Design and Implementation of Fiber Optic Interferometer for Protein Interaction Analysis

Capstone Report

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Abstract:

This capstone project particularly focuses on developing a Semi-distributed Interferometer made of optic fiber, geared to protein interactions. This work consists of the design of the interferometer, calibration, and functionalization steps. This will describe data processing on the acquired data using MATLAB and study the behavior of the protein through both static and dynamic measurements. This will thus lead to very high sensitivity of the interferometer, robust methodologies of calibration, optimized techniques of functionalization, and tools in data processing that will further allow the extraction of meaningful insight from interferometer data. Ultimately, the project targets performing the studies of protein interactions using the interferometer developed in the project and hence brings out a set of cellular processes and disease mechanisms into light.

Contents

Preface

This research project embodies the synergy of different fields such as optics, biology, data analysis, which are united in the pursuit of one goal: pushing the knowledge frontier and application.

Before embarking on this research journey, I would first like to acknowledge numerous scientists whose contributions toward optical interferometry, biosensing, and analysis of protein-protein interaction have laid our pathway and have been very frequently cited. We shall be thankful for their collective input.

This work is an example of the dedication of the students and mentorship by the faculty and unwavering support of the educational institutions committed to developing the students into the next generation of scientists and biomedical workers.

Thus, while we grapple with the fine details of interferometer fabrication and detection of CD44 proteins, it is with a constant perspective on the far-reaching implications our work holds. Ours is more than simply a capstone project; it's a journey toward a future where biosensing technologies will have progressed enough to allow breakthroughs in medicine, biotechnology, and more.

Throughout the following pages, we invite you to join us on this research, where curiosity, collaboration, and discovery come together to seek out new frontiers in science and technology.

Nazarbayev University, April 26, 2024

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Chapter 1

Introduction

Optical interferometry technique has shown tremendous growth in the recent past; hence, its use today is commonplace in important areas of science and technology, like precision measurements, remote sensing, and biophysics.

1.1 Background

Special emphasis has been laid on the area of refractive index sensing, which is the critical parameter for many studies involving various types of liquids. It finds a role in the analysis of substances like glucose, carbamide, and vitamins [1, 2]. These advantages have opened up the research and development of optical fiber sensors as a particularly valuable tool in refractive index sensing: immunity to electromagnetic interference, resistance to corrosion, lightweight construction, capability for remote sensing, and last but not least, flexibility [3].

A fiber optic sensor involves an instrument that ga detect and gauges many physical, chemical, and other conditions through the optical fiber transference of light from source to detection [4].

Optical fiber sensors promise much better corrosion and electromagnetic interference resistance compared to conventional sensors, life of over 20 years of lifetime exceeding, and with a good signal-to-noise ratio (SNR) [5]; therefore, they have drawn much recent interest in the field of pavement monitoring. Over the past decade, the fiber optic sensors have been used in the monitoring, which includes monitoring the data traffic between the structural health of pavements. Strain, temperature, and pressure measurements were realized in every layer of the pavement. This is because sensors had been embedded in the pavements. These sensors are of great help to design, maintain roads, and manage traffic.

While the technologies used in optical fiber sensors are numerous—optical grating, surface plasmon resonance (SPR), fiber interferometer [6], to just name a few—each of these methods has its limitations. Traditional optical grating has no

assurance on sensitivity; SPR technology is costly, while fiber interferometer sensors show low sensitivity and are substantially affected by environmental issues [7]. In contrast, fiber optic interferometer sensors possess the features of cost-effective fabrication, easy to fabricate, and high stability. In the last years, many new developments have been applied to this theory for the measurement of refractive index with very promising results, so that today there are several studies that propose sensors based on this theory with remarkable sensitivities [8, 9]. On the other hand, with these traditional, highly sensitive performances, the traditional optic interferometer sensors still pose great challenges. These traditional sensors have lots of drawbacks and yet with such performance levels; it does prompt researchers to work towards some sort [10, 11].

Indeed, this highly crucial diagnostic tool for all conditions related to the bladder is dominated by microelectromechanical systems (MEMS)-based pressure sensors [3].

Among these fiber optic sensors (FOSs), especially those belonging to the technique of extrinsic Fabry-Perot interferometer (EFPI), have come up as an alternative that is both small and sensitive [8, 9]. Another good combination for the EFPI sensors is the temperature sensor implemented using fiber Bragg gratings (FBGs), thereby offering a two-parameter configuration of the pressure/temperature sensing system with mutual compensations [12, 13].

Biosensors are viewed as a possible application in medical diagnostics. It provides applications that are label-free and in real time while detecting and measuring an analyte [6]. Optical technology, famous for presenting the highest sensibility and specificity of methods available to detect the amount of analyte with very low background noise [14], has emerged as the preferred tool for biosensing [15].

In this context, recent developments in biomolecular affinity have further expanded the spectrum of diagnostic and therapeutic utility of biosensors, particularly for monitoring disease progression citelin1997porous. Fabry-Perot fringes have appeared in the thin film of porous silicon semiconductors in protein interaction analysis and have thus indicated sensitivity to molecular binding events of proteins, oligonucleotides, and small organic molecules. Furthermore, chip-based interferometers with a porous Si layer allow a practical approach to the separation of biomolecules according to size with compensation for changes in matrix composition [7].

When combined with biosensing techniques, the integration of fiber optic interferometers to study the interaction of proteins opens the possibility of very promising developments. Specifically, this project outlines how to describe the design, fabrication, and calibration of a fiber optic interferometer using Fabry-Perot structures. The research methods are related to the functionalization of surfaces; the course work will deal with the research effects of interferometer spectra. It is based on the next main goals: 4) attachment of the CD44 protein on the surface

of the sensor and analysis of the interaction, and 5) processing the data obtained using MATLAB. Moreover, the project will 6) focus on the use of packaged interferometers in a microfluidic chip and be able to offer an exhaustive study of inventive strategies in the area of protein-interaction analysis.

1.2 Related works

1.2.1 Optical Interferometry in Biosensing

In addition, optical interferometry is a powerful technique that uses the wave nature of light to register measurements of extremely high precision over different physical quantities [17]. In this perspective, interferometry is shining as a star and is an expectant tool in biosensing research for biological interactions, showing incomparable sensitivity and possibility of real-time monitoring of molecular events [7, 16]. The use of interferometers can be seen in research on protein-protein interactions, DNA hybridization, and the study of cellular processes: through them, some breakthroughs in molecular biology and medicine have become possible.

