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FIBER OPTIC BIOSENSOR THAT DETECTS AND ANALYZES PROTEINS IN THE BLOOD USING PHASE-SENSITIVE INTERFEROMETRY

Princess Ehikhuemen

FIBER OPTIC BIOSENSOR THAT DETECTS AND ANALYZES PROTEINS IN THE BLOOD USING PHASE-SENSITIVE INTERFEROMETRY

by

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ABSTRACT

FIBER OPTIC BIOSENSOR THAT DETECTS AND ANALYZES PROTEINS IN THE BLOOD USING PHASE-SENSITIVE INTERFEROMETRY

Princess Ehikhuemen, B.S. Biomedical Engineering

The University of Texas at Arlington, 2019

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A current challenge in the healthcare field is the ability to continuously monitor blood analytes in real-time and with accuracy. The concentration of various blood analytes can change relatively quickly in critical care situations. The goal of this senior design project is to design a fiber optic biosensor that detects and analyzes specific proteins in the blood. In order to achieve this, a reservoir-receiver unit was designed to hold the fluid and the fibers were stripped, cleaved and spliced.

The fiber tips were finally coated with the specific capture chemistries and connected with an SC connector to a phase-sensitive analyzing system. The protein of interest was laminin. Epoxy was used to create the reference gap for the analyzing system and was also used to bind laminin. A functional system was obtained with a spectral

interference waveform that showed the binding of laminin to epoxy. This was visualized by an increase in the optical path length.

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CHAPTER 1

INTRODUCTION

1.1 Background and Significance

A current challenge in the healthcare field is the ability to continuously monitor blood analytes both with accuracy and in real-time. The concentration of various blood analytes can change relatively quickly in critical care situations. These changes in concentration could represent significant changes in the functioning of organs like the heart, lungs or kidneys. For example, blood pH levels could be an indicator that a patient is suffering from respiratory acidosis, and the lungs are not functioning optimally. Blood tests are usually ordered by healthcare professionals to get information that will help them more easily diagnose diseases, especially life-threatening ones [6].

Right now, a technician usually draws blood from the patient and the testing sample is taken to the laboratory. Then, trained technicians usually look for the information the healthcare provider ordered. Sometimes it could take days or weeks for the laboratory results to get back to the healthcare providers because they have numerous samples to test [6]. Closely monitoring the concentration of blood analytes, like proteins, is important for patients, especially those in the intensive care unit (ICU). Presently, the monitoring of glucose and lactate levels can be easily achieved bedside using single or multi use *in vitro* tests. Many of these *in vitro* tests are based on electrochemical sensors [5].

However, more recent technologies have started incorporating optical biosensors. Optical biosensors are preferred technology because they have ease of miniaturization

using modern optical fibers, the potential for long-term stability using ratiometric measurements at multiple wavelengths, and no transduction wires. In an optical fiber system, light is passed to and from a distal sensing site that contains immobilized dyes that are sensitive to the analyte of interest. This sensitivity is due to the optical fibers which measure the reflectance of the luminescence, either by fluorescence or phosphorescence, coming from the immobilized indicators at the fiber ends [5].

To successfully detect the protein of interest, it is necessary to immobilize antibodies to the glass surface that forms the fiber core. This aids the specific binding of the antigens to the antibodies in a region where the overlap with the guided mode of the fiber is strong. Antibodies cannot be attached directly to the glass surface, so the immobilized antibodies are attached on the surface with the use of silane and cross-linked layers. The silane layer is attached to the glass surface, then the cross-linker is attached onto the silane layer, and the antibodies are attached to the cross-linked layers [12].

The capture chemistries rely on these antibody-based capture agents. The capture chemistry is the antigen and the protein of interest in the antibody. A high-quality antibody exhibits high affinity and selectivity for its cognate protein and can detect analytes at a higher sensitivity than conventional macroscopic immunoassays. There are two kinds of antibodies that can be used: monoclonal and polyclonal antibodies. Polyclonal antibodies are a collection of antibodies from different B cells that detect multiple epitopes on the same antigen. Each antibody detects a unique epitope that is located on that specific antigen. A monoclonal antibody is a single, unique antibody-producing B cell that binds to one specific epitope [11]. The advantage of the monoclonal antibody over the polyclonal

is that is has a high degree of specificity to a single epitope. Hence, there is a reduced probability of cross reactivity. However, it is more expensive to produce.

Biomarkers are very important in the clinical setting because they supplement medical findings and patient history. For example, laminin is a cell adhesion molecule found in the basement membrane of animals. The basement membrane is a thin sheet of extracellular matrix (ECM) that underlies epithelial, endothelial, Schwann and adipose cells. Laminin is important for many physiological functions. It plays an important role in embryonic development, tissue repair and formation of organs [4]. Detecting the concentration of laminin in the blood can give information on those physiological functions.

The goal of this senior design project was to create a fiber optic biosensor equipped with protein-specific capture chemistries that will be able to detect and analyze proteins in the blood. The protein of interest is laminin. It was envisioned to be placed bedside in the ICU to monitor blood analytes continuously and in real-time. This should be able to eliminate the need for the current blood testing process in the ICU. This biosensor will operate using an analyzing system that incorporates spectral domain-phase sensitive interferometry.

