TAPERED OPTICAL FIBER PLATFORM FOR BIOSENSING APPLICATIONS

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TAPERED OPTICAL FIBER PLATFORM FOR BIOSENSING APPLICATIONS

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This study focuses on the design, fabrication, and characterization of tapered optical fibers for label-free, biomolecular sensing in both aqueous and gaseous environments. Single-mode fibers were tapered to a diameter of approximately 10 microns allowing for the propagation of multiple modes and creating an interference pattern in the output signal. Tapered regions serve as the sensing interface, such that the light propagating through/around the fiber interacts with molecules tethered to the tapered surface. Tapered regions are functionalized with biomolecules for capture and detection of analytes in both aqueous (antibody) and vapor phases (DNA, peptides). Molecular binding of analytes with recognition molecules changes the refractive index and the thickness of the biolayer on the fiber surface, which can be measured as a phase shift in the output spectrum. The sensing platform (fiber and Teflon flow cell) allows for fast and economical fabrication. The tapered optical fibers can be fabricated in array format for detection of multiple analytes in complex samples for biomedical (blood, saliva, breath), environmental, and homeland security applications.
This work is dedicated to my parents, Anna and Mark, and my grandparents, Rose and Joel, who always encouraged me to question everything. I would also like to dedicate this to the loving memory of my grandmother, Beverly.
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ABSTRACT ....................................................................................................................... iii

ACKNOWLEDGEMENTS .................................................................................................... v

LIST OF FIGURES ........................................................................................................ ix

LIST OF TABLES ........................................................................................................... xi

LIST OF ABBREVIATIONS AND NOTATIONS .............................................................. xii

INTRODUCTION ........................................................................................................... 1
  1.1 Background: ........................................................................................................... 1
  1.2 Optics theory: ...................................................................................................... 5
  1.3 Surface chemistry theory ................................................................................... 9

METHODS ..................................................................................................................... 11
  2.1 Aqueous flow cell construction: ........................................................................ 11
  2.2 Aqueous flow cell setup: .................................................................................... 13
  2.3 Tapered optical fiber fabrication: ...................................................................... 14
  2.4 Tapered optical fiber characterization: ............................................................... 15
  2.5 Biofunctionalization ......................................................................................... 15
2.6 VOC flow chamber: ................................................................. 17

RESULTS ................................................................................................. 19

3.1 Fiber Characterization: ................................................................. 19

3.2 Fiber Response in Solution: ......................................................... 21

3.3 Biofunctionalization of SM Fibers: .............................................. 21

3.4 Biofunctionalization of PM Fibers: .............................................. 26

3.5 Reusability: .................................................................................... 28

3.6 Repeatability ................................................................................ 33

3.7 Thresholds: .................................................................................. 34

DISCUSSION .............................................................................................. 36

BIBLIOGRAPHY ....................................................................................... 40

APPENDIX A: FIBER FABRICATION .......................................................... 45

Vytran LDC-200 Fiber Cleaver Protocol .............................................. 45

Vytran GPX-3000 Glass Processor Startup Protocol .......................... 47

Vytran GPX-3000 Normalization Protocol ........................................... 48

GPX-3000 SMF Splicing Protocol ....................................................... 49

GPX-3000 PMF Splicing Protocol ....................................................... 50

GPX-3000 SMF/PMF Tapering Protocol .............................................. 51

APPENDIX B: FLOW CELL FABRICATION ................................................. 52

APPENDIX C: FIBER TESTING SETUP ..................................................... 53
APPENDIX D: BUFFER AND CHEMICAL PROTOCOLS ........................................... 54

2% Amino Silane Preparation ........................................................................... 54

Antibody Solution Preparation ........................................................................ 55

APPENDIX E: SURFACE CHEMISTRY PROTOCOLS ........................................ 56

Fiber Surface Protein Chemistry Procedure .................................................... 56
LIST OF FIGURES

Figure 1. Design of single mode optical fiber................................. 1
Figure 2. HE$_{11}$ electric field in tapered fiber.............................. 7
Figure 3. IgG structure............................................................... 9
Figure 4. Schematic of flow cell.................................................. 11
Figure 5. PTFE flow cell schematic............................................. 12
Figure 6. Assembled flow cell.................................................... 13
Figure 7. Layout of GPX-3000 glass processor............................... 14
Figure 8. Flow cell connected to tunable laser.............................. 15
Figure 9. VOC adapter for flow cell.......................................... 17
Figure 10. VOC flow system setup............................................ 18
Figure 11. Dimensions of tapered fibers...................................... 19
Figure 12. Dimensions of tapered fibers 2.................................. 20
Figure 13. Spectra of tapered fibers in air.................................. 20
Figure 14. Spectra of tapered SMF in water................................ 21
Figure 15. SMF 1 biofunctionalization spectra............................. 22
Figure 16. SMF 2 biofunctionalization spectra............................. 23
Figure 17. SMF 3 biofunctionalization spectra............................. 24
Figure 18. SMF 4 biofunctionalization spectra............................. 25
LIST OF TABLES

Table 1. Average Peak Wavelength Shifts due to Antigen Capture .................................. 33
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>APTES</td>
<td>Aminopropyltriethoxysilane</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G (antibody)</td>
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<tr>
<td>IR</td>
<td>Infrared</td>
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<tr>
<td>MFC</td>
<td>Mass Flow Controller</td>
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<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEEK</td>
<td>Polyether ether ketone</td>
</tr>
<tr>
<td>PMF</td>
<td>Polarization-maintaining fiber</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene (Teflon)</td>
</tr>
<tr>
<td>SMF</td>
<td>Single-mode fiber</td>
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<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
</tr>
<tr>
<td>TIR</td>
<td>Total Internal Reflection</td>
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<tr>
<td>VOC</td>
<td>Volatile organic compound</td>
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CHAPTER 1
INTRODUCTION

1.1 Background:

Detecting the presence of specific biomolecules has applications in a wide variety of settings, such as medical clinics\(^1\), industrial factories\(^2\), and in homeland security\(^3\). As such, a diverse number of sensors have been designed for these various applications. These sensors greatly vary in their method of detection, utilizing calorimetric\(^4\), electrochemical\(^5\), piezoelectric\(^6\), or optical techniques\(^7\). In particular, the use of optical biosensors has grown immensely in the past 50 years through the development of fluorescence techniques as well as the inventions of both the laser and the optical fiber.

