measured transmission dip T_{dip}, through $A(\lambda^* = 650\ \mathrm{nm}) = T_{\mathrm{dip}}\ [\mathrm{dB}]/10$.

Figure 2 shows the transmission spectra of two 20-cm SP-PCFs, one filled completely with a 5-μM Cy5-labeled DNA Oligo solution and one with pure water. The inset shows the derived absorbance spectrum with a maximum value of 1.24 at $\lambda^* = 650$ nm. As described above, only a fraction of the optical field propagates in the evanescent field. Multiplying the nominal absorbance by γ compensates for the reduced overlap between the light and the sample. This corresponds to introducing an effective interaction length $L_{\mathrm{eff}} = \gamma L$ with 100% overlap between light and sample. The expression for the absorbance in the PCF-based sensor is then $A = \epsilon c L_{\mathrm{eff}}$, which emphasizes an advantage compared with standard cuvette measurements. Long effective interaction lengths can be achieved by use of submicroliter sample volumes. Given the dimensions of the fiber, a 20-cm-long section of the fiber holds a sample volume of only 0.6 μL. With 5.2% penetration of the optical field into the air holes, the effective length of this fiber section equals 1 cm. The molar extinction coefficient of the Cy5 molecule at $\lambda^* = 650$ nm is $\epsilon_{\mathrm{Cy5}} = 250{,}000\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$, and the expected absorbance for a 20-cm-long fiber filled with a 5-μM solution of Cy5-labeled DNA is 1.3, which is in good accordance with the measured value. The sensitivity of the PCF-based sensor evidently depends on the effective interaction length. With the current measurement setup we are able to detect an absorbance down to $A = 0.04$. For a fiber length of 30 cm the minimum concentration we can detect is then 0.1 μM Cy5-labeled DNA, which was confirmed experimentally.

We also performed a series of absorption measurements with a highly nonlinear photonic crystal fiber with a lattice pitch of $\Lambda = 1.6$ μm. This fiber has a γ value of 2.4%, and we are able to detect labeled DNA molecules in aqueous solutions placed in the air holes of this fiber. Compared with coupling light into the cladding crystal of the SP-PCF, it is significantly more difficult to couple light efficiently into the solid core of the highly nonlinear PCF. A solid-core PCF with a larger lattice pitch and thus a larger silica core is easier to couple light into, but because of the reduced penetration into the liquid it is not an interesting alternative. We conclude that the SP-PCF is superior to solid-core PCFs for applications within evanescent-wave sensing with respect to both sensitivity and handling issues.

In conclusion, we have demonstrated an efficient PCF-based evanescent-wave sensor for the qualitative detection of aqueous solutions of fluorophore-labeled biomolecules positioned in the air holes of the fiber. Light guided in the silica segments located between three neighboring air holes in the cladding crystal has strong penetration into the sample at frequencies in the visible range. The SP-PCF has the advantage that the sample positioned in all the air holes is interrogated, as opposed to solid-core PCFs, where the guided mode is strongly confined to the region surrounding the core and probes only the sample positioned in the first ring of air holes. The main advantage of the SP-PCF-based sensor is the possibility of achieving long effective interaction lengths while using submicroliter sample volumes. Finally, it is not necessary to remove the coating and cladding from the fiber, thereby making the device robust.

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