1.2 Spectral Doman-Phase Sensitive Interferometry

Interferometry works by correlating a change in phase of the optical path length with a substrate binding to the molecule of interest. The spectrum of light reflected from the sample creates the interference pattern. The signal obtained from the system has a frequency that correlates with the optical path length difference between the top and bottom

surface of the sensor. This causes a phase change which yields the optical thickness of the layer [2].

The analyzing system returns two waveforms with equations that show the spectral interference (Equation 1.1) and the change in phase (Equation 1.2) [3].

Equation 1.1:

$$S(k) = S_{ref}(k) + S_{samp}(k) + 2\sqrt{S_{ref}(k)S_{samp}(k)}.\mu(k).\cos(2\pi k\Delta p + \varphi_0)$$

Equation 1.2:

$$\varphi|_{z=n\Delta z} = \tan^{-1}(\frac{Im(F(S(k))}{Re(F(S(k))}) = \frac{2\pi n\Delta z}{\lambda_0}$$

Equation 1.1 shows the mixing of light from the reference path and sample path. Equation 1.2 shows the sub-wavelength changes in the path length by detecting the phase shift in the frequency component of Equation 1.1 [3].

1.3 Existing Similar Systems

There have been other applications of continuous monitoring of blood analytes. Medtronic has a Continuous Glucose Monitoring system (CGM), that helps diabetics monitor their glucose levels in real-time. The CGM system automatically receives the glucose readings from the user every five minutes which allows them to fingerstick less frequently. They have two applications of this system, the Smart CGM and the Professional GCM. The Smart GCM is used by the diabetic patients and the Professional GCM is used by the healthcare provider [7]. Another application of the continuous monitoring devices is the OptiScanner 5000/6000 which automatically monitors the glucose and lactate levels of ICU patients using an integrated blood centrifuge and spectrometer. This also allows for real-time monitoring [10].

1.4 Objectives

This device is to be used in the ICU to monitor protein concentration in the blood of patients. An automated extraction system was created by Dr. Davé's 2018 senior design team which will be used to draw blood from the patient. This system will place the blood into a reservoir and a functionalized fiber will be inserted into the blood sample in order to detect the proteins in the blood and return the concentration. The protein of interest used in the testing procedure was laminin.

1.5 Distinguishing Features

The CGM is like an insulin pump site in that it attaches directly to the patient's body and must be changed every 3-7 days depending on the exact model [7]. The device designed differs from the CGM in that it will not be required to be attached to the patient's body. The reaction between the protein and capture chemistry combined with spectral domain-phase sensitive interferometry will allow the device to detect more than just glucose levels. It will not just be specific to a single protein; it will be specific to the capture chemistry coated on the fiber tips.

CHAPTER 2

DEVICE SPECIFICATIONS

2.1 Functional Specifications

According to the project guidelines provided by Dr. Digant Davé, the biosensor should be able to detect protein in a pseudo blood sample and determine the concentration of protein in the sample. A gap needs to be created on the fiber tip so that it can be used as the reference path length. Having a reference path length allows the system to recognize a change in that optical path length. Furthermore, the optical fibers must be able to be inserted quickly and easily, while being secure in the sample.

2.2 Physical Specifications

The device should be small and portable enough to be placed near a patient's bed in an ICU room. The aim for this device is to monitor the patient's blood within the ICU room itself, without the need for transportation to a laboratory. Therefore, it is essential that the device fit in the room without taking away space needed for other vital ICU devices or personnel. The device should be simple enough for most technicians to operate with little training. The reservoir should also be leak-free to ensure that the blood components are trapped in there. This means that the insertion and removal of the fiber optic into the sample should be very quick without minimal loss of blood sample.

2.3 Applicable Standards

By FDA standards, this fiber optic biosensor will be classified as an *In Vitro* Diagnostic (IVD) Device. These are medical devices that analyze human bodily fluids like

blood and urine, to provide information for prevention, diagnosis or treatment of a disease. It would qualify as a Class I device, which means it would be exempt from premarket notification procedures. It is a low-moderate risk device and would be under general controls. The analyte specific reagent would have to be determined and would have to go through all the approval processes in the FDA's IVD regulation policy [14].

When dealing with blood, there is the possibility for the formation of biofilm. The reservoir was made disposable to avoid that. That way, the reservoir will be changed out after every fluid sample. There are also precautions for healthcare workers who handle blood. They must follow Occupational Safety & Health Administration (OSHA) standards. They must use gloves, masks and gowns. They must also take precautions for Other Potentially Infectious Materials (OPIM). The complete standards for blood handling can be found in OSHA's universal precautions for blood handling [9].

CHAPTER 3

METHODOLOGY AND COMPONENTS

3.1 Reservoir

The reservoir was designed and fabricated by the team. Using SolidWorks, the original reservoir was designed and converted to an STL file. This file was then taken to the UTA FabLab and printed using the uPrint SE 3D printer. This printer was chosen because of its ability to print using ABSplus material, along with support material. The support material was used because of the hollow portions of the reservoir. It is important that the walls of any 3D printed model are appropriately thick to ensure structural integrity. After the reservoir was printed, it was placed in a lye bath to dissolve the support material for 4 days. This reservoir has a volume of 13.8 mL.

