

# Selective detection of antibodies in microstructured polymer optical fibers

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**Abstract:** We demonstrate selective detection of fluorophore labeled antibodies from minute samples probed by a sensor layer of complementary biomolecules immobilized inside the air holes of microstructured Polymer Optical Fiber (mPOF). The fiber core is defined by a ring of 6 air holes and a simple procedure was applied to selectively capture either  $\alpha$ -streptavidin or  $\alpha$ -CRP antibodies inside these air holes. A sensitive and easy-to-use fluorescence method was used for the optical detection. Our results show that mPOF based biosensors can provide reliable and selective antibody detection in ultra small sample volumes.

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**OCIS codes:** (060.2370) Fiber optics sensors; (230.3990) Microstructure devices; (170.6280) Spectroscopy, fluorescence and luminescence, (120.1880) Detection.

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## 1 Introduction

Microstructured Optical Fibers (MOFs) based on either silica [1], or polymers, such as poly(methyl methacrylate) (PMMA) [2], have received an increasing interest over the last 5 to 10 years. This class of fibers is characterized by having a number of air holes running along the entire length of the fiber. The optical properties of the fiber are primarily determined by the relative position, size and shape of the air holes and MOFs exhibit a number of unique properties compared to conventional solid optical fibers. In addition to the effort invested in the utilization of these novel optical properties within the telecommunication industry, a number of MOF-based sensor applications have been demonstrated, especially within biosensing [3-6]. The majority of these applications take advantage of the unique possibility to position a given biological sample inside the air holes in close proximity to the fiber core [4-6]. The sample may hence be probed by the optical field from light propagating through the fiber without removing the fiber coating and cladding, thus maintaining the robustness of the fiber. In contrast, standard fiber based evanescent-wave devices are often very fragile since the un-protected fiber core is exposed to the surroundings in order to obtain an overlap between the optical field and the sample. When analyzing biological samples using fluorescent probes attached to the biomolecules selective detection is achieved by using either selective labeling of the biomolecules, or by ensuring that a signal is solely obtained from the molecules the sample is analyzed for. In this paper we present a biosensor concept based on a selective capture of specific antibodies in a sensor layer immobilized on the side walls in the air holes of a mPOF. The functionality of the mPOF based fluorosensor is illustrated by detecting two different Cy3-labeled antibodies,  $\alpha$ -streptavidin and  $\alpha$ -CRP, in microliter volumes of aqueous solutions injected into the air holes of the fiber. The demonstrated selectivity is high and the procedure used to define the sensor layer for the capture of these particular antibodies includes only a few steps.

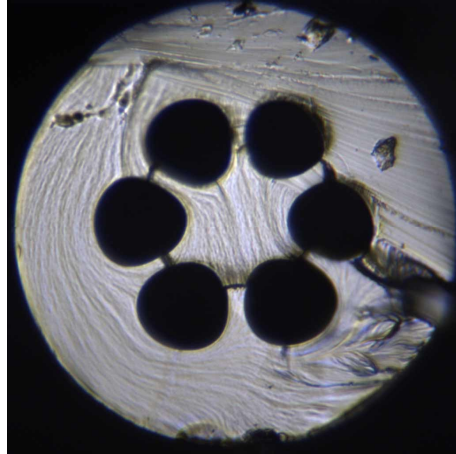


Fig 1. Micrograph showing the end-facet of the mPOF used in the biosensor experiments. The outer diameter is  $300\mu\text{m}$  and the air hole diameter is  $60\mu\text{m}$ . The bridges supporting the core broke during the cleaving of the fiber. The fracture only extends a few millimeters into the fiber.

## 2. Microstructured Polymer Optical Fiber

Figure 1 shows a micrograph of the multi-mode mPOF used in our experiments. The fiber core is defined by the 6 air holes arranged in a circle. The fiber preform was fabricated by drilling the holes in a PMMA rod with a diameter of 20 millimeters. The preform was then drawn to a fiber with an outer diameter of  $300\mu\text{m}$  and an air hole diameter of  $60\mu\text{m}$ . Due to the relative large width of the bridges supporting the fiber core, there is a significant leakage of the optical field from the fiber core. In the experimental setup presented in this paper, the length of the mPOF sections is limited to 20 centimeters, and the leakage induced loss is of little importance. The large diameter of the air holes ensures that samples flow easily through the fiber at the expense of a relatively large sample consumption. A 20 centimeter long piece of fiber hence holds a sample volume of  $3.4\mu\text{L}$ . The air hole diameter and thus the required sample volume can be reduced significantly without raising the flow-time to an unreasonable level. Similar to a solid glass rod immersed in an aqueous sample [7], a fraction of the optical field propagates as an evanescent field. This part of the optical field penetrates into the captured layer of biomolecules at the walls of the air holes, thereby allowing the fluorescence emitted from the laser excited Cy3-fluorophores to tunnel into the core modes and be guided along the fiber for subsequent detection.

