“Fibre optics immunosensors: A novel approach to identify cancer biomarkers.”

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Declaration

I hereby declare that the following work has been done with the humble assistance of Zhandos Yegizbay, a master's student from school of Science and Humanities, Nazarbayev University. All the experiments regarding the project 'Fibre optics immunosensors: A novel approach to identify cancer biomarkers' were conducted in the laboratory of Biosensors.

Additionally, I attest to the originality of this work and affirm, to the best of my knowledge and belief, that it has not been previously submitted for any degree or diploma at Nazarbayev University or any other institution, either in its entirety or in part, at any time before or during its submission.

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Abstract

Cancer is still one of the most significant worldwide health risks, and one of the reasons why is the imperative need for cutting-edge diagnostic technology to enable early, accurate detection. The current research addresses the development of fibre-optic immunosensors with SPR technology for specific detection of ALDH1A1, a biomarker highly associated with cancer stem cells, cancer malignancy, and drug resistance. The work focuses on optimizing the sensor's analytical performance using advanced functionalization methods and thorough spectral characterization, with the goal of creating a reliable tool for clinical cancer diagnosis. The experimental protocol involved the fabrication of SPR sensors, which were subsequently functionalized with ALDH1A1-specific antibodies utilizing protein G in order to preserve the proper antibody orientation. Comprehensive calibration using sucrose solutions over a range of concentrations was employed to calibrate the sensors' refractive index sensitivity. Detection capabilities were evaluated systematically through exposure to ALDH1A1 protein concentrations between 10 femtomolar and 100 nanomolar. The results showed significant differences in sensor response properties, with some sensors exhibiting high reproducibility and stability and others showing more unpredictable performance, suggesting the influence of functionalization quality and experimental conditions. The findings of this research indicate the great potential of SPR-based fibre-optic biosensors as a very sensitive and convenient platform for cancer biomarker detection. The capability of the technology to attain low detection limits with specificity is a key strength over other conventional diagnostic tools. Future research directions include the development of multiplexed detection systems and full clinical validation studies to bring this technology more towards practical realization in the medical setting, the potential to change early cancer detection and patient monitoring.

Keywords: Cancer biomarkers, ALDH1A1, optical biosensors, surface plasmon resonance, fibre-optic immunosensors, protein G, cancer diagnostics.

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List of Abbreviations

ALDHs: Aldehyde dehydrogenases

HER2: Human Epidermal Growth Factor Receptor 2

CTCs: Circulating tumour cells

CVD: Cardiovascular Diseases

APTMS: Amino propyl Trimethoxysilane

CEACAM: Carcinoembryonic antigen-related cell adhesion molecule

LDH: Lactate Dehydrogenase

CEA: carcinoembryonic antigen

CSCs: cancer stem cells

PSA: prostate-specific antigen

SPR: Surface Plasmon Resonance

PD-L1: Programmed Cell Death Ligand

Chapter 1- Introduction

1.1. Background

1.1.1. Cancer and its effects

Cancer is a disease that affects people all over the world and has a high fatality rate. According to the present studies and the current tendencies, cancer may become the leading cause of untimely deaths surpassing CVD in various countries during this century. Indeed, governments have to consider these shifts when synthesizing cancer strategies for the endemic profile of a certain region century [1].

Interventional methods may be applied to conventional cancer detection and tracking techniques. Many biomarkers in cancer are alarm signals, which tell doctors to investigate further. These tumour indicators may increase the outcome of therapy and recovery by helping in the early cancer diagnosis. Advancements in nanotechnology and molecular biology and health research have made it possible to design portable sensors to detect cancer biomarkers. It is important to note that they can find, monitor and treat other diseases in addition to cancer [2].

1.1.2. Cancer biomarkers

A quantifiable trait that predicts the likelihood, occurrence, or course of cancer as well as patient outcomes is called a cancer biomarker. These traits may be physiological, cellular, molecular, or imaging-related. These biomolecules are either created by cancer cells or represent a reaction of healthy cells to malignancy. They are present in tissues or bodily fluids. Examining tumours or bodily fluids to find changes in DNA, RNA, proteins, or other biomolecules is known as cancer biomarker testing[3]. These tests yield vital data for cancer diagnosis, prognosis, precision medicine guidance, therapy response prediction, and cancer progression tracking. To differentiate it from cancer biomarker testing, genetic testing, on the other hand, focuses on finding germline genetic abnormalities connected to cancer susceptibility, hereditary malignancies, or cancer-related disorders [4].

1.1.3. Types of Cancer Biomarkers

There are different kinds of biomarkers for different cancer types. For example, carcinoembryonic antigen (CEA) is used for the identification of colorectal cancer, Lactate Dehydrogenase (LDH) elevated in aggressive cancers due to increased glycolysis (Warburg effect), HER2 in breast or ovarian cancer, PSA in prostate cancer [5]. **Table 1-1** shows an overview

of clinically relevant cancer biomarkers, the respective cancer types, and their biological roles in diagnosis or prognosis. These biomarkers, being proteins (e.g., ALDH1A1, HER2), enzymes (e.g., LDH), and antigens (e.g., PSA, CEA), play critical roles in early diagnosis, treatment response monitoring, and disease prognosis prediction[6]. Their utilization in biosensor-based systems, as explored in this study, illustrates the potential for non-invasive and high-sensitivity diagnostic tools.

Table 1- 1 Different biomarkers in various cancers and their clinical roles

Biomarker	**Full Name**	**Associated Cancers**	**Clinical Role**
ALDH1	Aldehyde Dehydrogenase 1A1	Breast, ovarian, lung	Stemness marker, therapy resistance
HER2	Human Epidermal Growth Factor Receptor 2	Breast, gastric	Targeted therapy selection
PSA	Prostate-Specific Antigen	Prostate	Screening and recurrence monitoring
CEA	Carcinoembryonic Antigen	Colorectal, pancreatic	Metastasis detection
LDH	Lactate Dehydrogenase	Lymphoma, melanoma	Prognostic indicator for aggressive tumours

1.1.4. Role of ALDH1

Because cells expressing aldehyde dehydrogenase (ALDH) have the capacity to self-renew, differentiate into different lineages, and form tumours in xenograft models, it has been suggested that ALDH expression or activity in breast carcinomas serves as an indication for cancer stem cells. Furthermore, poor survival has been strongly linked to the presence of ALDH-positive cells in proactive breast tumours [7].

Aldehyde Dehydrogenase1 (ALDH1) is regarded as a biomarker for detection of cancer. It has a major impact on tumour genesis, development, metastasis, and resistance to therapy. It is especially linked to cancer stem cells (CSCs). ALDH1, a member of the aldehyde dehydrogenase family, is involved in retinoic acid metabolism, oxidative stress response, and intracellular aldehyde detoxification. ALDH1's association with therapy resistance is another important function; tumours that express a lot of ALDH1 frequently show resistance to radiation and chemotherapy. This resilience is explained by CSCs' capacity to repopulate the tumour and avoid traditional therapies. ALDH1 is being investigated as a potential therapeutic target due to its critical involvement in cancer stemness and medication resistance. Research is currently being conducted to find ways to block its activity and increase the effectiveness of treatment [8].