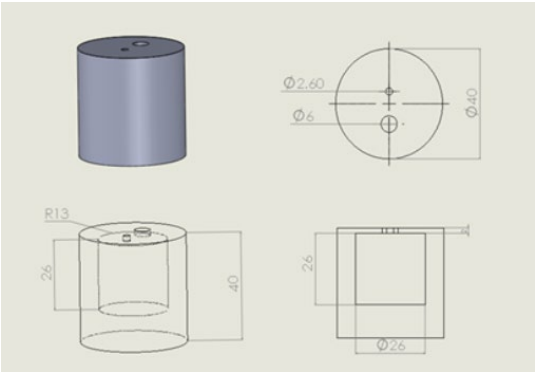

Figure 3.1: Schematic diagram of first reservoir showing all dimensions in [mm]

The second, smaller reservoir was also designed in SolidWorks and converted to an STL file. It was printed with the PolyPrinter 229 3D printer. However, this reservoir was printed without support material. This was done to avoid the time-consuming process

of dissolving the support material. Also, one of the holes at the top was larger and the reservoir was smaller so that could reduce the need for the use of support material. The second reservoir was designed to be smaller in height than the first because it would avoid the need to make a longer, more fragile fiber. Also, one of the holes was made bigger so that it is easier to insert fluid into the reservoir. This reservoir has a volume of 5 mL.

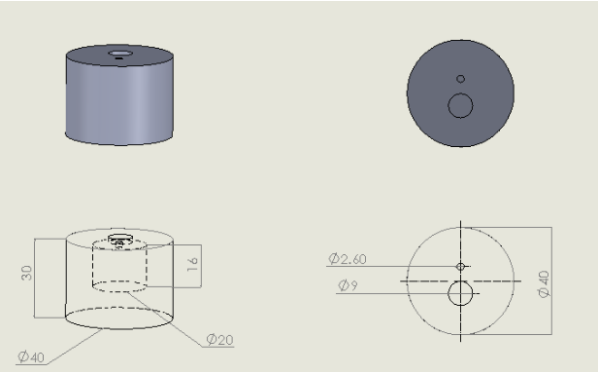

Figure 3.2: Schematic diagram of final reservoir showing all dimensions in [mm]

3.2 Receiver Port

The receiver port for the fiber connector is made up of an altered SC mating sleeve. The SC mating sleeve needed to be altered because it was purchased as a two-sided mating sleeve. The mating sleeve is used as a receiver for the connector and allows the functionalized fiber in the connector to reach the fluid in the reservoir more easily. The alterations were done by cutting the bottom half off with an electric jigsaw. Once cut, the half to be used was manually sanded until smooth. It is important to avoid sanding too far as it could alter the port that was made to fit the SC connector. It should be able to fit like a lock and key.

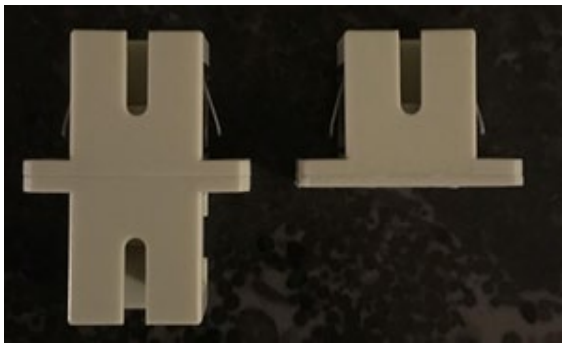

Figure 3.3: Two-sided SC mating sleeve (left) and altered SC mating sleeve after sanding (right)

Figure 3.4: Alteration process of SC mating sleeve with jigsaw

3.3 Prepared Fiber

The fiber used in this design consists of a glass core and a cladding. The core is what the light passes through and the cladding is what keeps the light inside the core. To prepare a fiber for functionalization, it must be stripped, cleaned and cleaved.

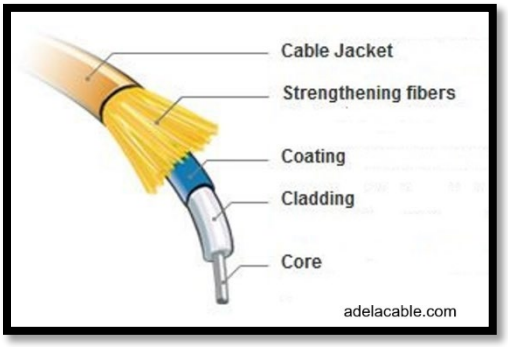

Figure 3.5: Enhanced view of glass fiber

3.3.1 Stripping

Stripping is done to remove the other coating of the fiber. The fiber is first placed in a clamp to hold it down and expose the tip that will be used. The clamped fiber is then placed in a heat stripper which melts the coating off the fiber and uses a blade to scrape the coating off. This exposes the core of the fiber.

