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Suspended core optical fibers for biological applications using UV wavelengths

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ABSTRACT

We have demonstrated the use of suspended core optical fibers as dip sensors for biological applications based on fluorophores operating at UV wavelengths for the first time. In this paper, we have demonstrated the use of suspended-core fibers to measure the fluorescence of 7-Amino-4-methylcoumarin (AMC), which is used as the transduction for the standard enzyme activity assay of PC6, a biomarker of women's uterine fertility. Concentrations down to 500 nM have been measured using a 2.1 μm core diameter fiber.

Keywords: Microstructured optical fibers, fluorescence, biological sensing, fertility

1. INTRODUCTION

Optical fibers are a promising technology for biological sensing, with benefits such as multiplexing, and their small size allows them to be minimally invasive probes[1]. In particular, microstructured optical fibers (MOFs), which contains air holes along their length, provide the additional advantage of being both a sensitive intrinsic sensor and a small volume (nL-μL) sample collector[2]. By chemically or biologically modifying the internal surface of the MOF it can be made sensitive to specific molecules or proteins of interest via fluorescence based measurements[3]. A challenge is that many biological assays employ UV absorption or fluorescence, which is not within the highest transmission region of silica fibers. However, this is not necessarily an issue when using short probe lengths and thus we have demonstrated, to the best of our knowledge, the first UV excited fluorescence sensing using suspended-core optical fibers, in this case for the particular application of diagnosing women's uterine fertility.

Women's infertility is an increasing problem due to pregnancies occurring later in life. The mean age at first birth in the United States has increased by 3.5 years from 1970 to 2000[4]. While many causes of infertility are understood some are not, and the receptivity of the endometrium (the inner lining of the uterus) is believed to be a significant factor[5,6]. There is a dearth of tools available to assess the receptivity of the endometrium for diagnosing infertility and assisting decisions on IVF implantation, particularly ones that will not affect the endometrium[5]. A MOF sensor is attractive for this application due to the small sample volumes required and thus the potential to not damage the endometrium.

In this paper, we investigate the suitability of suspended-core silica MOFs as a platform for harnessing UV excited fluorescence-based probes for fertility biomarkers. In particular, we consider the enzyme proprotein convertase 5/6 (PC6), which is elevated in the secretory (luteal) phase of fertile women and reduced in a subgroup of women with unexplained infertility[7]. As many biological fluorescence applications, including this one, use UV excitation we demonstrate the feasibility of UV fluorescence sensing with silica suspended-core fibers and characterize, experimentally and theoretically, the role of core size on the probe's sensitivity.

2. SENSING MECHANISM

PC6 can be measured via its enzymatic activity[7]. Previous work measured PC6 activity via the cleavage of a fluorogenic peptide substrate (pERTKR-AMC) in solution as indicated in Fig. 1(b)[7]. In this reaction, the PC6 releases a quenched fluorophore (AMC) from a peptide substrate, which then becomes fluorescent. Here we demonstrate a platform suitable for conducting this measurement based on a suspended-core fiber (Fig. 1(a)). Interaction of the portion

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of the fiber's guided mode that is located in the MOF holes with the AMC will then allow the fluorescence to be measured, as has been previously demonstrated for other biological applications[3,8]. In this paper, we focus on the demonstration of the use of AMC within the suspended core fiber platform, which is a first step towards developing a PC6 probe.