It is estimated that among ALDH genes, 19 functional ALDH genes, including members of the ALDH1 family, exist in humans. An important member of this family is ALDH1A1. The ALDH1A1 gene is situated on the long arm of the 9^{th} chromosome in sub region 13 of region 21. Different tissues have homotetrameric cytoplasmic proteins, which are produced by this gene. ALDH1A1’s high substrate specificity for retinal isomers (all-trans and 9-cis) underpins its role in retinoic acid synthesis, a pathway critical for cell differentiation and proliferation. Dysregulation of this process, as seen in ALDH1A1-overexpressing tumours, promotes stemness and chemo resistance[9]. Recent studies also implicate ALDH1A1 in modulating oxidative stress responses, further protecting cancer stem cells from apoptosis[10].

In over a decade, scientists have found that ALDH1A1 is essential for both physiological and pathological processes in several systems, including the neuron system, as well as for discomforts which are involved in inflammation and metabolism[11, 12]. Increased involvement in diabetes, obesity, and other health issues has been linked to elevated expression of ALDH1A1[13, 14]. To maintain the structure and functionality of ALDH1A1 several molecules are involved. These molecules are mentioned in Fig.1-1.

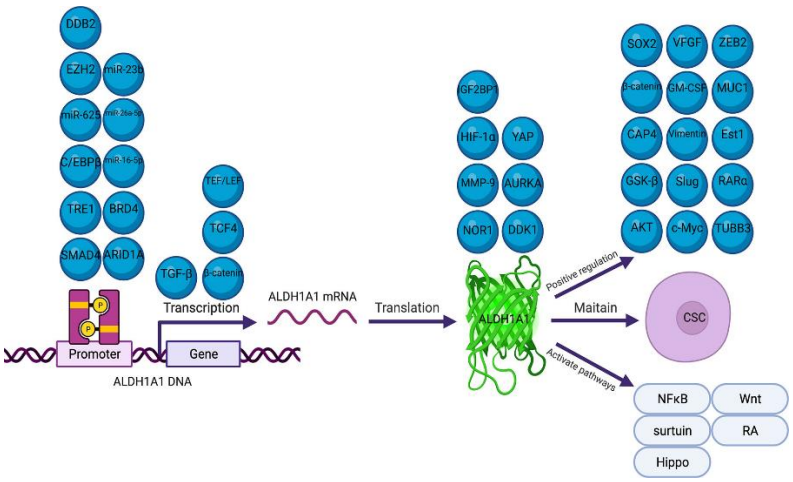

Figure 1- 1 *Regulation of ALDH1A1 in terms of involvement of molecules [10].*

1.2. Biosensors in cancer

Biosensors are effective instruments for detecting cancer. They provide quick findings, excellent sensitivity, and specificity. Biosensors use a transducer in conjunction with biological recognition components, such as enzymes or antibodies. They can identify minute alterations in biological samples as a result. They are useful in the treatment of cancer because they offer precise and real-time diagnosis [15].

1.2.1. Advantages of Biosensors in biomarker detection

Biosensors are essential for cancer detection since they provide accurate, quick, and non-invasive diagnostic choices. In bodily fluids like blood or saliva, they can identify cancer biomarkers, like proteins or nucleic acids, at extremely low amounts. Because of this, they are quite good at detecting cancer in its early stages [15]. By examining exosomes, DNA, or circulating tumour cells, biosensors provide vital information without requiring a tissue biopsy, hence avoiding invasive procedures. Their real-time monitoring features enable physicians to monitor the course of cancer, assess the effectiveness of treatment, and identify recurrence [16]. The main components of biosensors are shown in **Fig.1-2**[17].

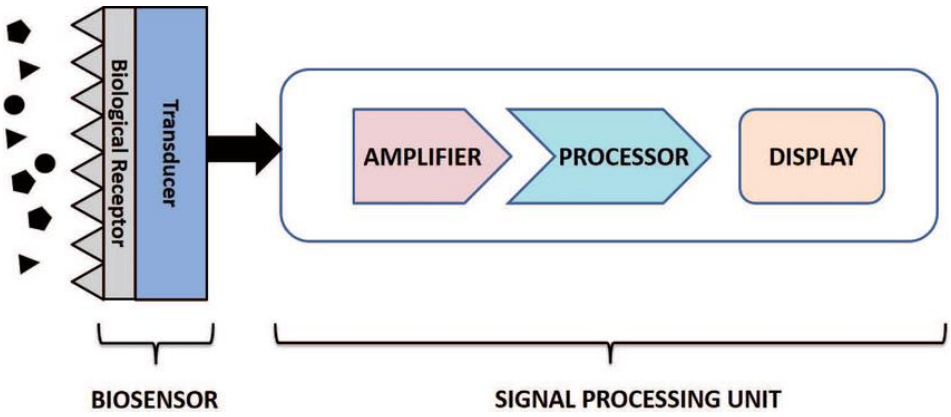

Figure 1- 2 *Components of a basic biosensor*

Comprehensive diagnosis and individualized treatment strategies are made possible by advanced biosensors that can profile many biomarkers simultaneously. Additionally, portable biosensors provide point-of-care testing, providing prompt outcomes in environments with limited resources [18, 19]. The cost-effectiveness, sensitivity, and specificity of these instruments make them a viable substitute for more conventional techniques like imaging or tissue-based testing. Consequently, biosensors are revolutionizing cancer diagnosis and enhancing patient results [4].

1.2.2. Types of biosensors used in cancer detection

Biosensors are revolutionizing cancer diagnosis by offering non-invasive, quick, and sensitive diagnostic alternatives. These instruments identify particular cancer biomarkers, which are frequently found in very small amounts in bodily fluids like blood, urine, or saliva and include proteins, nucleic acids, or DNA alterations [20]. They are quite useful for monitoring and detecting cancer in its early stages because of this ability.

Electrochemical biosensors are frequently employed to detect proteins linked to cancer or DNA alterations. They provide great precision and dependability by using electrical signals produced by the biomarker's interaction with the sensor [21]. Optical biosensors identify cancer biomarkers by using methods like surface plasmon resonance (SPR) or fluorescence [22]. The capacity of these biosensors to observe the real-time and label-free interactions makes them very beneficial.

Nanotechnology-based biosensors use Nanoparticles to improve sensitivity and specificity, enabling them to identify difficult-to-detect biomarkers such as exosomal RNA[23]. Multiplexing is another feature of these advanced biosensors that allows the simultaneous detection of several markers for thorough cancer profiling [24]. Biosensors can track the progression of cancer, assess

the efficacy of treatment, and identify recurrence thanks to their real-time monitoring capabilities. They are a useful and affordable substitute for more conventional techniques like imaging or tissue biopsies because of their portability and flexibility for point-of-care diagnostics. Because of their adaptability and effectiveness, biosensors are a key component of contemporary customized medicine and cancer diagnoses [25]. Figure 1-3. represents the schematic diagram of a biosensor's working principle [26].