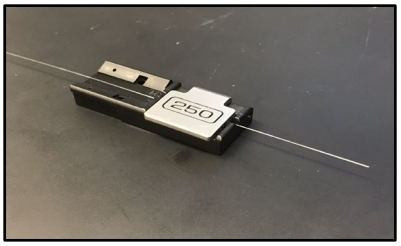

Figure 3.6: Clamped fiber to expose fiber tip that will be used

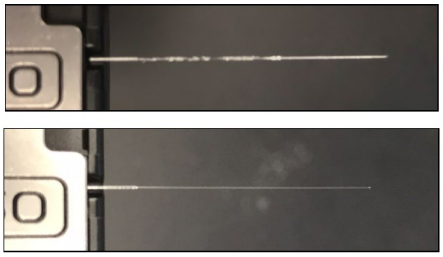

Figure 3.7: Stripped fiber before (top) and after (bottom) cleaning

3.3.2 Cleaning

Cleaning is done to remove the excess coating left after stripping the fiber. The clamped, stripped fiber is thoroughly cleaned with acetone and wiped with Kimwipes. It is very important that a fiber is cleaned properly to make sure it cleaves better.

3.3.3 Cleaving

Cleaving is done to cut the fiber to get a clean, straight edge. The clamped, stripped and cleaned fiber is placed in a cleaver, which is a device that uses a blade to cut a straight edge. It is important to be as accurate as possible with a cleave. The fiber must be cleaved to an angle closest to zero degrees, even one degree may cause problems with the splicing process.

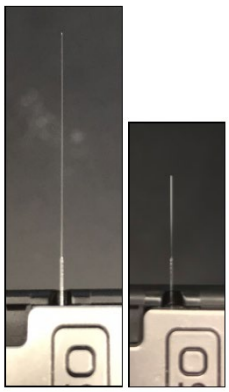

Figure 3.8: The fiber before cleaving (left) and after cleaving (right)

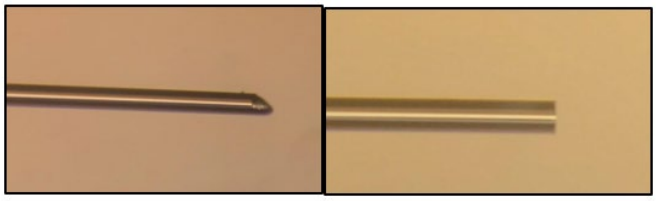

Figure 3.9: Magnified view of fiber before cleaving (left) and after cleaving (right)

3.4 SC Fiber Connector

The connectors were purchased from ThorLabs and assembled. The connector consists of the boot, the crimp eyelet, inner frame assembly, outer housing, and the dust cap. All these parts are not necessary, depending on the size of the fiber and the length it will be extended past the ferrule. For this experiment, a 250 µm fiber with a 125 µm core was used. Hence, all the SC connector components were not needed. The boot was slid onto the fiber, placed in the inner frame assembly and snapped into the outer housing.

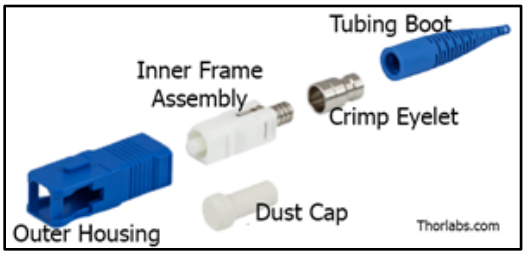

Figure 3.10: SC connector components

Figure 3.11: Fiber extending through connector

3.5 Coating Tray

The coating tray was designed to allow for the testing of multiple samples. It was designed in SolidWorks, converted to an STL file and 3D printed. The coating tray consists of a bottom housing unit that holds the fluid sample, and a lid where the mating sleeve will be epoxied onto. The lid was 3D printed using lime green ABS material, and the bottom was 3D printed using translucent white ABS material. Ten SC mating sleeves were fabricated using the procedure in section 3.2. The mating sleeves were then epoxied onto the lid. The lid was placed on the bottom housing unit and secured using tape. Tape was used because it is easily removable, which allows the inner housing unit to be visible during testing.

Figure 3.12: Coating tray with top view of various housing units

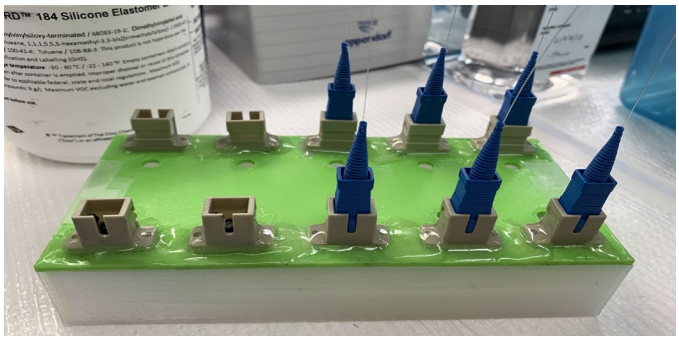

Figure 3.13: Coating tray with mating sleeves and connectors inserted

3.6 Functionalized Fiber Tip

Functionalizing the fiber tip is what makes it a biosensor. To functionalize a fiber, a gap must be created and then the fiber must be spliced.