Single mode optical fibers (SMF) were designed for low-loss transmission of information over long distances.\(^8\) These fibers are composed of a silica core surrounded by a cladding (Figure 1), the refractive index of which allows for total internal reflection of light through the core.\(^8\)

![Core and Cladding](image)
When the beam undergoes total internal reflection (TIR), some of it will exist outside of the core. A portion of the electric field permeates through the core/cladding interface and decays as a function of distance from the interface; this phenomenon is known as an evanescent wave. Evanescent light waves are the basis for surface plasmon resonance (SPR), a popular biosensing technique. In optical fibers, however, the cladding is much thicker than the distance it takes for the evanescent wave to decay to a negligible value, which is why they are useful for long-distance transmission. This low loss characteristic makes an untapered fiber an ineffective platform for sensing on its outer surface since light propagating through the fiber can only be measured in the core.

Optical fibers without cladding have been shown to exhibit measurable surface plasmon resonance, and therefore have been used as sensors. Biomolecule functionalization on tips of optical fibers has also demonstrated the capacity for molecule selectivity and detection sensitivity, the hallmarks of any successful biosensor. Optical fibers with biconic, tapered regions have been shown to act as a refractive index sensor. These biconic tapered fiber sensors have demonstrated the ability to detect protein-protein interactions, DNA hybridization, and to measure bacterial concentrations.

Tapering a fiber to a small diameter has distinct advantages for sensing volatile organic compounds (VOCs). First, thinning of the cladding allows the evanescent wave to extend outside of the cladding. Therefore, the light propagating along the fiber will interact with molecules that are bound to, or are near the surface of the fiber. The thickness of any layer of biomolecules that bind to the fiber surface would contribute to a
significant, measurable change in the effective refractive index of the layer and consequently increase the change in the output signal.

Tapered optical fibers have emerged as a biosensing technology, and have been reshaped and manipulated to allow for multiple methods to detect the presence of biomolecules\(^{10}\). Most commonly used for aqueous phase sensing, tapered fiber tips have shown the ability to detect antigen-antibody capture\(^ {16}\). Tapered fiber tips have also been used to collect chemiluminescent signals catalyzed by alkaline phosphatase\(^ {17}\). A variety of biconic tapered fiber sensors have also been designed: microfiber couplers, which consist of two intertwined tapered fibers with an adiabatic profile, exhibit a measurable interference signal\(^ {18}\). Other biconical optical fiber biosensors are tapered to a non-adiabatic profile, and are capable of detecting protein-protein interactions at the tapered surface\(^ {19}\).

While the majority of the optical fiber biosensor research has been conducted in aqueous environments, researchers are beginning to experiment with their capability of detecting volatile organic compounds\(^ {20}\). Volatile organic compounds (VOCs) are molecules that are readily found in the vapor phase at room temperature. VOC detection has been exhibited on fibers with deposited organometallic layers in place of a cladding\(^ {21}\). This device was capable of detecting different signatures from different drinks\(^ {22}\). The ability to recognize and differentiate between VOCs is of great value in the rapidly growing field of biosensing. A recent study detected the presence of breast cancer by simply measuring the VOCs in exhaled breath.\(^ {23}\) Phillips demonstrated that there was a statistically significant link between breast cancer and VOCs that were exhaled by subjects.\(^ {23}\) Other breath analysis techniques have arisen: exhaled breath VOC analyses (in
a study by Phillips) had a demonstrated detection rate in patients (histologically confirmed) for breast cancer that approached the specificity and sensitivity of mammograms.²⁴

These applications and most others require the development of sensors that can be deployed outside of a laboratory setting. A versatile and robust approach is to use optical fibers as the sensing platform. The fiber delivers light to a sample and also transmits the signal to a detector – both with negligible alignment of the fiber.

This study focuses on the design, fabrication, and validation of the tapered optical fibers for low level detection of analytes in aqueous and vapor phases.
1.2 Optics theory:

Figure 1 illustrates the typical design of a single mode fiber (SMF). Generally they consist of a small core region of refractive index $n_1$, surrounded by a significantly larger cladding of a lower refractive index $n_2$.

It is this index contrast that allows for total internal reflection (TIR), the mechanism by which light is confined within a fiber. The behavior of light propagating under TIR conditions is described using electromagnetic wave theory that identifies light as a discrete set of waveforms or modes moving inside the fiber. Being electromagnetic waves, the magnitude of the electric and magnetic fields vary over space and time. The spatial oscillation in the direction of propagation, quantified by the propagation constant ($\beta$), takes on a specific value depending on the modes. This oscillation also occurs in the transverse direction as well resulting in distinct rotationally symmetric field patterns. These types of discrete oscillations are quantified with the parameter $\nu$. With the two quantities known other parameters specific to the mode can be determined such as field shape, velocity, and power to name a few.