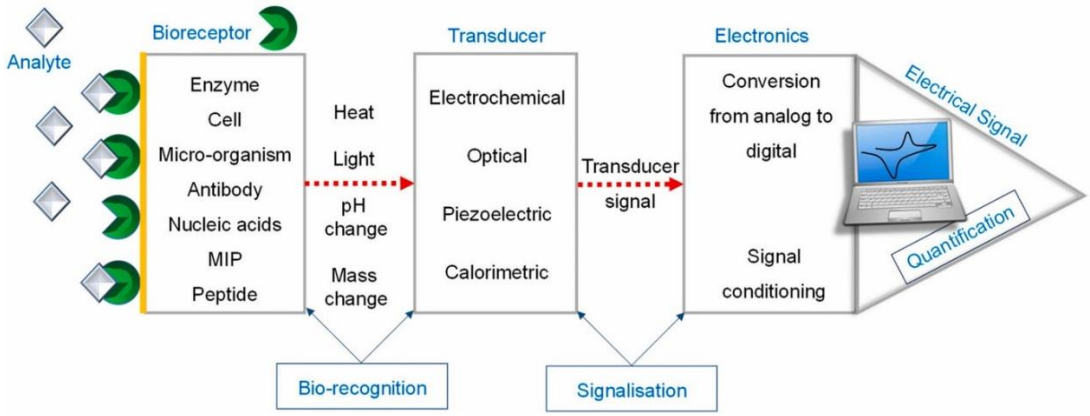

Figure 1- 3 *MIP, molecularly imprinted polymer, a Schematic representation [23].*

1.2.3. Optical biosensors

Optical biosensors are essential for detecting cancer because they use light-based methods to detect and measure cancer biomarkers with high specificity and sensitivity. Fluorescence, absorbance, and refractive index changes are examples of optical signals that they use to identify molecular interactions (such as antigen-antibody binding). These non-invasive biosensors provide real-time monitoring and are perfect for detecting recurrences, assessing therapy response, and early cancer diagnosis [27].

Types of optical biosensors: There are different types of optical biosensors which are used in cancer detection. Some of the types are as follows: Fluorescence-based biosensors are highly sensitive for detecting cancer biomarkers [28], such as circulating tumour DNA or proteins like HER2. They do this by using fluorescent dyes or labels. Label-free detection of biomarkers such as PSA or PD-L1 is made possible by surface plasmon resonance (SPR) biosensors, which assess changes in the refractive index at a metal-dielectric interface [29]. Biosensors based on Raman spectroscopy use Raman scattering to generate molecular fingerprints, which enables them to detect chemicals unique to cancer in fluids or tissues [30]. Evanescent wave biosensors employ the evanescent field produced on the surfaces of optical fibres or waveguide to detect biomolecules such as exosomal proteins. Interferometric biosensors ultimately quantify phase shifts in light resulting from

molecular binding events, providing remarkable sensitivity for the detection of nucleic acids or proteins. Diverse optical biosensors significantly enhance the precision and efficacy of cancer diagnosis [31].

1.2.4. SPR Biosensors

Surface Plasmon Resonance (SPR) biosensors are label-free optical biosensors that measure changes in the refractive index near a metal-dielectric contact to see how molecules interact in real-time [32]. Surface plasmon waves, which are electron oscillations made when polarised light hits a thin metal plate (usually gold or silver) in certain ways, enable them to operate [33]. Because these biosensors are very sensitive and specific, they are perfect for finding signs of cancer. They study how biomolecules interact, keeping an eye on how the diseases progress. With SPR biosensors, you don't need fluorescent or radioactive labels. This makes the discovery process easier and less invasive, which is especially helpful for finding cancer and making new drugs [34].

1.2.5. SPR biosensors in cancer detection

SPR biosensors show efficacy in the detection of cancer biomarkers and in the identification of novel ones. The fundamentals of SPR are shown in Figure1.4. Some of these recently identified markers show significant potential for precise diagnosis. Rac1b is very sensitive and specific for the detection of non-small-cell lung cancer, whereas aromatase is a promising biomarker for bladder cancer [35]. Some biosensors examined in the reviewed research have been carefully evaluated, while others have only received preliminary assessment [36].

Cancer biomarkers are becoming increasingly essential instruments for the early and precise identification of tumours. SPR-based techniques facilitate the non-invasive detection of biomarkers. Because of their numerous advantages, including non-destructiveness, ease of miniaturization, excellent selectivity, reliability, and cost-effectiveness [37].

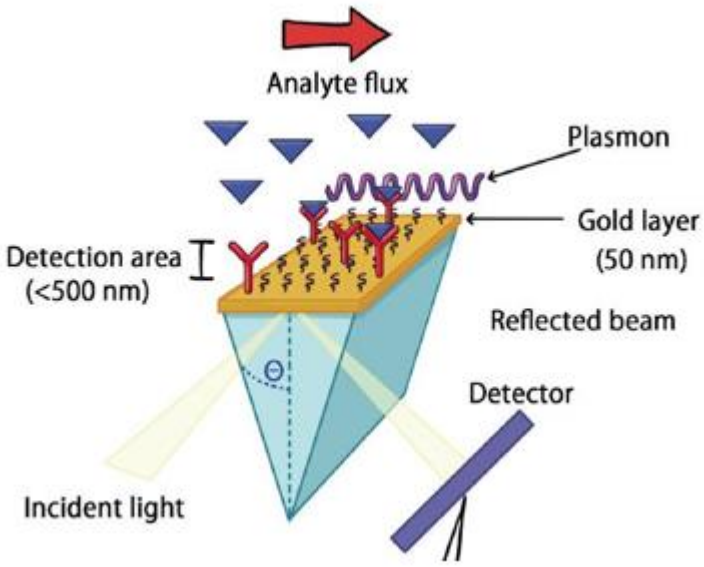

Figure 1- 4 *Fundamentals of SPR [1].*

1.3. Literature Review

The development of optical biosensors for cancer detection has come a very long way from the early demonstrations of surface plasmon resonance (SPR) as a method for analysing biomolecular interactions. Early prism-based SPR systems established the fundamental advantages of label-free and real-time measurements but were limited in miniaturization and clinical translation[38]. This made the transition to fibre-optic platforms inevitable, where surface plasmon resonance SPR sensors emerged as a highly commendable configuration due to their inherent sensitivity against refractive index variations and miniaturization with optical configurations[39]. Immobilization of biorecognition components is of utmost significance for biosensor performance. Protein G-directed antibody orientation is presently widely used because it allows for precise antibody orientation through Fc region binding to achieve 60-80% functional binding efficiency compared to 30-50% with random covalent coupling[40]. Concentration optimization has in all studies established 1 μg/mL to achieve maximum surface density free from steric hindrance, with minimal optimization likely to be required to compensate for differences in diameter and curvature of the fibre[22]. The cross-link conjugation with glutaraldehyde facilitates stable conjugation with preserved antibody function, but alternative methods using DNA scaffolds or nanobodies have applications to consider[41].

Advances in signal processing have overcome some of the long-standing limitations of SPR biosensing. Reference gratings for thermal drift elimination have reduced baseline jitter to <0.3 pm/h, while advanced denoising schemes can raise signal-to-noise ratios by >12 dB in multicomponent media[42, 43]. Machine learning algorithms have been found to be particularly

powerful in discriminating between specific binding and nonspecific adsorption, with classification accuracies of >95% even at femtomolar concentrations[43]. These computational advances have proven essential in maintaining reproducibility in low-abundance biomarker detection when traditional peak-tracking methods fall short.

Despite these technologies, there remain great challenges in clinical translation. Variability in batch-to-batch fabrication continues to create reproducibility problems, with sensitivity changes up to 30% even in controlled settings [44-46]. Complexity from biological matrices is introduced, serum constituents inclining to reduce signal intensity by 40-60% from buffer reading[47]. These problems have driven recent developments toward more stable surface chemistries and packaging approaches. The field is moving towards multiplexed systems for parallel biomarker detection, and several groups have demonstrated simultaneous detection of 8-12 analytes with arrayed fibre formats [48]. These limitations have driven recent work toward more robust surface chemistries and packaging solutions.