Versatility of Biosensors: The biosensors, developed by automation as a cost-sensitive and specific tool, are being used in diverse approaches to detect biomarkers in many avenues, such as in the biomedical analysis, environmental monitoring, and food manufacturing industry. Being highly sensitive, these sensors find the presence of biomarkers in body fluids with minimum invasiveness and can be very well placed for point-of-care applications in clinics, hospitals, and home use citelakhera2022recent. Based on the bio-receptors and the transducers, one can classify biosensors and thus show their versatile application fields. Owing to their simplicity, reliability, and portability, electrochemical biosensors have been considered efficient in point-of-care diagnosis.

One article describes a fiber optic sensor for the detection of formations of blood clots. The basis of the sensor is a Fabry-Perot interferometer with two types of tips. Their functioning was monitored by spectrometric observation of shifts in the clot-formation-indicating sensor spectrum, induced by changing amounts of thrombin reagent introduced into the blood. The sensor was very sensitive to the spectrum, with the micro-tip fiber type at about 7 nm/uL, while the SMF type was at 8.7 nm/uL, showing promise for potential fields like health, medical monitoring, and biological sensing [18].

1.2.2 Interferometer Types and Applications

The interferometers can be of varied types, especially designed for a particular application. Common interferometers include the likes of Michelson, Mach-Zehnder, and Fabry-Perot [8]. Advanced Mach-Zehnder interferometers are now one of the

highlights of biosensing, among most Mach-Zehnder interferometers, because they suit being integrated with microfluidic systems and are able to detect very small changes in the refractive index taking place during molecular interactions [15, 19]. These interferometers can be applied to label-free detection as well, and eliminate the usage of fluorescent or radioactive markers that might otherwise disturb the biological sample [1, 6].

The interferometers mentioned form a necessary part of practically any fiber-optic photoacoustic gas sensing system. They boost sensitivity to the system through the detection of changes in the optical path length occasioned by acoustic waves in transit. Especially, the Fabry-Perot interferometer has a high sensitivity with a compact structure, which is quite useful for many weak acoustic signal detections [20].

One important contribution in this field is detailed herein, whereby a carefully designed analysis is reported of the fiber optic refractive index sensors based on a semi-distributed interferometer. It is in this SDI that a cavity is exploited between the cleaved fiber tip and the high-scattering interface of the single-mode fiber doped with Mg-silicate nanoparticles. The fabrication process of SDI is simple, rapid, and has a mechanism of splice-and-cleave technique, resulting in a spectral fingerprint akin to an interferometer-shaped envelope. The value is as high as 787.0 dB/RIU, and its applications range from the chemical to biochemical and biological domains of SDI. These make the SDI device attractive for in-situ refractometry and biosensing applications due to ease of fabrication and even increased sensitivity [21].

Xie et al. [22] also reported the same fabrication of SDI sensors. The interferometric measurement sensing element is the construction of Sagnac and Mach-Zehnder interferometers, spectrum slicer. In the present work, the light from a broadband optical source modulated with an electro-optic modulator for radio frequency modulation subsequently passes through a dispersion compensation fiber and is recovered by a photodetector for analysis. Temperature sensing is used as a demonstration to showcase the proposed technique's enhanced sensitivity and tunability in fiber optic interferometer-based sensing systems [22].

1.2.3 Functionalization in Interferometry

The most sensitive issue on biosensing in optical interferometry is functionalization. Generally, functionalization considers the modification of the sensor surface, which would allow the immobilization of biological molecules such as proteins, antibodies, or DNA strands [9, 23]. This modification allows specific binding of target molecules, a very important modification for the success of any biosensing platform [14]. Researchers have employed various functionalization strategies, including self-assembled monolayers (SAMs), biotin-avidin systems, and covalent

immobilization , to enhance the selectivity and sensitivity of interferometric sensors [10, 11].

1.2.4 Protein Interaction Analysis

Protein interaction is one important key for the understanding of how cells work. Knowledge of interaction will help in finding possible disease mechanisms, cellular processing, and maybe drug production. CD44 is a cell surface glycoprotein receptor [12, 13]. CD44 is a biomarker for cancer, and it has been implicated as an adhesive molecule for cell-cell interaction of cancer cells, as well as their motility and signaling. A biomarker is any entity that gives an indication of the biological state regarding the subject and has an application in many medical fields. Thus, study of CD44 interactions with other proteins can give an idea of a number of cellular phenomena, beginning with cancer metastasis and regulation of immune response. This makes optical interferometry invaluable in the addition of a label-free, non-destructive toolkit probing these interactions in the realm of protein interaction analysis [3].

Cancer Detection: In light of such a formidable global health challenge posed by cancer, one of the most significant determinants for effective treatment has to be that of early detection of premalignant or pre-metastatic tumors, allowing for improvement in the survival rate of patients. There are a number of varied types of cancers occurring in different parts of the body, and so the focus of the biomedical field of study has shifted to timely, sensitive, and successful diagnosis of cancer. In this context, the biomarkers are being identified and for continual detection; they must improve the diagnostic results [10].

CD44 Protein as a Cancer Biomarker: The role of CD44 protein in the diagnosis of cancer largely lies in the fact that CD44 is a transmembrane glycoprotein expressed mainly in embryonic stem cells, and it is overexpressed in cancer stem cells. These CSCs play a very essential role in the development, relapsing, and metastasis of the cancer because of their capability to self-renew and oncogenesis [19]. In CSC, the level of CD44 is high and associated with some specific types of tumors, e.g., stomach, breast, colon, ovary, pancreas, head, and neck area. On the other hand, CD44 is found in biological fluids and expressed from the cell as a secreted protein. It implied the research of levels of CD44 protein in the blood serum in such an order to try to find a correlation with different kinds of cancer, stages, and outcomes in patients [24]. If such a direct link could be established between the levels of CD44 and cancer activity, metastasis, besides disease stages, and poor survival in addition to response to treatment, it would have potential value as a diagnostic or prognostic biomarker. For example, hypersensitive detection of biomarkers would enable the analysis of many body fluids, such as saliva, urine, or sweat, from minimal sample volumes and thus expand the diagnostic

capabilities of such small material volumes [19].

1.2.5 Packaged biosensors in the microfluidic device

Nevertheless, successful real-life use to the measure of their huge potential still poses a challenge: complicated and demanding automation and advances in miniaturization. Microchips take a key part in advances in biosensor automation and miniaturization [25].