For tapering and sensing applications, of primary concern is the class of modes known as hybrid modes. Although the single mode core supports only the fundamental mode, $HE_{11}$, higher order modes are able to propagate in the cladding assuming the index outside the fiber ($n_3$) is smaller than $n_2$. When tapering these higher order-cladding modes, $HE_{1\ell}$ $EH_{1\ell}$, are the only modes that can exchanges power with the initially excited $HE_{11}$.$^{25,26}$

In a sensing regime, a tapered region serves two purposes. The first is to allow for power to be exchanged between the $HE_{11}$ mode and the higher order modes $EH_{1\ell}$ and
HE\_1\_l modes. The process that regulates this power exchange is dependent upon mode shape and phase differences between modes. In the case of bi-conic fiber tapers, it has been demonstrated analytically and experimentally that the power output \( I \) resembles that of a two-arm interferometer\(^{27}\)

\[
I = \sum_m I_m + \sum_m \sum_n 2\sqrt{I_m I_n} \cos(\varphi_{mn})
\]  

(1)

where the subscript \( m \) denotes the HE\_1\_1 mode and \( n \) denotes the next nearest hybrid mode. The term \( \varphi_{mn} \), defined in Equation 2, is governed by the phase difference accumulated from the beginning of the taper to the end of the waist region \( L \).\(^{27}\)

\[
\varphi_{mn} = \int_0^L (\beta_n - \beta_m) dz
\]

(2)

The second purpose of the taper is to drive a sufficient amount of the field outside the fiber. When a change occurs on the surface of a fiber, such as a refractive index change, the propagation constants of the modes will change accordingly. The result takes the form of shifts in the interference pattern, which is the very effect that bi-conic fiber sensors seek to exploit. The magnitudes by which the propagation constants are altered depend upon the magnitude of the refractive index change as well as the field strength in the tapered sensing region, where the diameter of the core is negligible.\(^{27}\) In the case of biconic sensors, this would be the evanescent field which is outside the fiber region. As a fiber is tapered, the field inside the core is pushed farther and farther outside of the core. Eventually, the field will cease to be guided by the core, and becomes cladding-guided
instead. In this way, a large portion of the evanescent field can reach the sensing region on the fiber surface.

A visual example of the fibers electric field distribution at a small waist is shown in Figure 2. Although the HE\textsubscript{11} field is mostly confined within the fiber, a significant portion of the electric field extends beyond the surface of the fiber with decay at greater distances. This property is often quantified using the penetration depth ($d_p$), or length at which the field reaches ($1/e$) times the value at the boundary. When the refractive index contrast between the fiber and the external medium is small, this parameter is well approximated by the equation\textsuperscript{27}

$$d_p = \frac{1}{\sqrt{\beta^2 - k_0^2 n_3^2}}$$

(3)
where $\beta$ is the beam propagation constant, $k$ is the free-space wave number, and $n$ is the refractive index. As the radius of the fiber becomes smaller, the value of $\beta$ will approach $k_0^2 n_3^2$ resulting in an enhanced penetration depth.\textsuperscript{22} Since higher order modes have progressively smaller propagation constants they are also more sensitive to index changes than lower order modes.\textsuperscript{22,29} This is important because the phase shift term, $\varphi_{mn}$ in Equation (2) is based upon the difference between propagation constants. It follows then that interferometric sensors, like biconic tapers, also become increasingly more sensitive as they taper down to smaller radii.

It is important to note that the minimum radius of a biconic fiber sensor is limited by the mode cutoff higher order modes. Should the fiber be tapered down too far it will again support a single mode. Any and all power carried by the higher order modes will be radiated out of the fiber and lost to the environment. For our 10$\mu$m diameter fiber segment the propagation is well within the multi-mode operating regime (the normalized frequency – the $V$ parameter – is of order 30 for the tapered fiber immersed in water).\textsuperscript{20,22,26}
1.3 Surface chemistry theory

The tapered optical fiber platform is a sensor that is capable of detecting changes in the refractive index adjacent to the tapered fiber surface. This sensing capability requires the attachment of a molecular sensing element to the tapered surface. Aptamers – short sequences of DNA or protein that bind to a molecular analyte – were considered for this experiment, but were not used because of their small size. DNA hybridization – the joining of complementary single-strands of DNA – was also considered, but to demonstrate proof-of-concept a larger molecule was desired. Immunoglobulin G (IgG) antibodies – proteins derived from the immune systems of mammals – were selected as the recognition element for their innate selectivity and larger relative size.

IgG molecules (Figure 3) are Y-shaped molecules composed of multiple amino acid chains that are joined by cysteine-cysteine disulfide bonds represented by the number 6. The fragment antibody region is represented by the number 1. The tip of this...
The hypervariable region, represented by the number 5, is known as the hypervariable region. The hypervariable region is the part of the molecule that is designed with a short amino acid sequence that will allow it to bind specifically to certain molecules. The molecules that it binds to are known as antigens. In the immune system, these antigens may be foreign viruses, bacteria, proteins, or even cells and non-foreign molecules that the body itself creates. In this experiment, a second IgG molecule from a different source was selected as the antigen to demonstrate an effective doubling of the thickness of the biolayer.

IgG molecules are approximately 28 nm in diameter, as shown by atomic force microscopy. The approximate spacing of hydroxyl groups on the surface of silica in the form of our tapered fiber is approximately 10 groups/nm². A uniformly distributed tapered fiber surface with two perfectly conical, 5mm taper transition regions and a perfectly cylindrical tapered waist 20mm long was assumed for calculations. The total tapered fiber surface area was estimated to be 0.1397 cm² and would be saturated by 37.7 fM of IgG.
2.1 *Aqueous flow cell construction:*

A flow cell was constructed to hold the tapered fiber and to deliver solutions to the tapered region. The flow cell consists of two pieces of sheet polytetrafluoroethylene (PTFE) and sealed with a silicone gasket (Miami Valley Gasket, Dayton OH). These materials were chosen because they were inexpensive, easily machined, chemically resistant, and reusable. The design of the flow cell is shown in Figures 4 and 5.