The field is now moving toward multiplexed systems capable of parallel biomarker detection, with several groups demonstrating simultaneous measurement of 8-12 analytes using arrayed fibre configurations [49]. Microfluidic integration has alleviated some severe sample handling issues while enabling automated sensor regeneration, although the design of flow cells must balance optical and hydrodynamic demands with caution[50]. There is also growing interest in hybrid detection approaches combining SPR with supplementary techniques like electrochemical sensing to improve diagnostic fidelity[51]. Such developments are creating new opportunities for complete cancer profiling without sacrificing the optical detection's sensitivity and specificity advantages.

Chapter 2 - Materials and Methods

2.1 Materials

All the materials used in the experimentation were available in the laboratory. All the solutions were prepared and used in the laboratory. Sucrose solution for calibration was prepared beforehand while all other solutions used in functionalization were prepared a few minutes before using them. These solutions include:

- Piranha solution (H2SO4 & H2O2)
- APTMS solution (Methanol & APTMS)
- PBS solution
- GA solution (glycolic acid & PBS)

Other materials, which are mentioned in Figure 2-1 include fibre pigtails on which the fibres are attached, Wilfred fibre, which is used in the fabrication of sensors, Fujikura cleaver used to cleave the blunt ends of the fibres, pliers to remove the protective layers of the fibres, ethanol solution to clean the fibres.

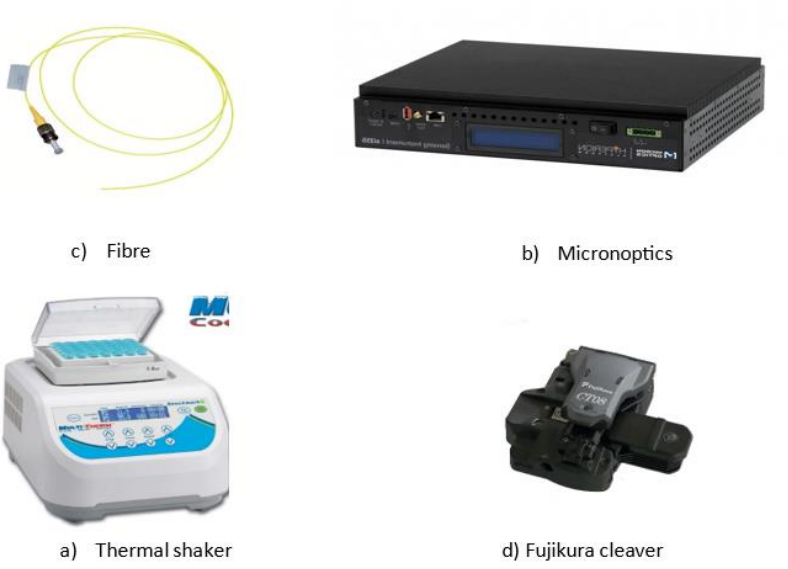

Figure 2- 1 *Devices used in the experimentation.*

Table 2- 1 List of materials used in the methodology.

Materials	Purpose
Surface plasmon resonance sensor	For fabrication
ALDH1A1 antibody	For functionalization of fabricated optical sensor
Piranha solution (sulfuric acid and hydrogen peroxide)	For functionalization of fabricated optical sensor
APTMS (3-Aminopropyltrimethoxysilane)	For functionalization of fabricated optical sensor
Glutaraldehyde (GA) solution	For functionalization of fabricated optical sensor
mPEG-amine	For functionalization of fabricated optical sensor
ALDH1A1 protein	For detection via optical measurement, using fabricated biosensor

2.2 Methodology

A simple flowchart of methods is shown in Figure 2.2. The methods used in the process of making the biosensors include 4 basic steps:

2.2.1 Fabrication

The fabrication of the sensor was done using Wilfred specialty optical fibres (SP1 to SP6) prepared for experimentation. Each fibre was treated with utmost care beginning with clean cleaving using a Fujikura fibre cleaver to maintain the best quality of the end-face for light transmission. The cleaved fibres were then spliced to standard fibre pigtails using a fusion splicer to have low-loss interfaces between the specialty and normal fibres. After splicing, all assemblies were cut again at a fixed distance from the joint location to produce the final sensor length. To finish preparing, all of the fibre ends were cleaned with ethanol to remove dirt and organic residue, leaving the optical surfaces clean and in good condition for subsequent procedures. This consistent approach to fabricating sensors yielded products with the same basic optical properties. This

provided a platform for subsequent antibody attachment and detection studies. Accurate cutting, consistent joining, and meticulous cleaning at each step resulted in each sensor performing the same way prior to the addition of biological materials.

2.2.2 Calibration

The calibration of the manufactured sensors is a crucial step to guarantee their proper functionality. It will be conducted using the Micronoptics system. This technology is selected due to its accuracy and compatibility with the sensors we are building. The sensors were subjected to sucrose solutions of increasing concentrations (5%, 10%, 15%, 20%, 30%, and 40% w/v) to evaluate their sensitivity. This testing simulated variations in refractive index to evaluate the sensors' efficacy in detecting these variations. This step is crucial for applications such as cancer biomarker detection.

The data gathered during these tests was analysed with MATLAB to process and interpret the optical spectra. The sensors' sensitivity was measured in decibels per refractive index unit (dB/RIU). Sensors exhibiting a sensitivity exceeding 120 dB/RIU, signifying high responsiveness, were considered appropriate. These high-performing sensors were selected for additional development and evaluation. This procedure guarantees the utilisation of only the most dependable sensors for various biological applications, including the early diagnosis of cancer.

2.2.3 Functionalization

Functionalization is the essential procedure of attaching specific antibodies to optical fibres, hence enhancing their selectivity and sensitivity for the detection of target analytes. This multi-step procedure includes cleansing, prepping the fibre surface, and immobilising the antibodies to guarantee reliability. The fibres were first decontaminated using a piranha solution, including sulphuric acid and hydrogen peroxide, for 15 to 30 minutes to eliminate organic impurities and reveal hydroxyl groups on the surface. This procedure guarantees a pristine surface for future applications. After decontamination, the fibres were meticulously cleansed with deionised (D.I.) water to eradicate any remaining piranha solution.

The following step involved salinization, during which the fibres were submerged in a 1% solution of 3-Aminopropyltrimethoxysilane (APTMS) for 60 minutes. This procedure put amino groups into the fibre surface, enabling subsequent chemical changes. Following salinization, the fibres were washed with methanol to eliminate unreacted silane and subsequently dried by the application of nitrogen gas. Dried fibres were subsequently heated in a preheated oven at 110°C

for one hour, which stabilized the silane coating and guarantees strong adhesion to the fibre surface.

Upon salinization, the fibres went through a cross-linking process. The fibres were incubated in a 25% glutaraldehyde solution for one hour at ambient temperature. Glutaraldehyde functions as a bifunctional linker, establishing covalent connections between the amino groups on the fibre surface and the proteins intended for attachment. Following incubation, we washed the fibres with phosphate-buffered saline (PBS) to eliminate surplus glutaraldehyde. The next step involved the immobilisation of protein G, which is essential for the proper orientation of antibodies on the fibre surface. Protein G attached to the Fc region of antibodies, therefore exposing the antigen-binding Fab regions for optimal target engagement. Fibres were divided into two groups one with G-protein (G+) and the other without G-protein (G-).Two solutions of protein G were formulated: one with 1 μg/mL of protein G in 999 μL of PBS and another with 0.5 μg/mL of protein G in 999.5 μL of PBS, both resulting in a total volume of 1 mL.