3.6.1 Creating the Gap

Creating the gap is important because it will be used as the reference path length so that the system recognizes a change in that path length as binding occurring. Several methods were tested to create the gap.

3.6.1.1 Testing Etch Cream

Initially hydrofluoric acid (HF) was to be used for etching but it could not be acquired. Hence, the etching cream, Armour Etch, was used in its place. The etching process is supposed to create a gap by removing a part of the core. First, the fiber tips were cleaned with acetone, then the cream was shaken and applied on the fiber tips with a Q-tip. Then the fibers were laid down and checked periodically at 30 minutes, an hour, 1.5 hours and two hours. The fiber tips were then cleaned again with acetone and water and observed under the microscope. No physical changes were observed so it was decided that new fiber tips would be coated for longer.

New fiber tips were coated for 24 and 48 hours to determine the maximum etching ability of the cream. Some of the fiber tips broke and upon viewing the rest under a microscope, it was determined that they etched partially. This partial etching was not enough to create the gap, so a new method needed to be devised.

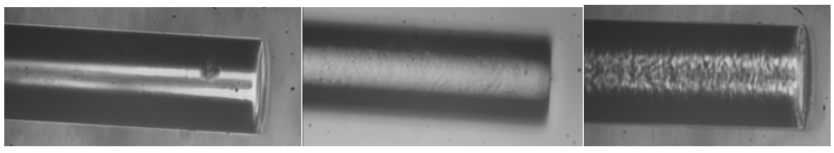

Figure 3.14: Unetched fiber (left), after 2 hours (middle), after 48 hours (right)

3.6.1.2 Testing Microbeads

Since the etching cream was not effective, microbeads were tested instead. The microbeads would work by attaching to the tip of the fiber using laminin and anti-laminin.

The microbeads were coated in anti-laminin and the fiber tip in laminin. Then, the fiber tip was used to touch the beads to see if they would attach via the laminin/anti-laminin chemistry. However, the microbeads would not stay attached to the fiber; they would either slide down the length of the fiber or fall off immediately when the fiber was moved. Hence, a new method needed to be devised to create the gap.

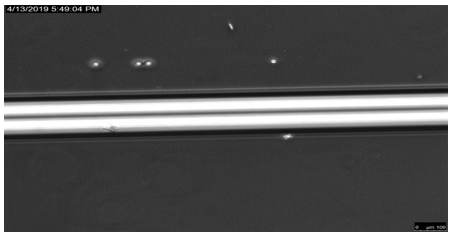

Figure 3.15: Magnified image of the microbeads attached to the fiber

3.6.1.3 Testing PDMS

Since the microbead method was ineffective, polydimethylsiloxane (PDMS) was tested to create the gap. To prepare the PDMS, 10 mL of SYLGARD 184 Elastomer Silicone Base and 100 µL of SYLGARD 184 Silicone Elastomer Curing Agent were combined and stirred vigorously for 10 minutes. This mixture was placed in a vacuum chamber to remove the bubbles formed during mixing. While the PDMS was still wet, the tip of each fiber was dipped in it and inserted into the coating tray to cure for 24 hours while placed upside down. The PDMS formed a bulbous shape near the tip of the fiber with a small gap, estimated to be 20 to 25 micrometers, at the tip. This gap is indicated in red in Figure 3.16 below.

Figure 3.16: Magnified image of the PDMS after curing for 24 hours and gap formed

3.6.1.4 Testing Epoxy

Epoxy was then tested to create the gap. The base and curing agent were mixed for 20 seconds, then the tip of each fiber was dipped into the epoxy. One fiber was inserted into the coating tray to cure upside down, and one fiber was left upright to cure. Both fibers were left to cure for 24 hours. The epoxy formed a similar bulb shape with a small gap at the tip.

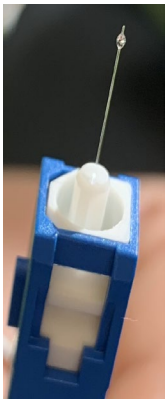
Figure 3.17: Fiber tip coated with epoxy

3.6.1.5 Further Testing of Microbeads

Using microbeads would create a fixed gap dependent on the size of the microbeads. Combining either the microbead and PDMS procedures or the microbead and epoxy procedures would allow for a more effective method of creating the gap. First, 20

µL of microbead solution was dropped onto a petri dish and allowed to dry for 1.5 hours. PDMS was mixed as described in 3.6.1.3 and the tip of the fiber was dipped into the PDMS. While still wet, the fiber was dipped into the dried microbeads. This was cured for 24 hours. Similarly, epoxy was prepared as described in 3.6.1.4 and the fiber tip was dipped into the epoxy. While still wet the fiber was dipped into the dried microbeads. This was also cured for 24 hours and the results can be seen in Section 4.2.1.

3.6.2 Splicing

Splicing is an important process to prepare the fibers for the phase-sensitive analyzing system. To get a successful splice, it is important to have a perfectly cleaved fiber. For accurate cleaving, the cleaver must be very clean, and the fiber must be thoroughly cleaned with acetone. The splicing process joins two fibers, the functionalized fiber and the analyzing system fiber, together. The analyzing system fiber uses an FC/APC connector. The splicing machine spliced only fibers with a cleaved angle closer to zero degrees. It is also important to look at the spliced fibers with an infrared viewer because that is the only way the presence of scattered light can be seen. A bad splice (see Figure 3.19) will have a lot of scattered light and that interferes with the fiber optics process that is vital to this project. The lights in the lab were turned off and the spliced fiber was looked at through the infrared viewer.