Figure 4. Schematic of flow cell. The flow cell is composed of silicone (orange) sandwiched between two pieces of machined PTFE (white). Model was made with Google Sketchup 8 software.
The top piece of the flow cell has an inflow port fitted to a PEEK P-235X flangeless 1/16 nut with a P-200X flangeless 1/16 ferrule (Upchurch Scientific) to allow the introduction of solution and analytes to the flow cell. The silicone gasket has a corresponding port connected the inflow port above to the fiber-containing flow cell area below. The gasket also served to seal the upper and lower flow cell components. The bottom piece of the flow cell contains a 1 ml volume flow area that houses the fiber. The bottom was machined with an outflow port from which waste can be collected as seen in Fig. 5.

![Figure 5. PTFE flow cell. The tapered region of the fiber rests across, and the smaller channels at each edge are used to secure and seal the fiber with PDMS (circled). Small troughs were made at the edges to support the optical fiber. The main trough of the flow cell was made significantly deeper than the fiber to ensure that the tapered fiber would not come in contact with the cell walls. The fiber was secured in place by gluing the two ends with 1:10 catalyst:base of Sylgard 184 silicone elastomer (Dow Corning) to the small troughs and heating/curing to 70°C for 30 minutes. Six screws were used to tighten the PTFE upper and lowers components to each other (with the silicone gasket in between) to make the entire trough watertight. The volume of the flow cell was approximately 1 mL.](image-url)
2.2 Aqueous flow cell setup:

Polyether ether ketone (PEEK) tubing #1531 (Upchurch Scientific) was used to connect the flow cell to a V-450 6-port medium pressure injection valve and syringe pump from (Figure 6).

Figure 6. Assembled flow cell (bottom) connected to 6-port injection valve (top) via PEEK tubing. A 500µL PEEK sample loop 1815 from Upchurch Scientific can be seen under the injection valve. Flow was created via syringe pump (back, left). Note: The cell does not contain a fiber in this figure.

Flow was introduced to the cell via the inflow port at the top. The injector valve sample loop was used to introduce solutions to the flow cell while maintaining a constant flow delivered by the syringe pump.
2.3 Tapered optical fiber fabrication:

Single mode optical fibers (SMF, Fibertronics Inc.) with 9/125µm core/cladding diameter and pigtail connectors were used. The ends of the fibers were cleaved at a 90° angle with the Vytran LDC-200-G optical fiber cleaving system. These cleaved fibers were spliced using the Vytran GPX-3000 graphite filament, fusion fire-polishing system. Once spliced, the GPX-3000 was used to taper a region of the fiber to a diameter of approximately 10 microns. A schematic of the GPX-3000 is shown in Figure 7 below.

As the filament was heated, the optical fiber would soften which allowed for it to be tapered. Tapering was accomplished by motorized clamps on both sides of the fiber applying tension and pulling at the heated section in a pre-programmed manner. The up-taper and down-taper regions of the fiber were each programmed to be five mm long, and the 10µm diameter tapered waist region was programmed to be 20 mm long.

Figure 7. Layout of GPX-3000 glass processor. A horseshoe-shaped, graphite filament surrounds the fiber and was used to polish the fiber by introducing an electrical current to the filament. To prevent degradation of the filament via oxidation, it is supplied by argon gas, which flows at 2 liters/min when the filament is heated.
2.4 Tapered optical fiber characterization:

Tapered optical fibers were placed on a motorized translation stage connected to a motion controller and viewed under a light microscope. The motion controller was programmed via a custom LabVIEW program that allowed the user to step down the axis of propagation while taking measurements of the fiber diameter. Step size was 100µm. Characterization was repeated three times for each fiber and the results were averaged.

2.5 Biofunctionalization:

Figure 8. Flow cell connected to tunable laser. Fiber is set into bottom piece of PTFE flow cell then connected to tunable laser source for biofunctionalization.

The tapered optical fiber was secured to the flow cell and sealed as described above. The ends of the fiber were connected to an Agilent 81682A tunable laser source and an Agilent HP81532A Power Sensor with output power of 1mW (Figure 8). A custom LabVIEW program was written to allow for the measurement and analysis of spectral output.

All chemicals were purchased from Sigma (St. Louis, MO) unless otherwise noted. The flow cell was rinsed with deionized water, then purged with ethanol for deposition of the molecular recognition layer. A 2% N-(2-aminoethyl)3-aminopropyl
triethoxysilane solution in ethanol was introduced to the cell and incubated for 30 minutes at 25ºC. The cell was then purged with ethanol to remove excess silane and rinsed with deionized water. To activate the silane layer on the surface of the fiber, 12.5% glutaraldehyde in 50mM phosphate buffered saline (PBS) at pH 7.4 was introduced to the cell and allowed to incubate for 2 hours at 25ºC. The glutaraldehyde was removed from the cell by purging with 1x PBS.

Three immunoglobulin G (IgG) antibodies were used in this experiment. The primary antibody that was bound covalently to the silane layer was Alexa Fluor 488 – conjugated Affinipure goat, anti-rabbit (Jackson ImmunoResearch Laboratories, Inc.). The antigen negative treatment (non-specific) was IgG from human serum and the antigen positive treatment (specific) was IgG from rabbit serum. All protein solutions were made to a final concentration of 50 µg/mL. One mL primary IgG solution was introduced to the activated silane layer and incubated at 4ºC for one hour. The flow cell was rinsed with 1x PBS and after antibody exposure three spectral sweeps from 1475-1565 nm were taken over 15 minutes of this rinse period. The process was repeated for the negative IgG treatment, and then the positive IgG treatment.