In fact, the integration of biosensors with microtechnology has set the ground for point-of-care diagnostics, where wearable and miniaturized biosensors can shift the entire paradigm of healthcare delivery by providing diagnostic services that are fast and inexpensive. However, the major challenge that arises is the biocompatibility of this synergy and how to make a seamless integration of the biosensors with the microfluidic elements and electronics. The system of microfluidics and optical fiber sensors, respectively in a catheter, increases their precision and gives diagnostic ability, showing new quality in diagnosing in urology with full biocompatibility [26]. It becomes very important from this perspective that the designing and implementation of various types of biosensors must be so cost-effective in terms of production and deployment. The principal approaches to microfluidic chip fabrications are Stereolithography (SLA), 3D Printed circuit board (PCB) printing, and their substrates can be Silicon (Photopolymer resin, Polydimethylsiloxane (PDMS), or Polymethyl Methacrylate). In reality, the microfluidic designs are laid out using a more ordinary design software, thereafter applied in the production of 3D blueprints. The blueprints then guide a laser scribe on exactly how to go about replicating the planned microchannel design on the surface of the printer. The design and fabrication of channels to enable the flow of glucose solutions and deionized water usually involve several steps. The only disadvantages linked with this device are slowly working, low resolution, and finally, post-processing chores have to be done [27].

In accordance with research of different groups, a point-of-care diagnostic test for myocardial infarction could be developed using this biosensor. The biosensor is not bound to any kind of special equipment; it is small in size, compact, portable, and very simple to use. The authors point to the encouragement of the development of wearable, miniaturized biosensors for point-of-care diagnostics. Most attractive of all are microchip-based biosensors that could be produced massively and cheaply. Real-time continual monitoring of patients might be done with needle catheter-based biosensors. Furthermore, some problems have been referred to in the literature on microchips, such as how to protect the biosensor from the environment so that it is biocompatible and how to be able to integrate it with other microfluidic and electronic devices [28, 29].

In another study, the false biosensor is integrated into the microfluidic chip,

involving an optical fiber that is coated with a thin gold layer. In this regard, antibodies against a specific biomarker—e.g., a protein secreted onto the surface of cancer cells—have been prepared. The microfluidic chip was small enough to be implanted in the patient's body and hence can be used to monitor the tumor response to chemotherapy in real-time. It even considers the possibility of packaging a biosensor in a needle catheter [30].. This would make a biosensor that could be used for collecting a section of tissue from a patient and then measure the level of biomarkers of the tissue at the point of care.

1.2.6 Current Challenges and Limitations

Current problems and disadvantages: Having said all this, the promising potential of optical interferometry in bio-sensing and protein-protein interaction analysis, there are still quite a number of drawbacks and challenges. They include the requirement for accuracy in calibration, functionality of sensor performance, and effective robust data processing [24, 21]. Another related study covered further investigation of the problem of relatively low sensitivity presented due to the low thermo-optic coefficient and coefficient of thermal expansion of silica material, needing further improvement [31]. The next challenge relates to how to assure the stability and reproducibility of such a sensor, especially in harsh environments where many external disturbances and pollution create problems for them. There are process-related problems: the fusion splicing of different fiber types and optimization of parameters to the best interest of the sensor [31]. These challenges have to be addressed, and it is only by surmounting them will the full potential in the life science area through optical interferometry be realized.

1.2.7 Gantt Chart

Table 1.1: Gantt Chart

Tasks	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Literature Review	✓	✓	✓	✓			✓	✓	
Fabrication of interferometers	✓	✓	✓	✓	✓	✓	✓		
Calibration of sensors		✓	✓		✓	✓	✓		
Functionalization of sensors			✓	✓	✓	✓	✓		
Printing microfluidic chip				✓	✓	✓			
Cell measurements			✓	✓	✓	✓	✓		
Writing a report			✓	✓	✓		✓	✓	✓

Chapter 2

Methodology

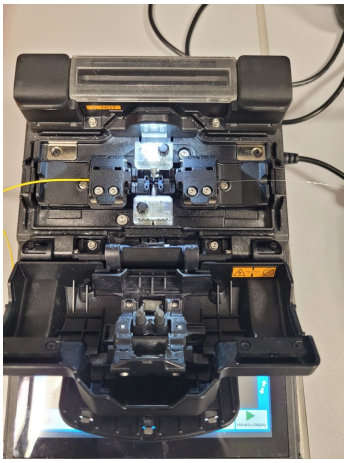
2.1 Fabrication of Semi-Distributed Interferometers (SDIs)

The Semi-Distributed Interferometer (SDI) is created by establishing a space between a cut fiber tip and the reflective surface at the junction of a single-mode fiber and a high-scattering fiber, the latter having a core infused with Mg-silicate nanoparticles [21].

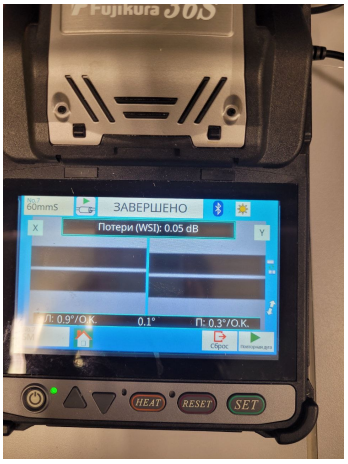
The procedure for creating SDI sensors is shown in 2.1 and involves a two-step process referred to as "splice-and-cleave." The materials and equipments used are depicted on 2.1(a) Initially, an enhanced backscattering fiber (EBF), acting as a reflector, is spliced to a standard single-mode fiber (SMF-28) using a Fujikura splicer) (2.1(b) and 2.1(c)). Due to the presence of scattering centers, the interface between SMF and EBF yields a minute reflectivity. To create a cavity, the EBF is manually cleaved with a Fujikura CT-08 cleaver at a short distance (<1 mm) from the splice section (2.1(d)), forming a tip mirror through Fresnel reflection at the EBF-outer medium interface. The EBF's internal reflections, induced by numerous scattering centers, generate distributed reflections with random intensity and phase due to defect inhomogeneity. This randomness imparts a semi-stochastic spectral fingerprint to the Fabry-Perot spectrum.

The sensing mechanism is entirely encoded in the EBF fiber, with the Fresnel reflection at the tip-side interface providing an RI-dependent mirror and the high Rayleigh scattering at the distal side producing the reflectivity for cavity formation. Throughout its length, the continuous set of distributed reflections creates intermediate layers influencing the output spectra [21].