The fibres, designated based on the protein G concentration utilised, are incubated in their corresponding solutions for one hour at room temperature and subsequently rinsed with PBS. The next stage was the binding of antibodies. A solution of 2 μg/mL antibody in 498 μL of PBS was produced, resulting in a total volume of 500 μL. The fibres were divided into two groups; one with antibodies (Ab+) and the other without antibodies (Ab-).The fibres which were to be treated with antibodies were incubated in the prepared solution for one hour at room temperature, facilitating the attachment of antibodies to the surface by protein G. This duration guarantees enough antibody binding to the fibre tip, enhancing their efficacy in detecting target analytes. The group without antibodies was simply immersed in PBS.

The concluding phase of functionalization is blocking, an essential procedure to inhibit nonspecific binding during sensor operation. The fibres were incubated in a 1–3% Bovine Serum Albumin (BSA) solution for around 60 minutes at room temperature. BSA saturates the residual active sites on the fibre surface, diminishing nonspecific interactions and enhancing the sensor's overall selectivity. Following blocking, the fibres were rinsed with PBS to eliminate surplus BSA and are subsequently stored at 4°C in a refrigerator to maintain their functionality. Functionalized fibres must not be stored at room temperature to prevent the degradation of the attached biomolecules.

2.2.4 Measurement

Solution preparation: Total of 9 concentrations from C9 to C2 were measured with decreasing concentrations of ALDH1 protein while C1 being only PBS. Starting from C9 with

100nM to C2 with 10fM with a working volume of 280uL. The volume of the solution prepared was measured by the formula:

$$C1V1 = C2V2$$

2.2.5 Detection

The mirconoptics setup was installed for detection. The fibres were attached to the channels and then were measured for sensitivity. A total of 9 concentrations with 10 measurements each were used with mixing the solution after each 4th and 8th measurements. The results obtained from measurement procedure were then analyzed by using MATLAB. Lastly, a scanning electron or an atomic force microscope will be used to study the surface morphology of the biosensor.

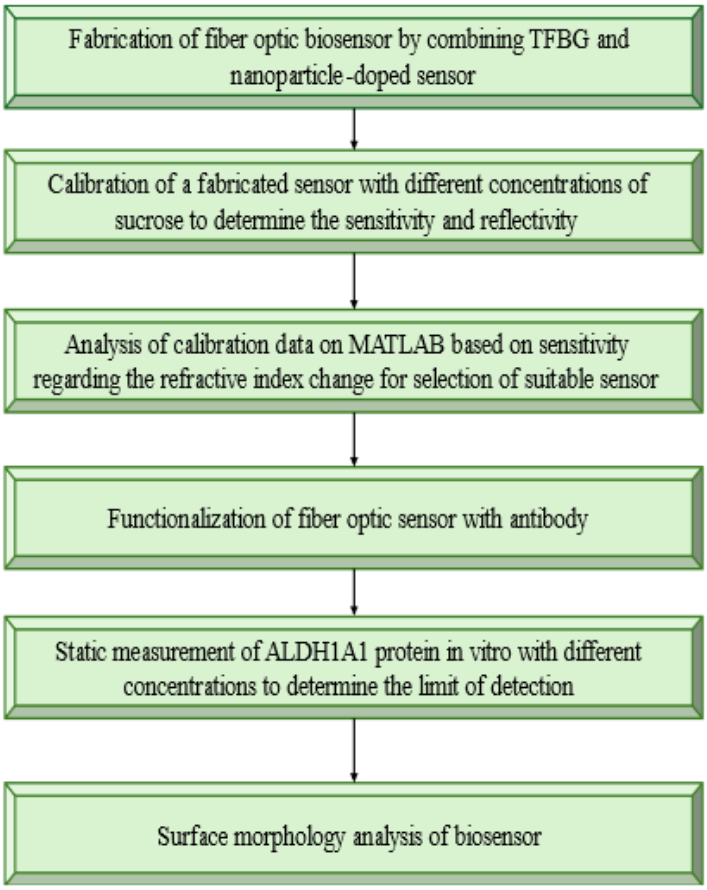

Figure 2- 2 *Flowchart of the steps followed for the experiment.*

Chapter 3 - Results and Discussion

3.1. With /without Antibody:

Figure 3-1 graph illustrates the reaction of various sensors when all of the sensors were used to detect ALDH1A1 using just antibodies, without Protein G to facilitate attachment. Although all three of the antibody-coated sensors (Channels 1, 3, and 4) picked up the cancer biomarker, they did not perform equally as well. Channels 1 and 3 registered steady, consistent readings, indicating the antibodies adhered to these sensors nicely and functioned as intended. Channel 4, however, had very erratic values, in which the signal randomly pinged up and down, which likely indicates that the antibodies did not bind evenly or well on this sensor. The control sensor with no antibodies (Channel 6) was relatively flat, indicating that the reactions within the remainder of the channels were indeed from antibody-ALDH1A1 binding and not from random sensor noise. These variations in channels - the erratic performance of Channel 4 versus the stable Channels 1 and 3 - are the reason why the simple antibody coating of sensors is not sufficient for reproducible results, paving the way for subsequent experiments that employed Protein G to enhance reliability through the facilitation of antibody binding in a more orderly fashion. The graph finally demonstrates that although the simple method functions, it must be improved if it is to be reliably used for cancer detection.