To test reusability of functionalized fibers, a 0.1M glycine·HCl elution buffer was introduced to antigen-exposed fibers for 30 minutes to remove the antigen from the tapered fiber. The cell was purged with PBS and the negative and positive IgG treatment protocols, above, were repeated.
2.6 VOC flow chamber:

The aqueous flow cell design was updated to allow for the introduction of analytes in vapor phase. The PTFE upper component for aqueous solutions can be replaced with an ABS plastic top that is compatible with a vapor generation system. This lid was designed in Google Sketchup and created with a Makerbot Replicator 2x 3D printer. The interchangeable flow cell parts allow the user to functionalize a tapered fiber with aqueous solutions, and then expose the same fiber to VOCs. In this setup, the bottom PTFE component that holds the fiber remains unchanged, however the top piece of PTFE from the aqueous system is replaced with the piece shown in Figure 9. The end apertures are used to connect and seal the flow cell to the vapor generation system (Figure 10).

![Figure 9. VOC adapter for flow cell (left) 3D printed flow cell vapor adapter. The ABS plastic adapter fits directly onto the fiber-containing piece of the PTFE flow cell as shown in Figure 3. The adapter allows for quick and easy switching between the introduction of vapor and aqueous analytes without the risk of damage to the fiber. (right) 3D model of VOC flow chamber.](image-url)
The vapor generation system is composed of two mass flow controllers (MFCs; Omega Engineering) that regulate the flow of compressed air to the VOC flow chamber (Figure 10). Compressed air enters through the tube on the left, and is split to two separate MFCs. The larger volume MFC passes the compressed air directly to the flow cell. The smaller volume MFC passes 1/10 the amount of compressed air to the sample bottle shown on the right. This bottle contains an analyte solution which is bubbled to create the vapor phase so that VOCs can be introduced to the main flow and to a fiber in the cell. The interchangeable pieces of the flow cell are shown in the foreground.

Figure 10. VOC flow system setup. Air flow is regulated by two mass flow controllers (center, yellow). Analyte introduced from solution in glass jar (right).
Briefly, the results discussed here include characterization, biofunctionalization, and biolayer detection.

3.1 Fiber Characterization:

The averaged diameter (n=3) of tapered, SM fibers are shown in Figure 11.

Figure 11. Dimensions of tapered fibers

The averaged diameter (n=3) of tapered, SM fibers are shown in Figure 11.
The near-IR spectra of the averaged output \((n=3)\) of four tapered, non-functionalized, dry fibers (not the fibers in Figure 11) are shown in Figure 13. The input power of the laser was 1 mW.

The spectra exhibit the sinusoidal modulation and the output power ranges from 0.15 to 0.61 mW. The standard deviations for these spectra are also plotted in the graph (error bars omitted for clarity) with the same respective color. The magnitude of all standard deviations was at least an order of magnitude smaller than the output, and these can be seen as the small-amplitude datasets plotted at the bottom of the graph.
3.2 Fiber Response in Solution:

Fiber response in solution (deionized water) exhibited similar spectra (Figure 14).

3.3 Biofunctionalization of SM Fibers:

For the biofunctionalization process of SM fibers, each analyte was introduced to the flow cell and excess analyte was removed by purging the cell with PBS. Spectra were recorded after each PBS rinse and recorded. Fast Fourier Transform (FFTs) of the amplitude and phase data were also recorded (Figure 15). For the phase plots, an algorithm was used to identify points of interest that correlated to amplitude measurements above a threshold specific to each spectrum. Where these labeled points appeared for all measured spectra on the same graph, the difference in phase between the treatments and the baseline was measured and added to the plot. Points marked by this threshold measurement that were below 0.012 cycles/nm were ignored to account for the resolution that was limited by the bandwidth of the laser. Note: Measured phase differences appended to the graphs are in degrees, y-axis plots are in radians.
Figure 15 shows the original spectrum and the amplitude and phase FFT plots of a functionalization on SMF, Fiber #1.

Figure 15. SMF 1 biofunctionalization spectra. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 1, shown in green on the amplitude plot.
Figure 16 shows the original spectrum and the amplitude and phase FFT plots of a functionalization on SMF, Fiber #2.

Figure 16. SMF 2 biofunctionalization spectra. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 2, shown in green on the amplitude plot.
Figure 17 shows the original spectrum and the amplitude and phase FFT plots of a functionalization on SMF, Fiber #3.

Figure 17. SMF 3 biofunctionalization spectra. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 3, shown in green on the amplitude plot.
For one functionalization, the antigen to the primary layer was introduced to the flow cell before the negative control treatment (Figure 18).

Figure 18 SMF 4 biofunctionalization spectra. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 3, shown in green on the amplitude plot.
3.4 Biofunctionalization of PM Fibers:

Changes to the spectrum became apparent when long, untapered sections of the single-mode fiber outside of the flow cell were brushed up against or moved. As such, the decision was made to switch to polarization-maintaining (PM) fibers to eliminate some of this noise. Furthermore, changes in the spectra also appeared after the introduction of the negative control treatment as shown in Figures 14 through 17. This was likely caused by direct binding of the negative control treatment (human IgG) to open moieties of the aminosilane surface that did not bind to the primary IgG layer (goat, anti-rabbit IgG). This resulted in increased density and thickness of the tapered fiber region, thus changing the spectra. Unbound aminosilane was saturated with a 50mM solution of Tris·HCl pH 7.4 for 30 minutes at room temperature. Spectra for the biofunctionalization of PMF with the blocking step are presented in Figures 19, 20, 22-24.
Figure 19 shows the original spectrum and the amplitude and phase FFT plots of a functionalization on PMF.