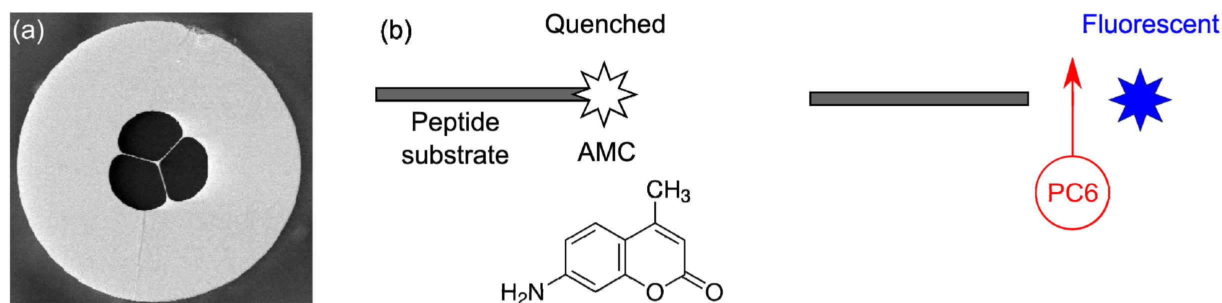

Figure 1. (a) A silica suspended-core microstructured optical fiber (IPHT, Jena). (b) The sensing mechanism. The enzyme (PC6) can release a quenched 7-Amino-4-methylcoumarin (AMC) molecule from the peptide substrate and allow it to fluoresce. The rate of fluorescence increase indicates enzyme activity.

As a first step and prior to fiber experiments (Sec. 3), the fluorescence properties of AMC were characterized in-cuvette. Previous experiments with AMC used an excitation wavelength of 355 nm[7], close to the absorption maximum. However, laser sources are more readily available at 375 nm and the transmission of silica is greater at this wavelength since it is further from the material's UV edge, thus it is desirable to know the loss in fluorophore efficiency in using this wavelength. A 1 cm x 1 cm cuvette fluorescence excitation spectrum was recorded using a Cary Eclipse Fluorescence Spectrophotometer and is shown in Fig. 2(a). In this experiment the AMC was dissolved in a pH=6.5 20 mM Bis-Tris buffer with 2 mM $CaCl_2$ at a temperature of 37°C, the same conditions used for PC6 measurements[7]. It is seen that the fluorescence intensity is still strong at 375 nm, at approximately 50% of the maximum.

AMC was then tested to determine its stability in lower buffer concentrations, which are desirable as MOFs can suffer from loss associated with salt precipitation[8]. Thus, 10X and 100X lower buffer concentrations were tested and Fig. 2(b) shows that AMC is stable for these conditions. In addition, the sensor will ultimately be used in-vivo and thus at 37°C, which is a known working condition for the PC6 assay. However, as MOFs can easily fill lengths of liquid beyond the length that will be in-vivo it is possible that the temperature cannot easily be maintained along the whole fiber. Thus, it is important to know the effect lower temperatures have on the assay. Fig. 2(b) shows that there is negligible difference in AMC fluorescence between 37°C and room temperature (25°C). Future experiments will need to measure any difference in enzyme activity between these temperatures before it is clear whether any form of temperature control will be needed in the device.

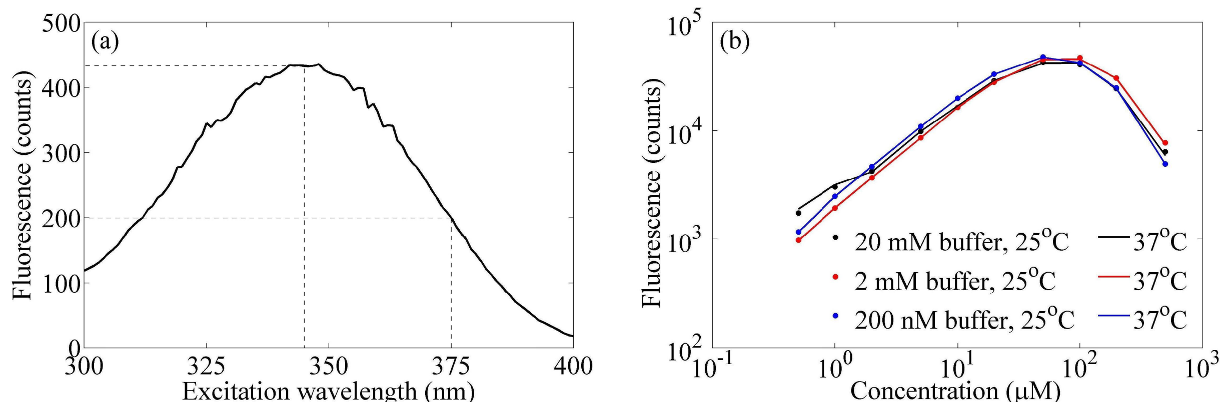

Figure 2. Cuvette measurements. (a) Fluorescence emission as a function of excitation wavelength. Dashed lines show peak fluorescence emission at an excitation wavelength of 345 nm, approximately 2 times greater than at 375 nm, the wavelength used for fiber experiments (Sec. 3). (b) Fluorescence as a function of concentration showing linearity below 100 μM and relative insensitivity to buffer concentration and operating temperature. For concentrations above 100 μM, reabsorption causes a reduction in measured fluorescence.