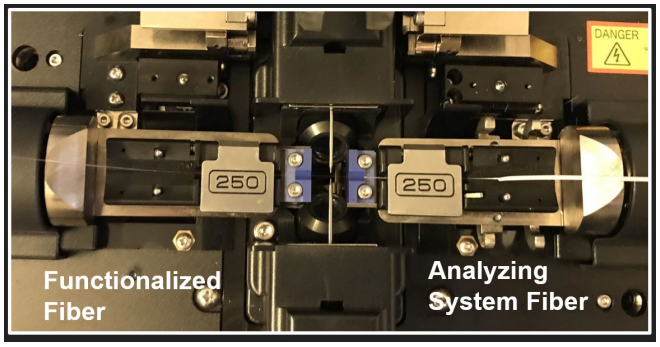

Figure 3.18: Splicing process joining functionalized fiber and analyzing system fiber

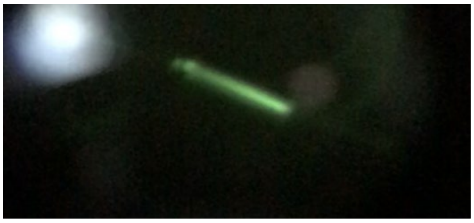

Figure 3.19: Infrared view of bad splice

3.7 Analyzing System Setup

The analyzing system used a broadband light source coupled with a single mode fiber (SMF) splitter (see Figure 3.20). A fiber isolator was inserted between the light source and input port of the SMF coupler. The other port of the fiber coupler was terminated with a FC/APC connector. Then, the light of the fiber port was focused on the sample. Lastly, the LabVIEW code is run to bring up the analyzing results.

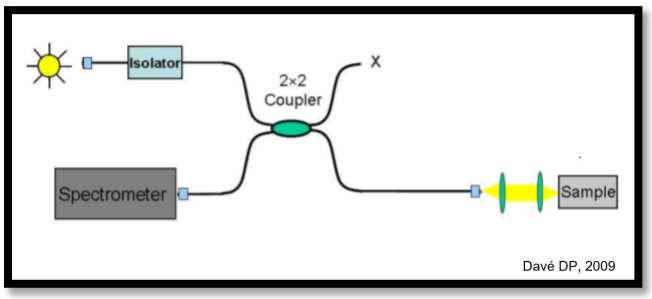

Figure 3.20: Fiber-based spectral domain-phase sensitive interferometer

3.8 Assembly

Finally, all major components had to be assembled. The fiber was placed in the SC connector, which was in turn, snapped into the mating sleeve. The mating sleeve was already epoxied onto the reservoir. It is important that when the fiber is placed in the connector, it is done very carefully because the fibers are very fragile. The SC fiber connecter equipped with the capture chemistry coated fiber easily and securely snaps into the SC mating sleeve receiver. The other end of the fiber will be spliced to an FC/APC connector that will be the connection to the analyzing system.

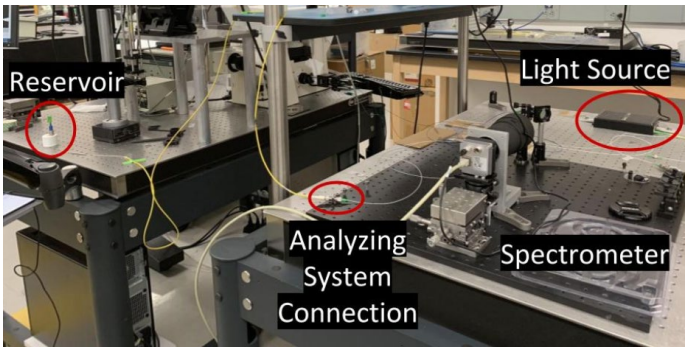

Figure 3.21: Complete system setup with reservoir and fiber-based spectral domain-phase sensitive interferometer

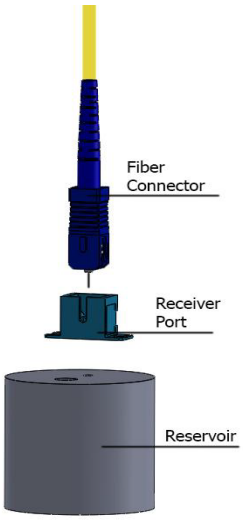

Figure 3.22: Exploded view of design assembly showing individual components

CHAPTER 4

TESTING AND RESULTS

4.1 Testing for Gap Formation

Testing for gap formation was done by splicing the SC connector that housed a PDMS-coated fiber to one of the analyzing system connectors. After splicing, the entire fiber was moved to the analyzing system and the AC end was screwed in and the system was turned on. The PDMS end of the fiber was inserted into an empty reservoir. Water was then inserted into the reservoir using a disposable pipette. The water was filled to the top to ensure that the fiber tip was covered. The waveforms were then observed while the fiber coupler was adjusted to change the amount of power being used. Once a stable graph was obtained, the signal was recorded for 10 minutes. After testing the gap of the PDMS-coated fiber, the process was repeated, this time with the epoxy-coated fiber. The formation of a gap was observed by inserting the fiber into the new reservoir and then adding 3120 μL of water before testing for protein binding. This number was calculated based off the length of the fiber and the size of the reservoir to ensure that the water would reach the tip of the fiber.