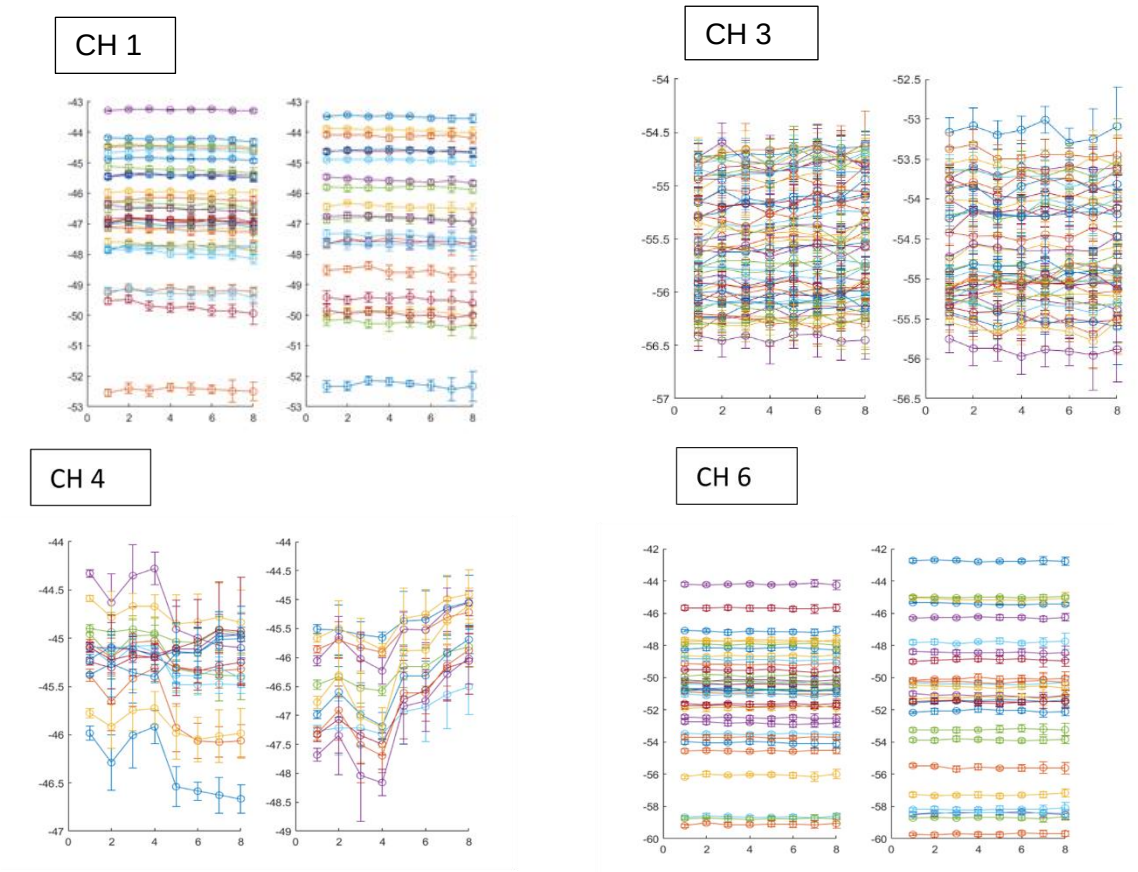

Figure 3- 1 *First detection with antibody. Channel1,3, 4: Ab+, Channel 6: Ab- (control).*

3.2. With/Without protein G

We tested four sensors' setups, with and without antibodies (Ab+/Ab-) and with and without protein G (G+/G-). The first measurements taken considered systems without protein G (Figure.3-1) with variable performance across channels. CH4 presented unstable results with huge leaps between measurements and big error ranges. CH6 presented steady results, like CH1 but with higher values. Both test samples (with antibodies) and control groups were present in all the channels. Results were measured for Ab+ G+, Ab- G+, Ab+ G- and Ab- G-. For first detection we did an experiment without G-protein and then with G-protein. The results were gathered for different channels of micron optics. In Fig.3-2. the graphs illustrate data from two channels (CH 4 and 6), each displaying various data series characterized by distinct patterns and degrees of variability. The plots contain one antibody positive and one control group. CH4 displays notable deviations, with certain data series demonstrating abrupt oscillations and increased error bars, signifying greater variability or instability within the system. Conversely, CH 6 exhibits generally stable and clustered patterns however with marginally elevated overall values and significant variability.

The graphs show obvious performance differences between Channel 4 (CH4) and Channel 6 (CH6) in detecting ALDH1A1. CH4 has significant signal variations with tremendous, uneven oscillations in the data. The larger error bars in the extended experiment, nearly as large as in CH6, indicate greater sensitivity to external conditions or protein G-supported antibody immobilization variability. CH4 was functionalized with protein G (10 µg/mL) but not antibodies (G+ Ab-), while CH6 had neither (G- Ab-). This indicates that protein G by itself can generate signal instability in the form of unbound molecules creating nonspecific binding sites.

CH6 shows stable baseline readings at higher signal levels than expected. Moderate error bars show stable reproducibility with minor fluctuations. This stability is seen in the absence of protein G and antibodies, demonstrating good consistency of measurement of the sensor. Minor changes in CH6 most likely account for system noise, rather than actual biological signals.

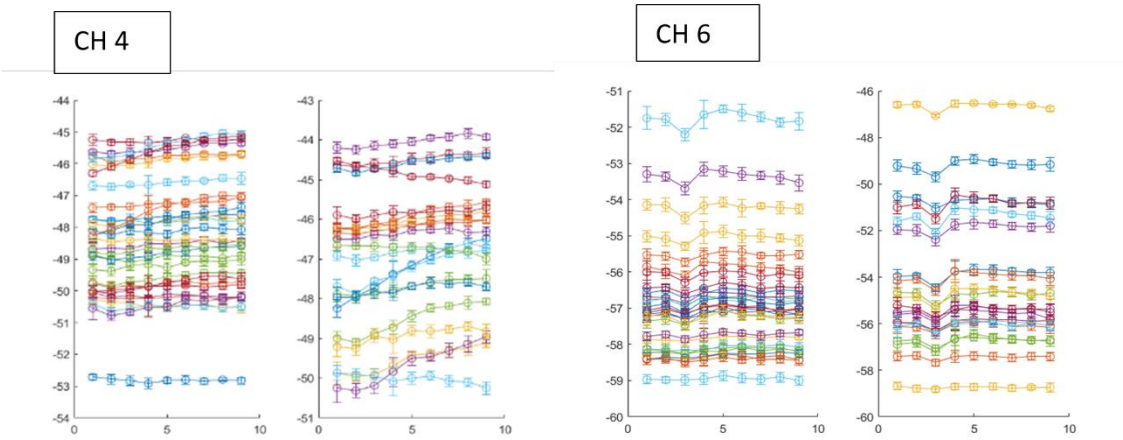

Figure 3- 2 *Detection with protein-G. Channel 4: G+ Ab-, Channel 5: G- Ab+ protein G concentration was 10ug/ml Antibody conc was 4ug/ml.*

The results show considerable disparity when we compare the non-protein G tests (Fig. 3-1) and the protein G experiments (Fig. 3-2). Most of all, Channel 4 (CH4) was much more unstable in the absence of protein G, with greater fluctuations in its readings. Channel 6 (CH6), on the other hand, still performed steadily under both test conditions, showing stable consistency whether protein G was present or not. Additionally, the groups that contained just the antibody tests (without the protein G) also exhibited different patterns compared to the protein G groups. This suggests that protein G has an important impact in modifying the way the sensor reacts. These comparisons help show how protein G affects the stability and consistency of measurements in our biosensor system.

3.3. With different concentrations of protein G

Protein G concentration greatly influences the performance of the sensor through the effectiveness of antibody attachment onto the sensor surface. Lower antibody concentrations (i.e., 0.5 μg/mL) can result in too much space between antibodies, decreasing the capacity of the sensor to bind ALDH1A1 and resulting in weak or unstable signals. Higher antibody concentrations (i.e., 1 μg/mL) allow antibodies to create even rows, enhancing detection consistency (such as Channels 1 and 3) by being able to create more binding sites. But excess Protein G would pack the surface and could inhibit some antibodies from binding to ALDH1A1 or enhance non-specific binding. Your experiments with intermediate amounts (e.g., 0.5 μg/mL) could indicate an optimal ratio—enough Protein G to stabilize antibody binding without causing steric hindrance—optimizing sensitivity and reproducibility for the detection of cancer biomarkers. Such a refinement would close the gap between the unstable behaviour of antibody-only sensors and the better outcomes with Protein G in subsequent figures.

The graphs shown in **Fig.3-3.** are not that different from the previous graphs. CH6 exhibits a predominantly stable trend, like CH1, although it presents periodic irregularities and moderate error bars, signifying that overall performance remains consistent, but with minor fluctuations. The four channels collectively indicate a stability gradient, with CH1 and CH6 exhibiting greater consistency, whereas CH3 and CH4 demonstrate increased variability, particularly in CH4.