3. SUSPENDED-CORE FIBER EXPERIMENTS

The ability to measure the fluorescence of AMC within a microstructured optical fiber was tested using suspended core pure silica fibers with two different core diameters; a 9.2 µm core fiber made at IPAS, Adelaide, and a 2.1 µm core fiber made at IPHT, Jena (effective core diameters). It is known that small core diameter fibers have a greater overlap between the guided mode field and fluid within the holes and thus can achieve improved fluorescence sensing performance[10]. However, there are practical advantages to using a larger core fiber such as better coupling stability and the larger hole diameters in such fibers, which increases filling speeds and also improves surface functionalisation coating processes as holes are less easily blocked. Thus, it is desirable to use the largest core diameter fiber that can yield a definitive result. The two types of fiber were filled with AMC solutions with various concentrations. Light from a 375 nm laser (Toptica iBeam Pulse diode laser, 60 µW) was reflected off a dichroic mirror (405 nm Semrock filter set) and coupled into the fiber using either a 10X (9.2 µm fiber, 5.2 µW coupled) or a 40X (2.1 µm fiber, 5.4 µW coupled) microscope objective. The backscattered fluorescence was then measured using a cooled CCD spectrometer (Horiba iHR550) both before filling and after filling the fibers. The background auto-fluorescence from the unfilled fiber was then subtracted from the measured AMC fluorescence signal and the results are shown in Fig. 3.

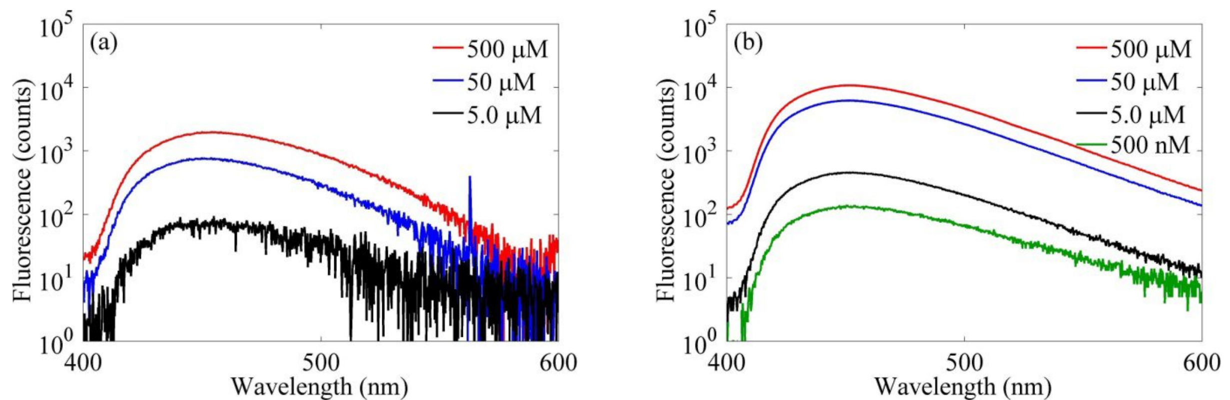

Figure 3. Fluorescence measured in suspended-core silica fibers filled with different AMC solutions (concentrations as per legend). (a) 9.2 µm core diameter fiber (IPAS, Adelaide) & (b) 2.1 µm core diameter fiber (IPHT, Jena). Spectrometer scan times were (a) 1.0 s and (b) 0.01 s, respectively, thus the data in (b) is 100X higher than in (a).

Fig. 3 demonstrates that the fluorescence of AMC can readily be measured using both suspended-core fibers. Observe that the signal is three orders of magnitude higher using the smaller core fiber (noting the spectrometer scan time). The data in Fig. 3 shows that a concentration of 500 nM was successfully measured using the 2.1 µm core diameter fiber while a concentration of 5 µM was measured using the 9.2 µm core diameter fiber.