4.1.1 Gap Results and Discussion

After 10 minutes, the recorded waveform for the PDMS fiber was observed on the screen and can be seen below in Figure 4.1. There were no distinct peaks in the waveform. The signal-to-noise ratio was not suitable for signal analysis. Therefore, PDMS did not form an effective gap on the fiber tip. This could be because the PDMS did not form a

thick enough layer on the tip or there was no PDMS on the tip at all. It is possible that not enough curing agent was added, or it evaporated too quickly when making the PDMS.

Figure 4.1: Spectral interference waveform of PDMS-coated fiber – Units: x axis - Time [s], y axis -OPL [nm]

After 10 minutes of stabilization of the epoxy-coated fiber in water, Figure 4.2 was produced and analyzed in MATLAB after filtering. To calculate the size of the gap formed, the produced waveform was compared with the waveform of a glass coverslip that had a predetermined gap size of 150 microns. The length of the cavity formed by epoxy was 25.2 μm (See Equation 4.1). The flat line on the middle peak is due to the spectral intensity exceeding the limit of the 12-bit camera. This results in saturation when the signal goes above 4095 pixels.

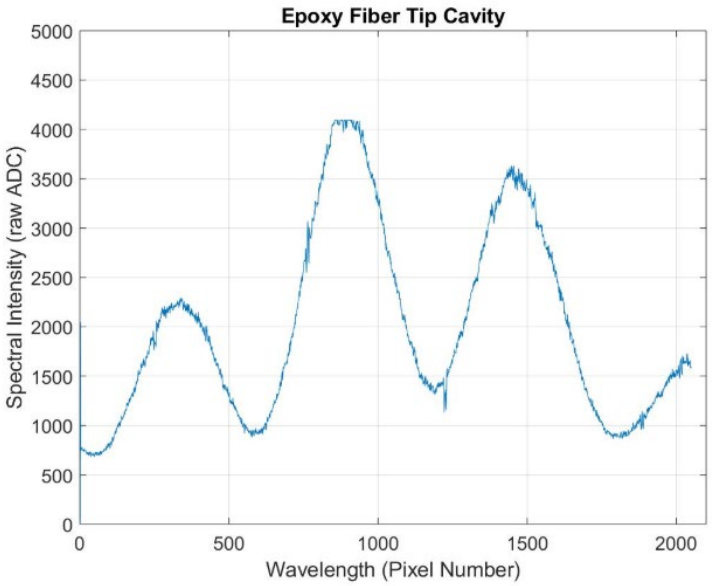

Figure 4.2: Epoxy fiber gap waveform

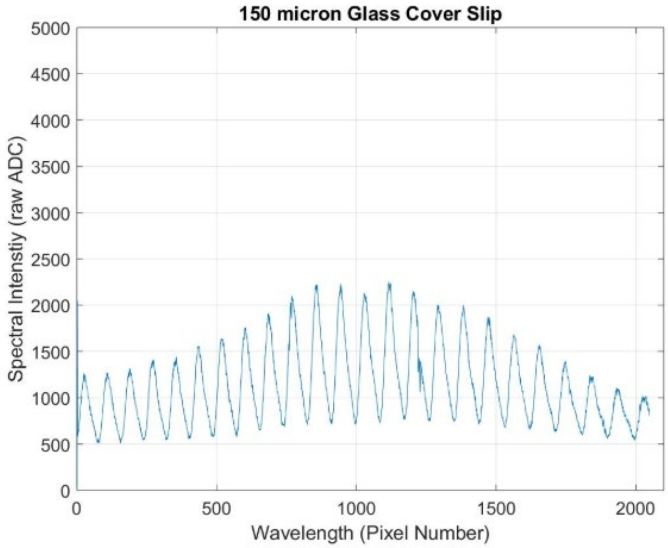

Figure 4.3: 150-micron glass cover-slip waveform

Equation 4.1:

$$frequency\ of\ glass\ cover\ slip = \frac{1\ peak}{90\ pixel\ number}$$

$$frequency\ of\ fiber\ tip\ cavity = \frac{1\ peak}{530\ pixel\ number}$$

$$150\ \mu m \rightarrow \frac{1\ peak}{90\ pixel\ number}$$

$$length\ of\ cavity = \frac{\frac{1\ peak}{536\ pixel\ number}}{\frac{1\ peak}{90\ pixel\ number}} * 150\ \mu m$$

$$= 25.2\ \mu m$$

4.2 Testing for Protein Binding

After confirming that a gap with a good signal-to-noise ratio was obtained, testing for binding could then begin. This was done by using a diluted concentration of laminin and deionized (DI) water. The laminin was first diluted to a concentration of 90.9 µg/mL. To touch the tip of the fiber, 3120 µL of DI water was added to the reservoir. The results were recorded for 2-3 minutes. This established the reference sample path length. Next, 880 µL of the diluted laminin was added to the reservoir. This gave a total volume of 4 mL, and a total diluted laminin concentration of 19.998 µg/mL. These results were recorded for 15 minutes to allow adequate binding for the sample path length. This was done for fiber tips containing epoxy plus microbeads, and PDMS plus microbeads.