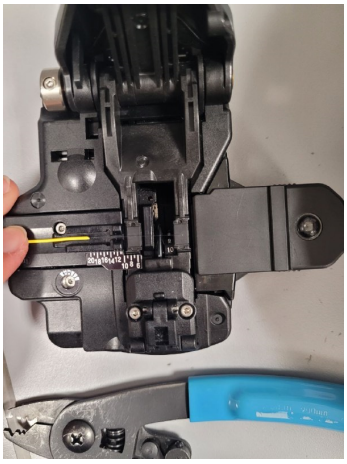
(a) Used materials for Fabrication SDI



(b) Splicing of SMF and EMF



(c) Successful splicing

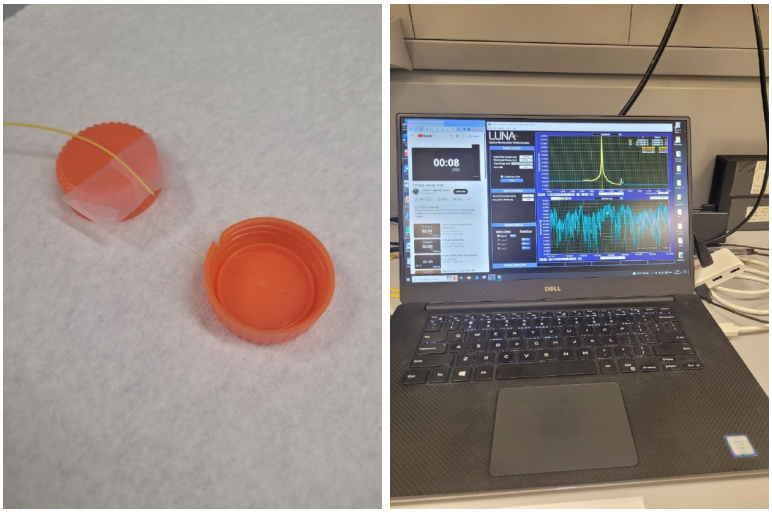


(d) Cleaving process

Figure 2.1: Fabrication stage

2.2 Calibration of SDIs in Sucrose or Ethanol Solutions

The calibration process after the fabrication of SDI is essential in order to find out the sensor's sensitivity. It is needed to decide if the sensor is appropriate for the functionalization or not (as functionalization process will be useless in case if the interferometer has bad sensitivity). The full experimental setup of calibration stage is shown in 2.2. The fabricated SDI was measured in air, water, Phosphate buffer saline (PBS) and different % sucrose solutions through Optical Backscatter Reflectometer (LUNA 4600 or Micron Optics) (2.2(b)).



(a) Immersing of SDI into sucrose solution (b) LUNA 4600 OBR measurements

Figure 2.2: Calibration stage

High sensitivity value (more than 75 dB/mm) to changes in refractive index (RI), as proven by calibration testing, led to the selection of manufactured spherical tip sensors for functionalization. To conduct calibration, the sensor was immersed in 6 ml of a 10% sucrose solution with an initial refractive index (RI) of 1.34860. Subsequently, 400 µl of 40% sucrose solution was incrementally added, reaching a total of 5 concentration points. The maximum point on the scale was achieved when the RI value reached 1.35826. A linear regression analysis was used to assess the sensitivity.

2.3 Glutaraldehyde (GA) Functionalization of SDIs

The next step was biological and chemical process. The surface of the manufactured optical fibers with spherical tips was thoroughly cleaned of organic residues using a Piranha solution, a 4:1 mixture of sulfuric acid and hydrogen peroxide conducted at room temperature (2.3). To do so, all fibers were identified and attached to glass rods, allowing them to be immersed in a beaker containing 30 ml of the Piranha solution. Following a 15-minute exposure, the optical fibers were thoroughly rinsed with deionized (DI) water, which was repeated several times, and then dried with nitrogen gas. To perform silanization, the pure optical fibers were treated for 20 minutes with a 1% solution of APTMS in methanol. Following post-treatment, the optical fibers were rinsed with methanol and heated in an oven for 1 hour at 110°C. The heat-treated optical fibers, complete with spherical tips, were incubated for one hour in a glutaraldehyde solution, which is often used as a cross-linking

agent for antibody attachment. The glutaraldehyde solution was used at two different concentrations: 25%. The glutaraldehyde content was varied to enhance surface chemical conditions. The optical fibers were then extensively cleaned in a phosphate-buffered saline (PBS) solution. After that, the glutaraldehyde-treated optical fibers were incubated for 1 hour with 500 l of CD44 monoclonal antibody at concentrations of 4 µg/ml. This incubation took place with constant agitation. Any unreacted aldehyde groups were blocked using a 10% solution of mPEG-amine after antibody immobilization. Additional PBS washing processes were carried out prior to and after the blocking treatments [24].

Figure 2.3: Functionalization process

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2.4 Microfluidic Chip Development and Measurements

After research, it was found that the best transparent photopolymer resin for the manufacture of a microfluidic device is “Anycubic High Clear Resin”, which has a transparency of 98%. The next important feature of this resin is its protection from yellowing, which contributes to its durability. The first important step was to prototype the chip on the CAD software 2.4.

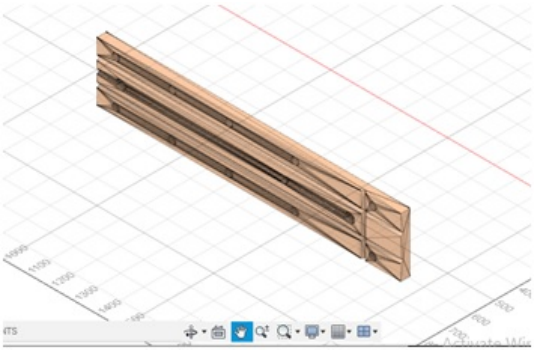

Figure 2.4: The 3D model of the microfluidic device (upper plate)

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The next step was 3D printing on "Anycubic Mono X" equipment. The necessary configurations for this type of photopolymer resin were set on the 3D printer (they can be found on the Anycubic website). The print duration was 25 minutes. The next step was washing the parts from the extra resin and curing with UV light for 4 minutes, in Fig. 2.5(A). To make the parts more transparent, a glossy clear lacquer spray was used, and the results can be seen in Fig. 2.5(B) – the spray assisted to make the chip highly transparent. Finally, the parts are connected by 10 bolts M3, and a layer of PDMS was applied between them to protect the lab on the chip from water erosion.

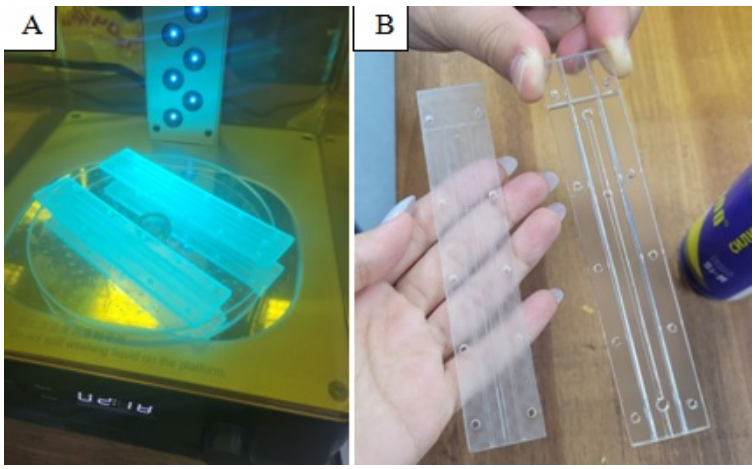

Figure 2.5: Stages of printing a microfluidic device. A. Microchip parts are in Anycubic Wash and Cure 2.0. B. After PDMS layering and using clear gloss lacquer spray parts are more transparent