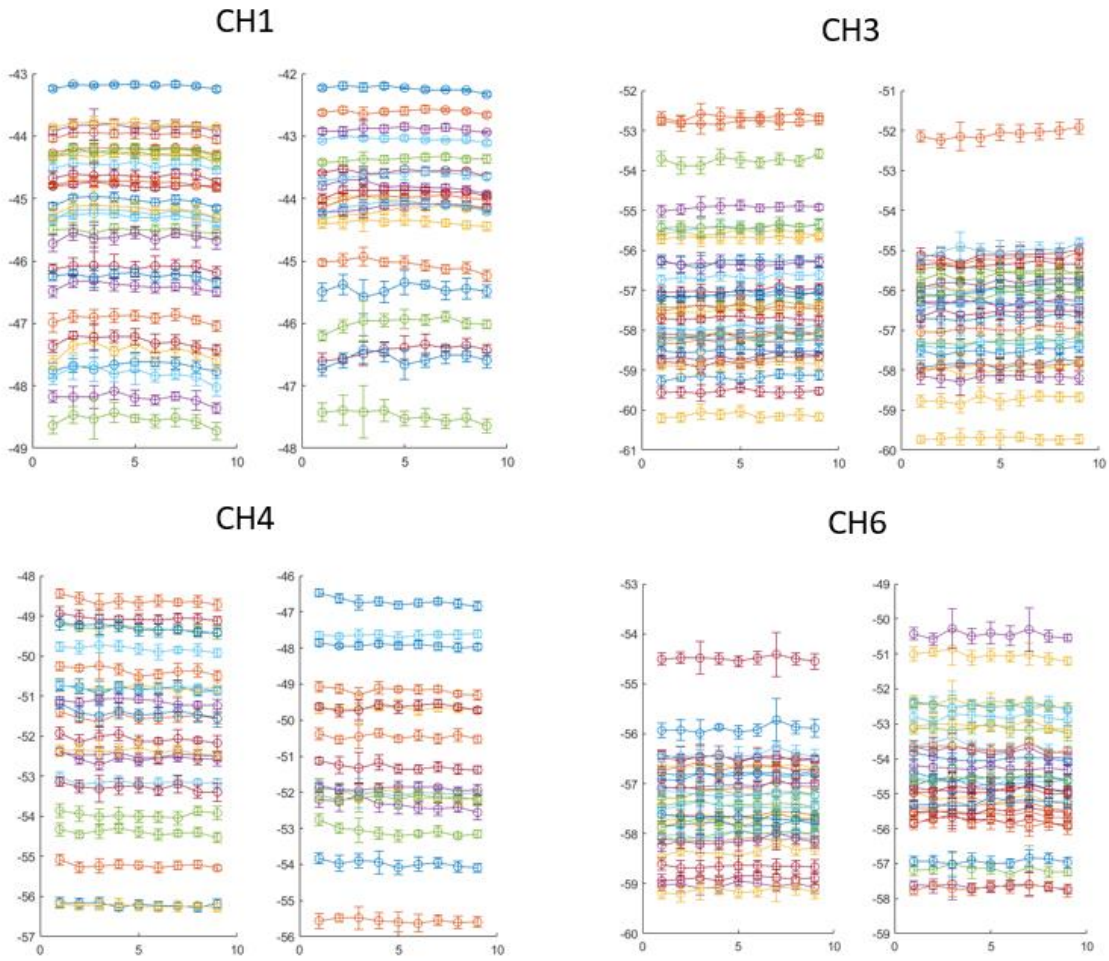

Figure 3- 3 *Channel 1: G1+ Ab+, Channel 3: G1+ Ab+, Channel 4: G1+ Ab+, Channel 6: G0.5+ Ab-. Protein G1 concentration was 1ug/ml. Protein G0.5 concentration was 0.5ug/ml. Antibody conc was 4ug/ml.*

3.4. Statistical and spectral evaluation:

The spectroscopy data reveal how ALDH1 reacts to light at different concentrations. As we test samples with differing concentrations of the ALDH1 protein (from very diluted to concentrated solutions), we observe distinct patterns in the way the sensor responds to different wavelengths of light. These patterns tell us two things that are key: first, how the strength of the light signal at certain wavelengths is altered in the presence of ALDH1, and second, how these effects are amplified at higher concentrations of ALDH1.

The information we gather tells us a lot about ALDH1's optical properties - basically how this cancer-associated enzyme influences light traveling through our sensor system. At the specific wavelength of 1526 nm (in the infrared range), we observe a very robust response that is linearly related to ALDH1 concentration. The fact that signal strength is linearly related to concentration

is especially important since it allows us to create a reference system whereby, we can deduce unknown concentrations of ALDH1 based on the light signals that we measure.

These findings form the foundation for the development of practical detection methods that can eventually be used in the clinic. By understanding exactly how ALDH1 affects light over a series of concentrations, we are better equipped to come up with sensitive tests that can potentially help doctors monitor this important cancer biomarker in patient samples.

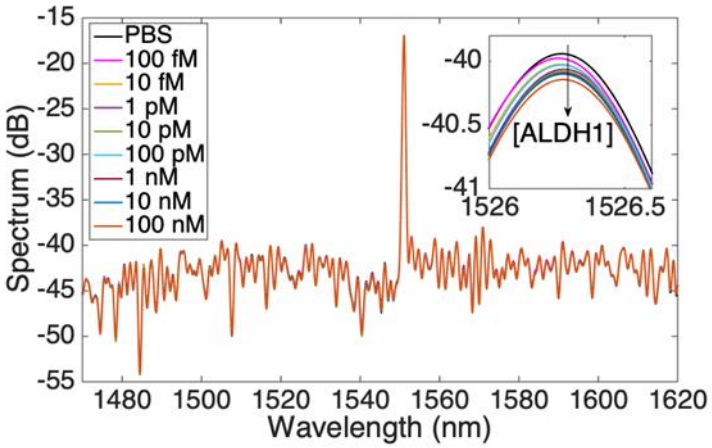

Figure 3- 4 *Spectrum of the SDI sensor for various concentrations of ALDH1; the inset shows one peal at 1526 nm.*

As the concentration of ALDH1 goes up, the graph shows how the spectral reactions change. Each concentration (for example, PBS, 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM) has its own unique spectral features. A Gaussian distribution is used to fit the data. The mean value (μ) shows the spectral answers' central tendency, and the two-sided standard deviation (2σ) shows how spread out or variable the data is around the mean (shown in Fig. 3-5).

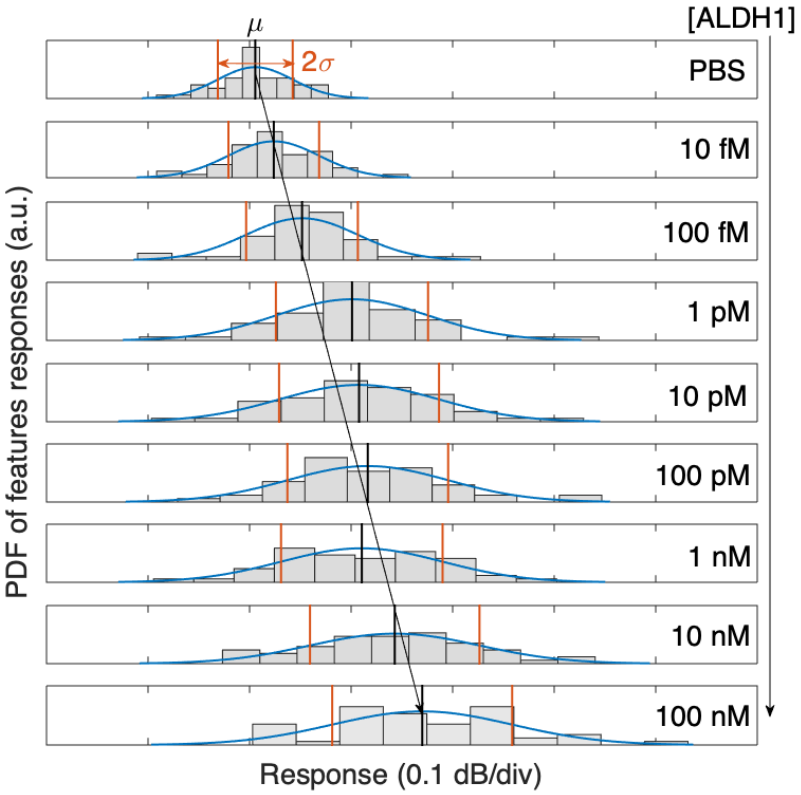

Figure 3- 5 *PDF of the 50 spectral peaks of the SDI spectrum, evolving with concentrations of ALDH1 from 10 fM to 100 nM, and its Gaussian distribution fit (μ = mean value, 2σ = two-sided standard deviation).*