4.2.1 Binding Results and Discussion

While performing the experiment there was no binding observed in the analyzing system live results. However, after analyzing the data in MATLAB, Figure 4.4 was obtained. This showed that the laminin attached to the epoxy and microbead-coated fiber. This means that the laminin was successfully detected from a fluid sample. The sharp peak seen just after 200 seconds is when the laminin was inserted into the reservoir. At 400 seconds a positive slope can be seen. This indicates an increase in optical path length, which means that laminin bound to the fiber. Negative slopes can be seen from 0-400 seconds and 600-1200 seconds. This is due to downward temperature drift which occurs when the epoxy shrinks.

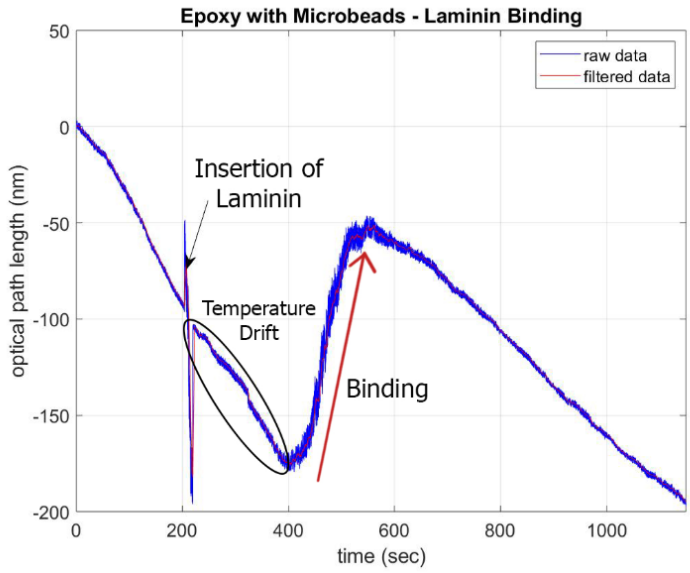

Figure 4.4: Sensorgram graph depicting change in optical path length

CHAPTER 5

DISCUSSION AND CONCLUSION

Etching the fibers with hydrofluoric acid would improve the formation of the gap in the fiber. Using epoxy does not seem to be the best means of forming the necessary gap due to the inconsistencies when mixing the epoxy and coating the tip of the fiber. The epoxy was only used due to its ease of handling and time constraints. But hydrofluoric acid has already been proven to be a successful and consistent way of etching fibers.

Originally, troponin protein levels were to be tested because they are the most common biomarker for detecting heart disease [8]. However, troponin could not be acquired due to cost constraints. It would be preferable to test troponin in the future because it has more applications in the healthcare industry.

Because the fiber needs to reach the sample, it had to be extended through the fiber connector. While this allowed the device to meet the design specifications, it caused the fiber to be brittle. To improve the quality of this device, a better way to make sure the fiber reaches the sample needs to be developed.

Right now, laminin successfully binds to epoxy. For the binding chemistry to be more specific, an antibody-antigen relationship would be more ideal. With antibody-antigen chemistry, the protein will only bind to its specific antibody. This ensures that only the molecule of interest is detected from the fluid sample. This will also allow for different proteins to be detected from the same sample.

Overall, based on the results of the gap, and binding procedures, it can be concluded that the device successfully detects laminin from a fluid sample. Creating a gap proved to be the most difficult and time-consuming part of the experiment. However, at the end, it was successfully created and used as the reference path length. When laminin was introduced into the fluid, there was a notable change in the optical path length because the laminin bound to the layer of epoxy on the tip of the fiber. Also, the reservoir successfully holds fluid without leakage, and the connector is secure. However, due to time constraints, the system was not calibrated to return the concentration of the protein detected from the sample. But, being able to detect protein binding is a huge success on its own.

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BIOGRAPHICAL INFORMATION

Princess Ehikhuemen graduated Magna Cum Laude in June 2015, from MacArthur High School in Irving, Texas. She started at The University of Texas at Arlington in August 2015 as a freshman Biomedical Engineering major. She was invited to apply to be a member of the UTA Honors College in her senior year of high school and joined as an incoming freshman.

She was also on the pre-medical track and was accepted into two medical schools. She decided to attend Texas Tech Health Sciences Center, Lubbock in Fall 2019 after graduation. She is expected to graduate Summa Cum Laude with her Honors Bachelor of Science in Biomedical Engineering in May 2019.

She is very passionate about the research field. She volunteered in a biomedical engineering professor's lab where they researched breast cancer tumor migration. She also participated in a joint research internship with the US Food and Drug Administration and the University of Maryland, College Park. There, she worked on the development of 3D-vascular structures to represent *in-vivo* environments. This led to a poster that was presented at the FDA Research Fair in August 2018.