**Figure 19.** PMF 1 biofunctionalization spectra. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 4, shown in green on the amplitude plot.
3.5 Reusability:

To test reusability, the antigen was eluted from the surface of the fiber, and a new aliquot of the antigen was introduced. The spectra of the first functionalization on this fiber were recorded in Figure 20. Blocking steps were used, control treatment spectrum not shown.

Figure 20. PMF 1 biofunctionalization spectra – first reuse. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 4, shown in green on the amplitude plot.
The raw spectral data for both biofunctionalizations on this fiber are plotted in Figure 21.

Figure 21. PMF 1 reuse overlay.
The process above was repeated twice with a different fiber (Figure 22).

Figure 22. PMF 2 biofunctionalization spectra. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 0.6, shown in green on the amplitude plot.
The antigen layer was eluted from the fiber and a new aliquot of the antigen was introduced to the flow cell. The cell was rinsed with PBS and the output was recorded in Figure 23.

Figure 23. PMF 2 biofunctionalization spectra – first reuse. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 0.2, shown in green on the amplitude plot.
The antigen was eluted again and a new aliquot of was antigen introduced. The recorded spectra are shown in Figure 24.

Figure 24. PMF 2 biofunctionalization spectra – second reuse. A – Measured output power of tapered fiber as a function of wavelength. B – FFT of fig. A showing amplitude measurements. C – FFT of fig. A showing phase measurements. The threshold to mark points of interest was 0.2, shown in green on the amplitude plot.
The recorded spectra of 22A, 23A, and 24A are shown in comparison to each other in Figure 25.

3.6 Repeatability:

A peak wavelength between 1.54 and 1.56µm in each of the raw spectra was measured before and after antigen capture for 10 fibers. This shift in wavelength was recorded in Figure 26 and Table 1.

![Figure 25. PMF 2 reuse overlay.](image)

![Figure 26. Shifts in peak wavelength across all individual experiments. Bars 1-3 were experiments conducted on SM fiber, bars 4-10 on PM fiber. Statistical analysis of each of these experimental groups is shown in Table 1 below.](image)

<table>
<thead>
<tr>
<th>Table 1. Average Peak Wavelength Shifts due to Antigen Capture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Peak Wavelength Shift (nm)</strong></td>
</tr>
<tr>
<td>SM Fibers</td>
</tr>
<tr>
<td>PM Fibers</td>
</tr>
<tr>
<td>All Fibers</td>
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</tbody>
</table>
3.7 Thresholds:

To assess the time frame for IgG interaction, a fiber was biofunctionalized with the primary layer IgG. One mL of 50µg/mL rabbit IgG was introduced to the over the course of an hour (Figure 27). Spectra were recorded every 15 minutes.

![Figure 27. Time response of tapered PMF. Output spectrum of antibody-antigen capture. Redshift of spectrum observed as biolayer thickens.](image)

A fiber was prepared to test the platform’s lower limit of detection using goat, anti-rabbit IgG as the primary biolayer. Rabbit IgG aliquots of increasing concentration were introduced to the flow cell. The fiber was exposed to a 1mL aliquot of rabbit IgG with a starting concentration of 500pg/mL for one hour. At the end of this hour, the spectrum was recorded, the concentration of rabbit IgG was increased tenfold and introduced to the cell. This process was repeated sequentially until the spectrum stabilized (Figure 28).
These results are also in agreement with the theoretical amounts of antibody the tapered fiber can facilitate. IgG molecules are approximately 28 nm in diameter, as shown by atomic force microscopy. The approximate spacing of hydroxyl groups on the surface of silica in the form of our tapered fiber is approximately 10 groups/nm$^2$. A uniformly distributed surface with two perfectly conical, 5mm taper transition regions and a perfectly cylindrical tapered waist 20mm long was assumed for calculations. The total surface area was estimated to be 0.1397 cm$^2$ and would be saturated by 37.7fM of IgG.

Figure 28. Threshold of detection for aqueous tapered optical fiber sensing platform. Spectral redshift is observed at concentrations as low as 500pg/mL (3.75fM). Spectrum continued to redshift as antigen concentration was increased through 50ng/mL. At 500ng/mL the spectrum exhibited a slight blueshift, suggesting that the surface has been saturated.
CHAPTER 4
DISCUSSION

Methods were devised to reliably taper both single-mode and polarization-maintaining optical fibers to approximately 7µm in diameter for biosensing applications. By tapering the fibers to this diameter, the light propagating through the fiber could interact with any molecules bound to the surface. The flow cell was built from a design that was both simple, relatively inexpensive, and customizable using sheet PTFE. The sensor platform showed a characteristic change in spectral output as a consequence of antibody-antigen capture. FFT analyses of spectral data provided a method that could be potentially used for computer automation of analyte detection.

In order to sense molecules on the surface of the fiber, the fiber itself needs to be tapered to a diameter of <10µm. At this diameter, the fiber is fragile, and a platform needed to be designed that both protected the fiber and allowed for the introduction of various solutions. The chemical resistivity and ease of machining of PTFE made it the obvious material from which to construct the platform. Through the use of 3D printing technology, interchangeable parts were printed from ABS plastic for eventual vapor sensing applications.
The flow cell platform, when connected to a flow controller and syringe pump can be used to introduce precise amounts of aqueous solution at varying flow rates without damaging the fiber. There is room for optimization of the system, as the volume of the flow channel is approximately 1mL, much larger than the tapered region of the optical fiber. By reducing this volume, the sensor platform may be able to resolve lower concentrations of analytes, as there would be surface area in the flow cell for the functionalized fiber to compete with. Flow cells with smaller volume flow channels have been designed and future experiments are planned.