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Experimental setup for dynamic measurements using microfluidic device: The main setup of the experiment is depicted on 2.6. In vitro measurement with an SP5 interferometer with a syringe pump was carried out using air, water, PBS and 2% ethanol (the corresponding RI values for them are 1.000, 1.33276, 1.334 and 1.33505). A syringe pump was used to inject 5 ml of a 2% solution of water and

ethanol through a tube with a diameter of 1 mm at a flow rate of 10 µl/min. A reactor with a microfluidic chip was made with the tube used for the study in order to fix the biosensor accordingly. A fiber-optic biosensor was inserted into the microchip to a certain point (on 2.6(C)). The reactor volume was calculated based on the flow rate * time spent on passing water through the chip. In total, using Micron Optics interrogator 15 measurements were carried out for each solution with an interval of 11 minutes for 2 hours and 47 minutes. The reactor volume is 1.67 ml.

For experiments, it was decided to use 10 µl/min flow rate (on 2.6(B)). When feeding liquids into the laboratory-on-a-chip system using a syringe pump, it is important to maintain an accurate flow rate, 10 µl/min, in order to reproduce physiological conditions and obtain accurate experimental results. The human body is a dynamic system in which different tissues and arteries carry fluids at different speeds. Blood and other body fluids, as well as interstitial fluids, constantly flow through organs and tissues. The flow rate of 10 µl/min is designed to simulate the micro-scale flow dynamics observed in the human body. As a result, the studies conducted in the "laboratory on a chip" system are guaranteed to be more accurate when modeling real physiological conditions [24]. The human body exposes its cells to a constant, regulated influx of oxygen, nutrients and signaling molecules. A flow rate of 10 µl/min helps to create a microenvironment on the chip that supports cell culture and other biological processes very similar to in vivo conditions.

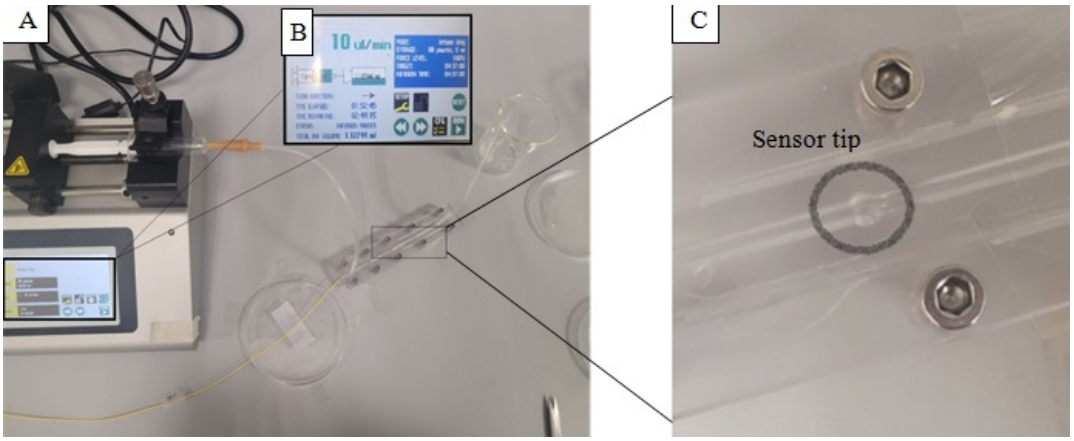

Figure 2.6: Experimental setup used for biomarker measurements. A. General setup showing syringe pump and microchip. B. Image showing syringe pump parameters used for the experiment. C. Microchip circle shows the tip of the fiber.

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2.5 CD44 Protein measurements and Data Analysis

Various concentrations of CD44 protein were prepared, ranging from 100 nM down to 42.9 aM. Human serum, diluted in a 1:10 ratio in a PBS solution, was used to prepare the proteins. The experimental setup for CD44 protein detection involved an OBR, a SDI optical fiber biosensor, and a manually crafted vial containing the analyte. During measurements, an optical fiber tip was immersed in a solution containing 250 µl of CD44 protein, and the other end was connected to the interrogator to capture reflected spectra. The binding event was monitored for 10 minutes with signal recording performed every minute [23].

The OBR, operating on optical frequency domain reflectometry principles, utilized a polarization beam splitter to separate light into S-polarization and P-polarization states. Changes in return loss and S/P-polarization were recorded and analyzed based on variations in surrounding RI when an analyte bound to the biosensor. The OBR parameters during experiments were set as follows: wavelength range of 1525–1610 nm, gain of 0 dB, integration width of 0.4 m, and spatial resolution of 0.1 m. Data was collected in 65,536 data points [23].

Spectral noise was filtered using a Chebyshev low-pass filter (Butterworth finite-impulse response, 5th order, 0.0014 cut-off). The Limit of Detection represents the CD44 concentration.

Chapter 3

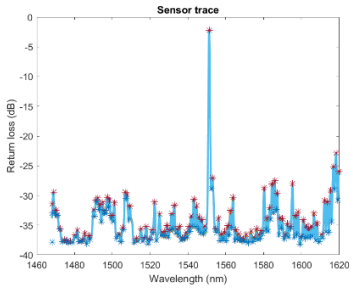
Results and Discussions

3.1 Results

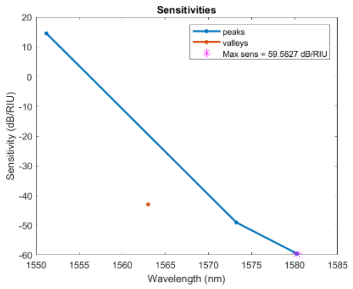
3.1.1 Data analysis of Calibration before Functionalization:

Table 3.1: Results for SDIs calibration before functionalization

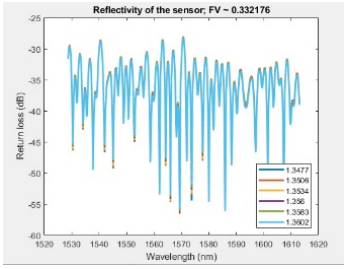
SDI	SP3	SP4	SP6
Number of valeys	29	52	37
Number of peaks	27	56	44
Fringe visibility	0.332176	0.361356	0.319690
Mean sensitivity (dB/RIU)	97.76	141.40	89.04
Mean R-squared value	0.975	0.974	0.969