4. NUMERICAL MODELLING

To understand the sensing performance of suspended-core fibers at UV wavelengths and the relative difference between the results from the two different fibers (Fig. 3), modeling was performed using a simple model for the fraction of power in the holes of the fiber. A step-index model with fully analytical vectorial solutions was used to determine the electric and magnetic fields all of the modes that can propagate in the fiber. The power fraction (PF) of light that propagates in the fiber holes is shown in Fig. 4(a), where the thick lines show the average PF assuming equal excitation of all guided modes while the thin lines show the result for the fundamental mode only. Fig. 4(a) indicates that at any particular wavelength there should be relatively little difference in power fraction between different core diameters if all modes are considered equally. However, coupling efficiency (CE) and, to a lesser extent, confinement loss (CL) reduces the fractional power in the higher order modes. Fig. 4(b) shows the effect of including the coupling of a linearly polarized Gaussian input beam with a waist of 0.65 x fiber diameter (as suggested by Buck for multi-mode fiber[9]) and confinement loss for a W-shape structure with an inner cladding diameter equal to 10 times the core diameter[10]. The results show a power fraction (relative to the laser power) of 11% at 375 nm for a 0.5 µm core diameter fiber or 0.1% for a 4.0 µm core diameter fiber. Using the parameters used in these experiments we predict that there should be an 18 times increase in sensitivity between the 2.1 µm core diameter compared to the 9.2 µm core diameter. This does not fully explain the three orders of magnitude decrease experimentally observed and thus future theoretical work will include additional factors such as the coupling configuration, the fiber geometry, and the fluorescence capture efficiency[10].

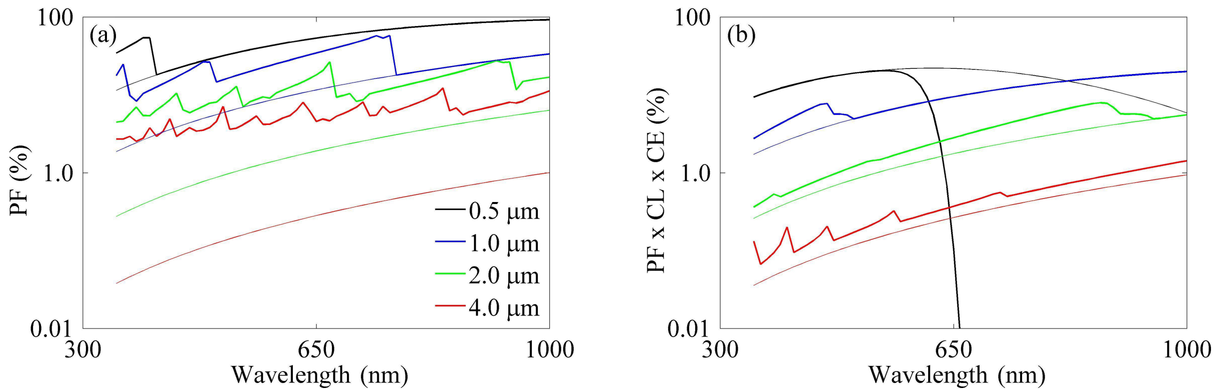

Figure 4. Power fraction (PF) of the fundamental mode (thin lines) and for all modes (thick lines) where (a) the modes are equally excited and (b) modes are scaled by the efficiency with which they can be excited using a linearly polarized Gaussian beam (waist = 0.65 x core radius) and confinement loss for a 30 cm fiber length. Colors refer to the step-index core diameter (black = 0.5 μm, blue = 1.0 μm, green = 2.0 μm, and red = 4.0 μm).

5. CONCLUSIONS

We have demonstrated the feasibility of using suspended core fibers for biological applications using UV wavelengths. We have investigated this for the particular application of probing the enzyme PC6, a biomarker of women's uterine fertility. This was achieved by measuring the fluorescence of AMC, which, when coupled to a suitable peptide, is used as the transduction for the standard enzyme activity assay of PC6. We have demonstrated that the evanescent field at 375 nm is sufficient to measure concentrations of AMC down to at least 500 nM samples when using a 2.1 μm core diameter fiber or 5 μM when using a 9.2 μm core diameter fiber.

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