The sinusoidal modulation seen in the measured spectra is caused by the interference of the two major modes propagating through the fiber which is tapered to a non-adiabatic profile. This modulation actually provides the basis for the detection and measurement of protein-protein interactions, as a redshift in the spectrum is exhibited when antigens are captured by the antibodies tethered to the surface of the fiber. This redshift of the signal is seen across multiple fibers with an average shift in peak wavelength of 1.42 nm. The spectra of tapered single-mode fibers were very susceptible to any physical disturbances to the non-tapered region of the fiber. Any slight bends or accidental movement of the non-tapered regions of the fiber changed the polarization states of the propagating light, resulting in shifts of the spectrum that were independent of the surface chemistry on the tapered region. To compensate for this, polarization-maintaining fibers were used instead that exhibited a static output so long as the fibers were not bent too much. This static output was sufficient to create a baseline for biosensing.
These fibers are also reusable, as the antigen can be eluted with a low-pH solution. The original antibody remains tethered to the fiber surface and is capable of capturing more antigen after the fiber is resuspended in PBS, as supported by the resulting redshift in the spectrum. Unexpectedly, when the eluted fibers were resuspended in PBS at pH 7.4, the spectrum did not return to an initial baseline. Instead, the fiber normalized to a different baseline, from which the redshift was observed again when antigen was reintroduced to the platform. This may be problematic, as a marketable biosensor must be able to return to baseline if it is going to compete with commercially available SPR.

Experiments to determine the lower limit of detection suggested a capture limit of >3.75 fM, as the system began to show a redshift when a 500pg/mL aliquot of antigen was introduced to the fiber functionalized with antibody. Additional experiments are planned to verify this threshold, but this preliminary result shows great promise, as it would exceed current commercial SPR detection thresholds by an order of magnitude. When working with larger concentrations of antigen, the system shows a spectral response within 15 minutes of exposure which is a reasonable response time for a commercial system.

The antibody-antigen capture can be measured by a simple shift in the peak wavelength at a local maxima or minima on the spectrum. Such a straightforward analysis is easily done by the human eye, which can quickly discern which peak best demonstrates the effect. However, if this process were to be scaled up to an array format technology that observes multiple tapered fibers at once or to be automated by a computer, the computer algorithm would have difficulty identifying the peaks of interest.
Fast-Fourier Transforms are a mathematical technique demonstrated in this paper that can break down a periodic function into the phase and amplitude components for each frequency. Using FFT, the primary frequencies of the signal (modes of the propagating light) were identified. The phases of each of these primary frequencies redshifted 45-90 degrees after an hour of exposure to the antigen. This method is one possible way of analyzing the spectral data in a way that a computer could easily differentiate. A recent study\textsuperscript{22} has used neural network algorithms in conjunction with primary component analysis, which might be useful to consider for future work on this project.

This tapered optical fiber system is capable of label-free detection of antibody–antigen interactions with a limit of detection that is comparable to, and might exceed the performance of existing commercial systems. More work will need to be done in order to meet this goal, and future experiments are planned to determine the limit of detection as a function of molecular size, as the biolayer in this experiment was approximately doubled as the antigen (rabbit IgG) was added to the flow cell. All of the work presented in this paper shows specific interactions, but arrays of fibers functionalized with a variety of nonspecific molecules have been shown to resolve different analytes. Thus, future work could be directed towards the detection of specific analytes of commercial significance or towards a more versatile sensor that can resolve a variety of analytes with less sensitivity. Furthermore, future experiments are planned to conduct preliminary testing for VOC sensing on the platform presented in this paper.
BIBLIOGRAPHY


APPENDIX

APPENDIX A: FIBER FABRICATION

Vytran LDC-200 Fiber Cleaver Protocol

- Wear proper PPE – safety goggles and gloves
- Turn on power supply unit
- Turn on power switch on back of LDC-200
- Ensure digital keypad readout displays cleave angle and diameter of fiber you wish to work with
- Remove protective 500µm jacket from approximately 10cm of fiber
- Remove protective 250µm jacket from approximately 3-4cm of fiber
- Use lens cleaning cloth with acetone to wipe down stripped fiber
- Ensure that 250µm-diameter fiber holding block is in position on the left
- Ensure that 125µm-diameter fiber holding block is in position on the right
- Press the start button to begin the vacuum
- The interface where the stripped fiber meets the 250µm jacket should be placed at the right-most vacuum hole in the left block, the stripped fiber should reach the 125µm block on the right
• 250µm jacket should not be resting on the black, ceramic v-block at the end of the fiber holding block. Gently close the fiber blocks, flip up the lever lock for each block, tighten the screws on top of each block to finger tightness

• Press start to cleave

• Use tweezers to remove and dispose of remaining fiber in right block

• Remove fiber-containing block and place on GPX-3000

Note: Fibers were purchased from Fibertronics, Inc. 9/125µm core/cladding diameter.
Vytran GPX-3000 Glass Processor Startup Protocol

- Open FFS3 software
- Turn on power supply unit
- Turn on vacuum switch (orange box)
- Turn on switch on the back of GPX-3000
- Press ‘initialize’ button (key icon) in software
- Loosen argon regulator valve – Argon tank is turquoise blue – pressure 9-12psi
- Press ‘purge’ button (gas tank icon) to purge filament with argon to prevent oxidization
- After purge, software should read 0.15 liters/min of argon flow
**Vytran GPX-3000 Normalization Protocol**