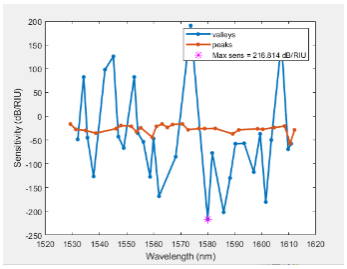
a) Return loss of SDI-SP2



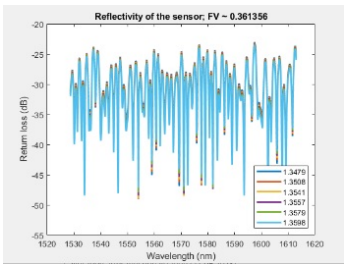
b) Sensitivity of SDI-SP2



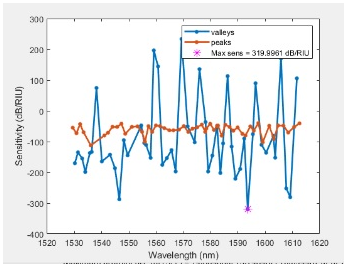
(a) Return loss of SDI-SP3



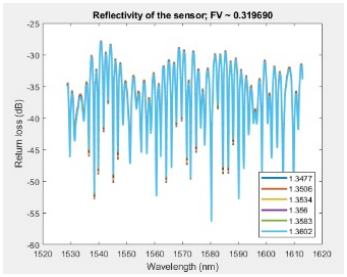
(b) Sensitivity of SDI-SP3



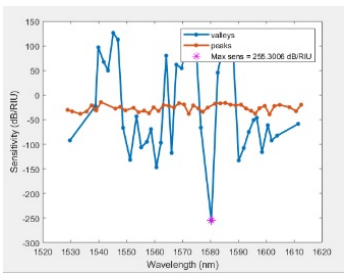
(c) Return loss of SDI-SP4



(d) Sensitivity of SDI-SP4



(e) Return loss of SDI-SP6



(f) Sensitivity of SDI-SP6

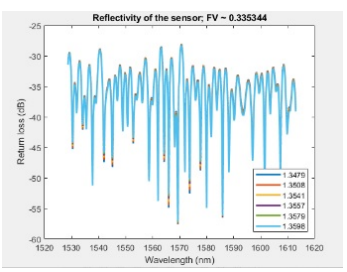
Figure 3.1: The data analysis of SDIs SP3, SP4 and SP6 interferometers calibration before functionalization.

3.1.2 Data analysis of Calibration after Functionalization:

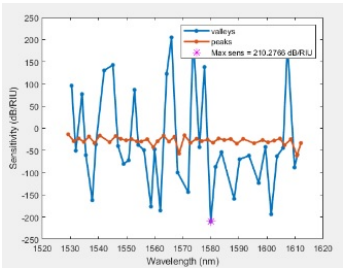
In total, 10 fibers were fabricated with 5 different EBFs (SP2,3,4,5,6). Two of them were functionalized and used for CD44 protein detection. Only SP3 and SP4 SDI sensors were functionalized.

Table 3.2: Results for SDIs calibration after functionalization

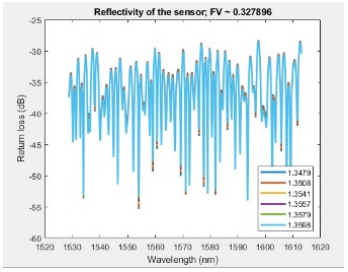
SDI sensor	SP2	SP3 before	SP3 after	SP4 before	SP4 after	SP5	SP6
Number of valeys	43	29	38	52	54	88	37
Number of peaks	60	27	41	56	59	87	44
Fringe visibility	0.36	0.33	0.335	0.36	0.328	0.01	0.319
Max sensitivity (dB/RIU)	59.6	216.8	210.3	319.9	234.9	36.4	258.3



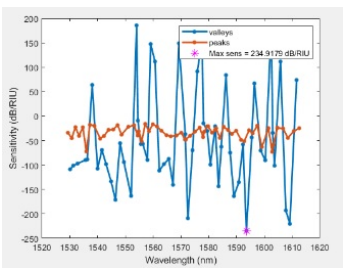
(a) Return loss of SDI-SP3



(b) Sensitivity of SDI-SP3



(c) Return loss of SDI-SP4



(d) Sensitivity of SDI-SP4

Figure 3.2: The data analysis of SDIs SP3 and SP4 interferometers calibration after functionalization.

3.1.3 Data Analysis of CD44 proteins measurements

The SDI SP3 biosensor was used to measure CD44 protein concentrations.

Concentrations: PBS, 10.93fM, 55.6fM, 0.33pM, 2 pM, 2.9pM, 77pM, 0.46nM, 2.78nM, 16.7nM, 100nM (300ul) stock.

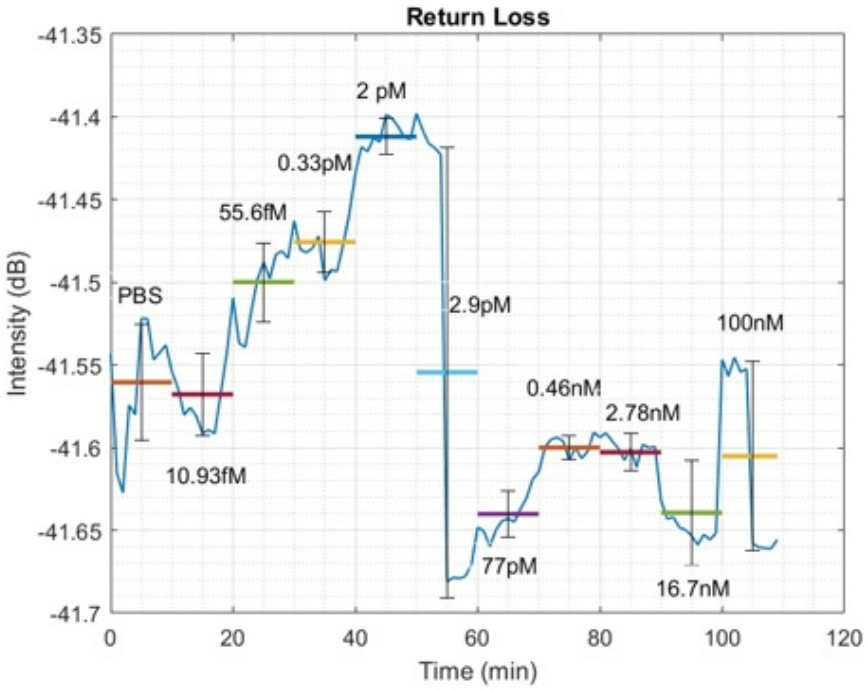


Figure 3.3: The Return loss of SDI-SP4 interferometer in CD44 proteins.