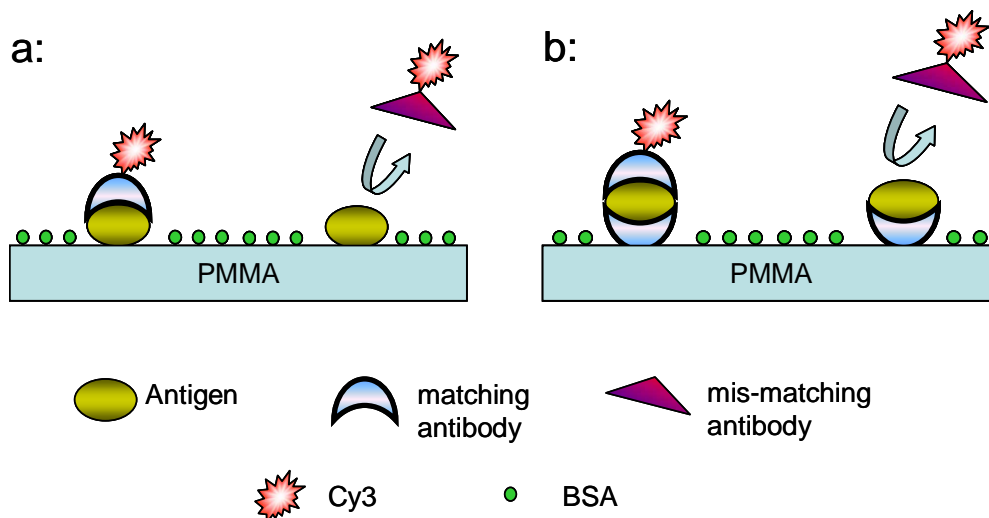


Fig 2. Schematic presentation of the capture processes utilized in the selective detection of the antibodies. a:)  $\alpha$ -streptavidin-Cy3 is captured by streptavidin molecules directly immobilized on the PMMA surface while the mis-matching  $\alpha$ -CRP-Cy3 molecules are washed out, b:)  $\alpha$ -CRP-Cy3 molecules are captured by the  $\alpha$ -CRP/CRP sandwich, while the mis-matching  $\alpha$ -streptavidin-Cy3 molecules do not bind and are washed out.

### 3. Definition of sensor layer

The sensor layers for the selective detection of the Cy3 labeled antibodies  $\alpha$ -streptavidin and  $\alpha$ -CRP were defined in different manners.  $\alpha$ -streptavidin antibody was captured by an antigen-antibody interaction with streptavidin. The streptavidin molecules can bind directly to the polymer surface while still being able to bind the antibody. The procedure for capturing  $\alpha$ -streptavidin is hence as follows. The PMMA surface is activated by exposing it to 1mg/mL streptavidin in Phosphate Buffered Saline (PBS) solution for 30 minutes. Any open sites on the PMMA surface are then blocked by a solution of 1% (w/v) Bovine Serum Albumin (BSA). The sensor layer is then ready and is subsequently probed for 60minutes by either 0,05mg/ml  $\alpha$ -streptavidin-Cy3 or 0,05mg/ml  $\alpha$ -CRP-Cy3 solutions. Figure 2(a) shows a schematic illustration of the selective capture of the  $\alpha$ -streptavidin-Cy3 molecules. After every step in the procedure there is a 1-3 minute wash with PBS. Similar to the  $\alpha$ -streptavidin capture, the  $\alpha$ -CRP molecules are captured by an antigen-antibody reaction with CRP. In the case of CRP, an additional binding step is required since the CRP molecules do not bind as effectively to the PMMA surface. This is probably due to a lower electrostatic affinity towards the polymer compared to the streptavidin molecules. Instead of activating the PMMA surface with CRP directly, the  $\alpha$ -CRP antibody was bound to the surface (1mg/mL  $\alpha$ -CRP in PBS for 30 minutes) and then CRP was captured (0.16mg/mL CRP in PBS for 30 minutes) as illustrated in Fig. 2(b). Before binding the CRP molecules, the open sites on the PMMA surface were blocked with the above mentioned BSA solution. The sensor layer is then probed for 60minutes by either 0,05mg/ml  $\alpha$ -streptavidin-Cy3 or 0,05mg/ml  $\alpha$ -CRP-Cy3 detector antibody solutions. After every step in the procedure there is a 1-3 minute wash with PBS. In both series of experiments the aqueous samples were pushed into the air holes of the mPOF by applying approximately 20kPa overpressure to the liquid. In the PBS-washes, pressure was applied for the full duration of the wash, while in all other steps, fresh liquid was pushed into the air holes every 10 minutes. After the last wash, the air holes are blown dry with nitrogen for 1 minute prior to the optical measurements.