- Run startup protocol
- Cleave two fibers, and place them into each of the fiber holding blocks on the GPX
- Open normalization file
- Move camera to ‘back’ or ‘front’ position
- Press ‘Load Fibers’ button (yellow forklift icon)
- Move each block into field of view using coarse adjustment for ‘left block’ and ‘right block’
- Press splice
- Read on screen prompts – percentage of expected result will appear, repeat as necessary until software tells you to stop. It should normalize to within 3% of 100%

Note: This protocol uses single-mode PANDA fibers from Fibertronics, Inc.
**GPX-3000 SMF Splicing Protocol**

- Run startup protocol
- Open SMF SMF file
- Place one cleaved fiber in each of the fiber holding blocks of the GPX-3000
- Press ‘load fibers’ button (yellow forklift icon)
- Move camera to ‘back’ or ‘front’ position
- Move each block using coarse adjustment until fibers enter camera’s field of view
- Press ‘splice’ icon (blue button icon)
- If necessary press ‘loss estimate’ (dB icon) to estimate splice efficiency
GPX-3000 PMF Splicing Protocol

- Run startup protocol
- Open PANDA PANDA file
- Place one cleaved fiber in each of the fiber holding blocks of the GPX-3000
- Press ‘load fibers’ button (yellow forklift icon)
- Move camera to ‘back’ or ‘front’ position
- Move each block using coarse adjustment until fibers enter camera’s field of view
- Press ‘splice’ button (blue button icon)
- Procedure may fail if fibers cannot be aligned – if so, press abort and try ‘splice’ button again. This may require multiple restarts.
- Alignment may not be possible in some cases due to orientation of fibers in fiber blocks. In these cases, just change the alignment of one of the fibers.
GPX-3000 SMF/PMF Tapering Protocol

- Open taper or SMF SMF profile
- If tapering fibers that were just spliced, remove spliced fiber from fiber holding blocks
- Press ‘load taper’ button (red forklift icon) – blocks will move farther apart
- Load fiber, ensuring that 125µm diameter fiber and not the 250µm jacket is resting on the ceramic v-blocks
- Check View>Tension monitor to ensure tension is between 130-145g
- Press ‘taper’ button (tapered fiber icon)
APPENDIX B: FLOW CELL FABRICATION

- Sheet PTFE is used as the base material for the flow cell
- PTFE can be machined in the machine shop
- 3D printer with ABS plastic may be used for vapor phase flow cell components, but is not chemically compatible with the aqueous phase
- Import 3D model to 3D printer
- Print off flow cell
- Silicone gasket is used to seal flow cell

Note: Sheet PTFE is purchased from Laird Plastics in Dayton. ABS Plastic and 3D printer are from Makerbot. Silicone is from Miami Valley Gasket.
APPENDIX C: FIBER TESTING SETUP

- 6-port flow controller is attached to syringe pump and flow cell
- Refer to Chromtech V-450 manual for setup of individual ports
- Flow cell with fiber is connected via fiber to laser source and photodetector

Note: This protocol uses equipment purchased from Chromtech, including a V-450 6-port flow controller, PEEK tubing, and ferrules. The syringe pump was purchased from Fischer Scientific.
APPENDIX D: BUFFER AND CHEMICAL PROTOCOLS

2% Amino Silane Preparation

- Aliquot 980µL ethanol to the number of centrifuge tubes you wish to use
- APTES must be opened under nitrogen, using glove bag in fume hood. Failure to do so can result in injury or death
- The glove box is found under the HPLC in SC341
- Place ethanol filled tubes inside glove box, as well as pipettes and pipette tips
- Connect the tubing on the glove box to the Nitrogen tank next to the hood
- Place the glove box with all necessary materials inside the hood.
- Turn on the nitrogen
- Open the APTES inside the glove box, adding 20µL to each tube
- Close all centrifuge tubes, place in Tupperware container with water absorbing beads
- Seal Tupperware container

Note: Chemicals acquired from Sigma (St. Louis, MO)
**Antibody Solution Preparation**

- All antibody solutions should be prepared to 50µg/mL aliquots in PBS solution
- PBS should be prepared to 50mM and pH of 7.4
- One liter of PBS can be prepared with the following recipe
  - (137 mM NaCl, 2.7 mM KCl, 10 mM Sodium Phosphate dibasic, 2mM Potassium Phosphate monobasic, pH 7.4)
- For 1 liter of 1X Phosphate-buffered saline (1X PBS buffer):
  - Dissolve in 800ml of distilled H2O:
    - 8.0g NaCl
    - 0.2g KCl
    - 1.44g Na2HPO4
    - 0.24g KH2PO4
  - Add H2O to 1 liter.
  - Adjust the pH to 7.4 with HCl or NaOH

Note: Chemicals acquired from Sigma (St. Louis, MO)
APPENDIX E: SURFACE CHEMISTRY PROTOCOLS

Fiber Surface Protein Chemistry Procedure

- Rinse fiber with DI water
- Acid wash fiber with 1:1 solution of H$_2$SO$_4$ and DI water
- Purge cell with H$_2$O
- Purge cell with ethanol
- Inject 2% APTES solution in ethanol, incubate 30 minutes at room temperature
- Purge cell with ethanol
- Purge cell with DI water
- Inject 12.5% glutaraldehyde in 50mM PBS @ pH 7.4 to activate silane, incubate for 2 hours at room temperature
- Purge cell with PBS
- Inject 1mL of 50µg/mL of primary IgG solution to activated surface, incubate for 1 hour
- Purge cell with PBS
- Inject 50mM Tris solution, incubate for 30 minutes
- Purge cell with PBS – Measure spectral output for baseline
- Inject 50µg/mL antigen solution to biofunctionalized fiber
- Measure spectral output

Note: Chemicals acquired from Sigma (St. Louis, MO)