3.1.4 Data Analysis of packaged biosensor SDI-SP5

After fabrication of SDI-SP5 sensor, it was calibrated in air, water, PBS and ethanol 2% solution using microfluidic device with Micron Optics (see 2.6)

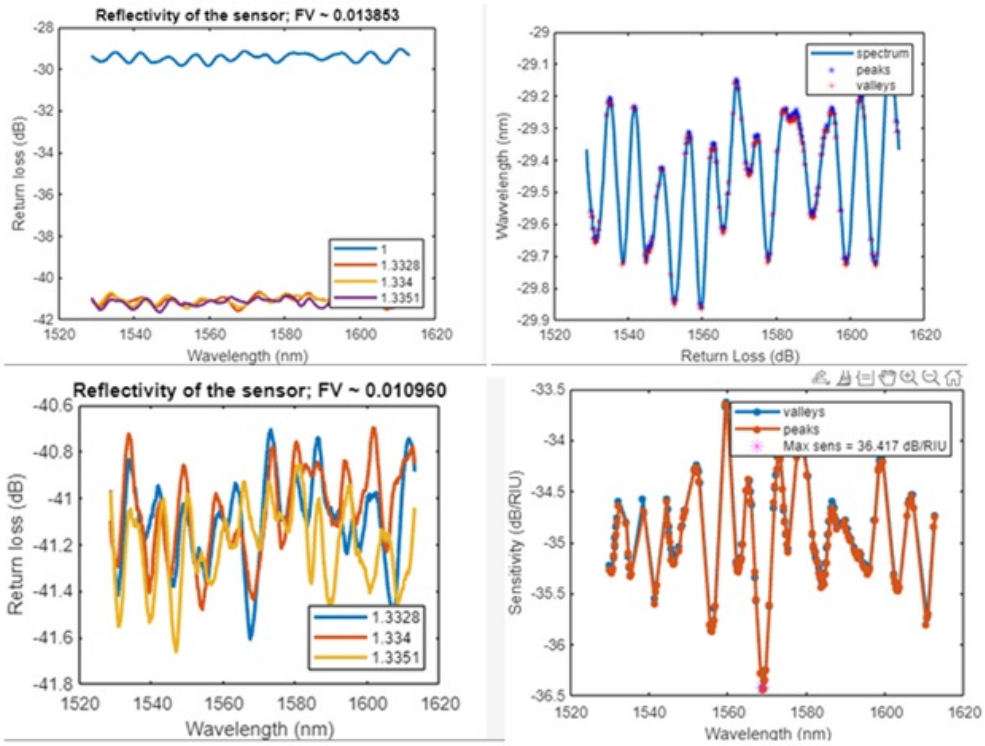


Figure 3.4: The data analysis of SP5 interferometer in microchip.

3.2 Discussions

3.2.1 Calibration of SDI

The results obtained from the calibration and functionalization of Fiber Optic Interferometers SP3-AB3 and SP4-AB1 provide valuable insights into the performance and sensitivity of these sensors in the context of protein interaction analysis.

In the calibration phase before functionalization, both interferometers exhibited reasonably high R-squared values, indicating a good fit of the sensor data to the expected model. This suggests that these interferometers were accurately calibrated for precise measurements. Additionally, the sensitivity values, whether in decibels per refractive index unit (dB/RIU) or nanometers per refractive index unit (nm/RIU), reflected the sensors' responsiveness to changes in refractive index. Higher sensitivity is desirable in biosensing applications as it allows for the detection of subtle molecular interactions.

Across different sensors, there is notable variability in maximum sensitivity. SDI-SP4 exhibited the highest sensitivity at 319.9 dB/RIU before functionalization, marking it as an exceptionally effective sensor. In contrast, SDI-SP3 displayed more stability in the number of peaks and valleys and provided sufficient sensitivity

for the detection of CD44 proteins. However, for dynamic measurements, this biosensor demonstrated poor performance in detecting the cancer biomarker, with a sensitivity range only from 41.4 dB/RIU to 49 dB/RIU. The poorest performance was observed in SDI-SP5, which also recorded a fringe visibility of zero.

However, it's noteworthy that the polarized light measurements (S polarization and P polarization) in SP4-AB1 showed varying levels of sensitivity and R-squared values. This indicates the importance of considering polarization effects when designing and calibrating interferometers.

After functionalization, the wavelength shift in both interferometers suggests changes in the refractive index at the sensor surface due to the attachment of biomolecules. These shifts are critical for monitoring specific protein interactions and represent a successful modification of the sensor surface.

SP4 consistently stands out as a high-performing sensor, both before and after functionalization. SP3 and SP6 also exhibit good performance, with slight variations observed after functionalization.

The sensors, especially SP4, appear suitable for accurate refractive index measurements and potential applications like CD44 protein measurements and microfluidic chip integration.

3.2.2 CD44 proteins measurements

According to Return loss graph (3.3), it can be seen that SDI-SP4 does not depict good results as expected. It is suggested to redo the functionalization stage again with proper conditions.

3.2.3 Packaged interferometer

The data analysis reveals valuable insights into the sensor data (see 3.4). With 142 identified peaks, the mean sensitivity of approximately 150.6 dB/RIU for valleys and 134.9 dB/RIU for peaks suggests a high sensitivity in detecting refractive index changes. Additionally, the consistently high mean R-squared values of 0.9996 indicate a strong correlation between the measured data and the fitted sensitivity curves. The fringe visibility (FV) of 0.013853 and the average Full Spectrum Analysis of 0.586361 nm underscore the precision and reliability of the sensor in capturing subtle variations.

Chapter 4

Conclusion

This project has successfully explored the design, fabrication, and calibration of semi-distributed interferometers (SDIs) for refractive index sensing. Semi-Distributed Interferometers with 5 different EBFs were designed and calibrated. SDI-SP3, SDI-SP4, SDI-SP6 are the most effective sensors, demonstrating the potential for high-performance in biomarker sensing. The calibration results before and after functionalization revealed the sensors' robust performance, with SP4 consistently demonstrating superior sensitivity and accuracy. The functionalization process introduced changes in the interference pattern, but the sensors maintained high performance. It is suggested to implement proper functionalization method that ensures better attachment of CD44 antibodies to the sensor's tip for effective protein detection. Moving forward, the project will extend its focus to the integration of SDIs into microfluidic chips, exploring their utility in packaged biosensors. Additionally, the CD44 protein measurements did not show promising results, which needs further protein analysis. Further research will emphasize improvements in the accuracy of functionalization and refining MATLAB codes for enhanced data analysis, solidifying the SDIs' position as versatile and reliable optical sensors in diverse applications.

Bibliography

[1] Ya-nan Zhang et al. "Study on the effect of carbon nanotube coating on the refractive index sensing sensitivity of fiber modal interferometer". In: *Optical Materials* 75 (2018), pp. 666–672.