#### 4. Optical detection of antibodies

The optical characterization was carried out using an easy-to-use fluorescence setup, where the mPOF is exposed from the side with a line-shaped laser beam [8]. The laser beam is focused down to a line width of a few hundred micrometers on the short axis of the fiber. Along the fiber the beam is expanded to cover 7 centimeters of the fiber in order to excite fluorophores on a large number of captured antibodies and hence obtain a strong fluorescence. The emitted fluorescence tunnels through the evanescent field into the fiber core and is guided through the fiber. At the fiber end, the fluorescence propagating in the fiber core is collected by a standard multimode optical fiber with a 50 $\mu\text{m}$  core diameter butt-coupled to the mPOF in one end and directly connected to a highly sensitive spectrometer (Ocean Optics HR2000) in the other end. The butt-coupling is optimized by launching white light into the distant end of the mPOF and reading the intensity of the collected signal from the spectrometer.

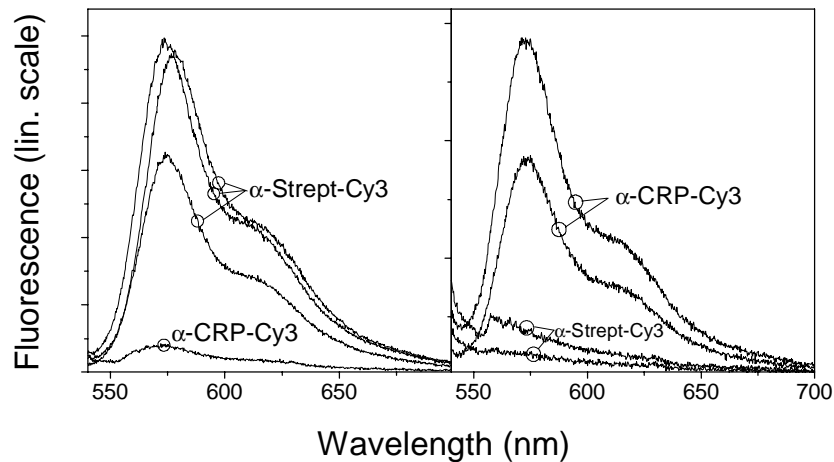


Fig 3. Fluorescence measurements demonstrating the excellent selectivity of the capture process in a fiber activated with a: a) a streptavidin-layer, and b:) a  $\alpha$ -CRP/CRP sandwich.

#### 5. Results

Figure 3(a) and 3(b) show fluorescence spectra of mPOFs activated with sensor layers to capture  $\alpha$ -streptavidin and  $\alpha$ -CRP, respectively. These results show that the selectivity of the antibody capture is excellent. Evidently there is some variation in the strength of the fluorescent signal from the fibers probed by the matching molecules, but there is a clear separation between the intensity from these fibers and the fibers exposed to samples containing the mis-matching antibodies. These observations were confirmed using epi-fluorescence microscopy. The spectra presented in Fig. 3 are all centered at 575nm, while the fluorescence spectrum of the Cy3 molecules attached to the antibodies has its maximum intensity at 560nm. This indicates that re-absorption of the emitted fluorescence takes place. As the fluorescence collected by the core-mode is guided along the fiber, a fraction of the optical field propagates as an evanescent wave inside the air holes and hence interacts with the Cy3 molecules. This results in absorption of the short-wavelength side of the fluorescence spectrum, and hence shifts the peak in the measured spectrum towards longer wavelengths. Selective detection of biomolecules using silica based MOFs has previously been demonstrated [9]. Compared to the work on the silica based MOFs, the definition of the sensor layer for the capture of  $\alpha$ -streptavidin antibody require only 6 steps, while the earlier presented procedure

based on silica MOFs require 10 steps. In chip-based sensor configurations using a MOF based sensor element, a limited number of steps in the full detection procedure including the definition of the sensor layer is desirable. A few-step procedure may allow for practical sensor systems, where the sensor layer is defined immediately before analyzing the sample. By doing so, the biomolecules used in the procedure can be kept under suitable conditions until immediately before use, and the limited lifetime of the biomolecules will not pose a problem.