The results of this study show that the spectral reactions depend on the concentration. The spectral peaks are clearer and more noticeable when the ALDH1 concentration is higher. A statistical picture of the data is given by the Gaussian fit, which shows how consistent and accurate the spectral readings are across a range of concentrations. This data is very important for figuring out how sensitive and how far the SDI method can find ALDH1.

Figures 3-6 and **3-7** display the performance and specificity of Spectral Domain Interferometry (SDI) sensors in measuring ALDH1 across a range of concentrations (10 fM to 100 nM). In **Figure 3-7**, responses of sensors are compared under four conditions: (1) in the absence of antibody (Ab) and protein-G, (2) in the absence of Ab but presence of protein-G (1 μg/mL), (3) in the presence of Ab (4 μg/mL) but absence of protein-G, and (4) in the presence of Ab (4 μg/mL) and protein-G (1 μg/mL). The results show that the combination of antibodies and protein-G provides the peak response, indicating enhanced binding efficiency and sensitivity. Slopes of the responses, calculated by log-linear regression, also approve the concentration-dependent response of the sensors, with error bars (± standard deviation) showing the reproducibility of the measurements.

Figure 3-7 deals with the specificity of ALDH1 detection at the highest concentrations (10 nM and 100 nM). It shows a comparison between the responses of sensors with ALDH1 antibody functionalization (with and without protein-G) and sensors without antibodies. The findings confirm that antibody-functionalized and protein-G functionalized sensors have the strongest and most specific response, while non-functionalized sensors either have weak or non-specific binding. This indicates the indispensable contribution of antibody functionalization and protein-G in achieving high sensitivity and specificity for ALDH1 detection. These values attest to the effectiveness of the SDI sensor platform as a measure of ALDH1, where the antibody and protein-G functionalization schemes significantly increased the sensitivity and specificity. The accuracy and reproducibility of the measurements, as indicated through the error bars, also support the effectiveness of this approach in detecting ALDH1 in biological systems.

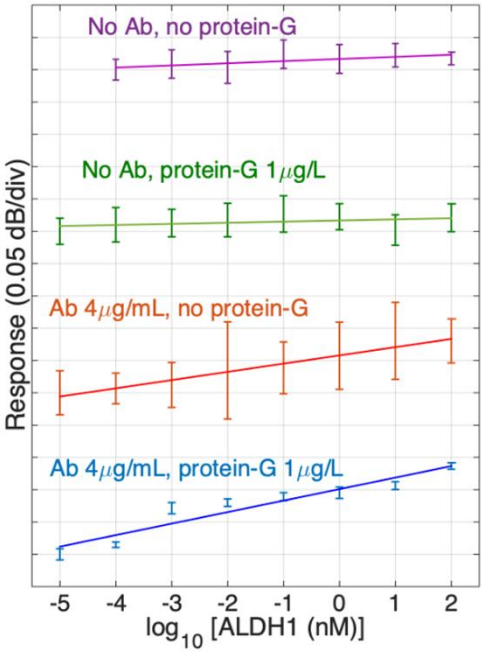

Figure 3- 6 *Response of different SDI sensors in ALDH1 concentrations from 10 fM to 100 nM, from the PBS reference. The data report sensor functionalized with ALDH1 antibodies (4 μg/mL) and without antibodies, and with protein-G (1 μg/mL) and without protein-G. Slo Slopes are estimated using log-linear regression (from bottom to top: 0.0178 dB, 0.0127 dB, 0.0017 dB, 0.0033 dB). Errorbars show ± standard deviation over 10 consecutive measurements.*

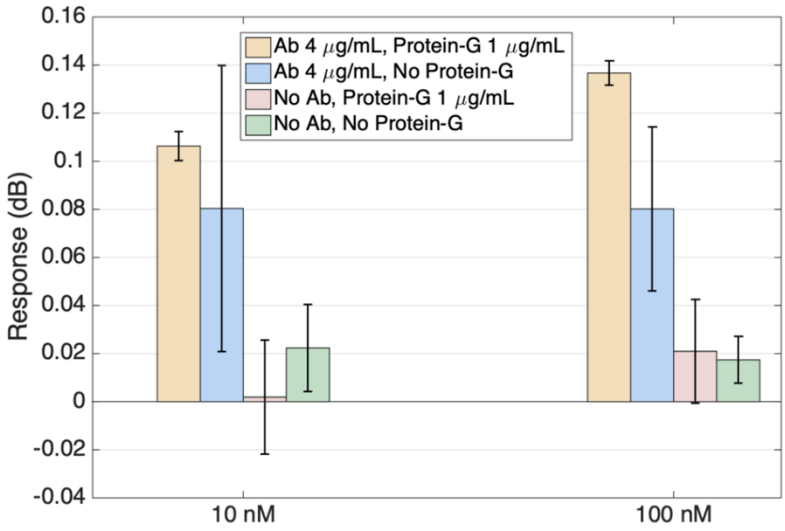

Figure 3- 7 *Specificity of the ALDH1 detection, comparing the response of sensors functionalized with ALDH1 antibodies (with and without protein-G functionalization) with sensors functionalized without antibodies. Data are reported at the highest concentration (10 nM, 100 nM).*

Chapter 4 - Conclusion

Our investigation has enlightened a new understanding of cancer diagnostics with the pioneering introduction of fiber-optic immunosensor technology as a novel analytical tool. Besides proving the technical feasibility of the detection of ALDH1A1 at medically relevant concentrations, our study also revealed insights into the detection. The challenges encountered during sensor optimization - notably concerning reproducibility and environmental stability - have provided valuable lessons that will inform future biosensor development.

In addition to the technical accomplishments, this study possesses significant implications for translational medicine. Femtomolar detection of ALDH1A1 indicates possible applications beyond primary diagnosis, such as treatment monitoring and detection of recurrence. The protein G-mediated functionalization strategy developed here may serve as a new template for detection of other clinically useful biomarkers. Our findings are part of growing evidence that confirms optical biosensors as viable substitutes for classical diagnostic methods, particularly in resource-limited settings where cost and infrastructure are significant issues.

Future avenues for the work include several promising ones. The integration of machine learning algorithms could introduce additional accuracy in detection based on sensor-to-sensor differences. Investigations of other biorecognition units such as aptamers or nanobodies could improve the stability of the sensor and reduce production costs. Above all, the transition from prototype laboratory instruments to clinic-grade instruments will require close collaboration among engineers, clinicians, and industry partners. At the intersection of photonics, nanotechnology, and precision medicine, this work is at the same time a valuable contribution to the literature and a stepping point towards realizing the full potential of optical biosensing in oncology.

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