[2] Daniele Tosi et al. "Optical fiber ball resonator sensor spectral interrogation through undersampled klt: Application to refractive index sensing and cancer biomarker biosensing". In: *Sensors* 21.20 (2021), p. 6721.

[3] Sven Poeggel et al. "Differential in vivo urodynamic measurement in a single thin catheter based on two optical fiber pressure sensors". In: *Journal of Biomedical Optics* 20.3 (2015), pp. 037005–037005.

[4] Madina Shaimerdenova et al. "Spatial-Division Multiplexing Approach for Simultaneous Detection of Fiber-Optic Ball Resonator Sensors: Applications for Refractometers and Biosensors". In: *Biosensors* 12.11 (2022), p. 1007.

[5] Jiawei Wang et al. "Applications of optical fiber sensor in pavement Engineering: A review". In: *Construction and Building Materials* 400 (2023), p. 132713.

[6] Semra Akgönüllü and Adil Denizli. "Recent advances in optical biosensing approaches for biomarkers detection". In: *Biosensors and Bioelectronics: X* (2022), p. 100269.

[7] Claudia Pacholski et al. "Biosensing using porous silicon double-layer interferometers: reflective interferometric Fourier transform spectroscopy". In: *Journal of the American Chemical Society* 127.33 (2005), pp. 11636–11645.

[8] Zhengyong Li et al. "Label-free detection of bovine serum albumin based on an in-fiber Mach-Zehnder interferometric biosensor". In: *Optics express* 25.15 (2017), pp. 17105–17113.

[9] Yasser M Sabry, Diaa Khalil, and Tarik Bourouina. "Monolithic silicon-micromachined free-space optical interferometers onchip". In: *Laser & Photonics Reviews* 9.1 (2015), pp. 1–24.

[10] Humaira Khan et al. "Cancer biomarkers and their biosensors: A comprehensive review". In: *TrAC Trends in Analytical Chemistry* (2022), p. 116813.

[11] P Lakhera et al. “Recent developments and fabrication of the different electrochemical biosensors based on modified screen printed and glassy carbon electrodes for the early diagnosis of diverse breast cancer biomarkers”. In: *Materials Today Chemistry* 26 (2022), p. 101129.

[12] Aida Rakhimbekova et al. “Fiber-optic semi-distributed Fabry-Perot interferometer for low-limit label-free detection of CCL5 cancer biomarker”. In: *Optics & Laser Technology* 168 (2024), p. 109953.

[13] Xiaoqi Ni et al. “A hybrid Mach–Zehnder interferometer for refractive index and temperature measurement”. In: *IEEE Photonics Technology Letters* 28.17 (2016), pp. 1850–1853.

[14] Ming Deng et al. “Miniaturized fiber-optic Fabry-Perot interferometer for highly sensitive refractive index measurement”. In: *2008 1st Asia-Pacific Optical Fiber Sensors Conference*. IEEE. 2008, pp. 1–4.

[15] Cheng Zhou et al. “Fiber optic sensor for simultaneous measurement of refractive index and temperature based on internal-and-external-cavity Fabry–Pérot interferometer configuration”. In: *IEEE Sensors Journal* 21.8 (2021), pp. 9877–9884.

[16] Victor S-Y Lin et al. “A porous silicon-based optical interferometric biosensor”. In: *Science* 278.5339 (1997), pp. 840–843.

[17] Aliya Bekmurzayeva et al. “Label-free fiber-optic spherical tip biosensor to enable picomolar-level detection of CD44 protein”. In: *Scientific reports* 11.1 (2021), p. 19583.

[18] Marjan Ghasemi et al. “Fabry–Perot Interferometric Fiber-Optic Sensor for Rapid and Accurate Thrombus Detection”. In: *Biosensors* 13.8 (2023), p. 817.

[19] Zhaniya U Paltusheva et al. “Highly Sensitive Zinc Oxide Fiber-Optic Biosensor for the Detection of CD44 Protein”. In: *Biosensors* 12.11 (2022), p. 1015.

[20] Chenxi Li et al. “Fiber-optic photoacoustic gas sensor with multiplexed fabry–pérot interferometric cantilevers”. In: *Analytical Chemistry* 95.48 (2023), pp. 17477–17485.

[21] Sakengali Kazhiyev et al. “Semi-distributed interferometers fiber-optic sensors for high-sensitivity refractive index detection: Design and sensitivity analysis”. In: *Measurement* 220 (2023), p. 113327.

[22] Tongtong Xie et al. “Frequency down-converted and sensitivity enhanced fiber optic sensing system based on multi-passband microwave photonic filter by cascaded fiber optic interferometers”. In: *Journal of Lightwave Technology* (2023).

[23] Zhannat Ashikbayeva et al. “Green-synthesized gold nanoparticle-based optical fiber ball resonator biosensor for cancer biomarker detection”. In: *Optics & Laser Technology* 161 (2023), p. 109136.

[24] Aliya Bekmurzayeva et al. “Ultra-wide, attomolar-level limit detection of CD44 biomarker with a silanized optical fiber biosensor”. In: *Biosensors and Bioelectronics* 208 (2022), p. 114217.

[25] Natalya V Zaytseva et al. “Development of a microfluidic biosensor module for pathogen detection”. In: *Lab on a Chip* 5.8 (2005), pp. 805–811.

[26] Madhusudan B Kulkarni, Narasimha H Ayachit, and Tejraj M Aminabhavi. “Biosensors and microfluidic biosensors: from fabrication to application”. In: *Biosensors* 12.7 (2022), p. 543.

[27] Jian Qu et al. “Microfluidic Chip with Fiber-Tip Sensors for Synchronously Monitoring Concentration and Temperature of Glucose Solutions”. In: *Sensors* 23.5 (2023), p. 2478.

[28] Yuhki Yanase et al. “Development of an optical fiber SPR sensor for living cell activation”. In: *Biosensors and Bioelectronics* 25.5 (2010), pp. 1244–1247.

[29] Wenchao Zhou et al. “Ultrasensitive label-free optical microfiber coupler biosensor for detection of cardiac troponin I based on interference turning point effect”. In: *Biosensors and Bioelectronics* 106 (2018), pp. 99–104.

[30] Shu-Yu Fan et al. “Percutaneous fiber-optic biosensor for immediate evaluation of chemotherapy efficacy in vivo (Part II): In vitro and in vivo characterization”. In: *Sensors and Actuators B: Chemical* 222 (2016), pp. 579–587.

[31] Siyu Lin et al. “Tip-packaged high-temperature fiber-optic sensor based on parallel Fabry-Perot interferometers and the Vernier effect”. In: *IEEE Sensors Journal* (2023).