## 6. Discussion

With the present configuration we have a detection limit of 80nM when analyzing a sample volume of 27 $\mu$ L. This limit is determined by a number of parameters involving both optical and biological issues. The optical aspects include the strength of the evanescent field in the sensor layer. It seems reasonable to assume that the fluorescence from the excited Cy3 molecules is emitted in all directions with an isotropic intensity distribution. The fiber core only collects the part of the emitted fluorescence that couples to the evanescent field of the core modes and a relatively large fraction of the fluorescence is lost. Lee and coworkers [10] have previously shown that the intensity of the collected fluorescence is proportional to the strength of the evanescent field in the sensor layer. By improving the fiber design with optimized overlap between the evanescent field and the sensor layer the sensitivity of the sensor can be increased significantly. The interaction length is also important for the sensitivity of the sensor. A longer interaction length corresponds to collecting fluorescence emitted from a larger number of captured antibodies and thus a better sensitivity. In our setup the interaction length is determined by the length of the fiber section that is exposed to the laser beam. In the present configuration the laser beam expose a 7 cm long section of the fiber. This length and hence the sample volume required for the analysis can be reduced at the expense of a lower sensitivity. In addition to the exposed section, we need approximately 3 cm of fiber for alignment relative to laser beam and coupling to the spectrometer. In total we hence only need 10 cm and the total sample volume used for the detection can be reduced to 10 $\mu$ L when using the fiber presented in this paper. By careful design of the air hole pattern of the fiber, the sample volume required to fill 10 cm fiber can be reduced to 100nL, while maintaining the area of the region where the optical field interrogate the sensor layer and hence the sensitivity. Reducing the number of times a fresh sample is injected into the air holes from the 6 times used during the experiments presented in this paper will furthermore reduce the sample consumption, but in some cases also the sensitivity.

Competing optical fiber based detection techniques, such as the Surface Plasmon Resonance (SPR) technique, has been used to detect biomolecules from 200fM solutions [11]. Another label-free highly sensitive detection method based on interferometry was presented by Markov and co-workers [12]. In addition to these label-free methods, a large number of other techniques for the detection of biomolecules have previously been demonstrated. RIAs (Radioimmunoassay) and enzyme immunoassays like ELISA (Enzyme-linked immunosorbent assay), LIA (luminescent immunoassay), and FIA (fluorescent immunoassay) are widely used in research, drug discovery and diagnostics for highly specific and cost efficient detection of analytes not detectable with other techniques. The antibodies are here labeled with radioisotopes (e.g. 125I), fluorescent dyes (e.g. FITC) or enzymes (e.g. HRP or AP) which catalyze fluorogenic or luminogenic reactions. The sensitivity of these techniques lies in the order of 1-100 pM depending on the affinity of the antibodies. The sensitivity can be increased even further by using Surface Enhanced Raman Scattering (SERS), which in combination with coherent anti-Stokes Raman scattering can make detection on a single-molecule level [13]. The sensitivity and the sample volume required to make a reliable analysis using the method presented in the present paper cannot compete with the performance of these methods. The advantage offered by our method is that we potentially can make the analysis *in vivo*. Surface Plasmon Resonance based devices can in theory also be used for this application. This technique requires that the coating and cladding of the fiber is removed thus making the device very fragile. The mPOF based biosensors on the other hand

operate by positioning the sample in the air holes. There is hence no need for removing the protective coating and highly robust devices can be fabricated.

The mPOF based sensor has so far only been tested for applications as a single-use component. Using the sensor for analysis of several samples with respect to antibody-antigen detection will most likely be possible assuming that the surface immobilization chemistry is resistant to acid treatment (Glycine pH 2.8). The present concept may be used for a range of biodetection events involving recognition events. It has been demonstrated that it is possible to detect acute phase proteins (e.g. C-reactive protein) in whole blood [14] using capture antibodies. We believe that it will be possible to use the present technique for detection of some blood proteins (markers for disease). It would also be possible to use the technique for detection of nucleic acid molecules as depicted by Regensberg and co-workers [15]. Furthermore it may also be useful for detection of minute amounts of unwanted molecules, plant protection agents and derivatives thereof, in drinking water

## **7. Conclusion**

In conclusion, we have demonstrated selective detection of fluorescently labeled antibodies,  $\alpha$ -streptavidin-Cy3 and  $\alpha$ -CRP-Cy3, from aqueous samples interrogated by a sensor layer immobilized inside the air holes of a microstructured Polymer Optical Fibers. The procedure used to define the sensor layer for the detection of these antibodies is fast compared with the more complex procedure applied when the biomolecules are probed by a silica MOF.