

Design and Development of Portable IoT-Integrated Optical Fiber Biosensors for Biomarker Detection

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**Submitted in fulfilment of the requirements
for the degree of Master of Science
in Electrical and Computer Engineering**



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March 2026

Declaration

I hereby declare that this manuscript, entitled “Design and Development of Portable IoT-Integrated Optical Fiber Biosensors for Biomarker Detection”, is the result of my own work except for quotations and citations which have been duly acknowledged.

I also declare that, to the best of my knowledge and belief, it has not been previously or concurrently submitted, in whole or in part, for any other degree or diploma at Nazarbayev University or any other national or international institution.

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Date: March 19, 2026

Abstract

Traditional optical biosensors are still hindered by the large instrumentation, limited portability and low compatibility with digital health management. To overcome such limits, this study presents a portable smartphone-based plastic optical fiber biosensing platform compatible with biomarker detection in non-invasive biological fluids.

The study was centered on the fabrication, calibration and functionalization of POF sensors with different sensing geometries - D-shape, cylindrical and notch. The flashlight and camera served as the main source and detector in the proposed setup. Image acquisition was managed through a custom Android application. Functionalization comprised a multistep protocol including KOH treatment, silanization with APTMS followed by immobilization of an antibody. Progressive morphological changes were confirmed by AFM as indicative formation of functional sensing layers.

In the reported study, functionalized sensors were utilized to detect IL-8 in artificial saliva and VEGF in artificial tear. Compared with cylindrical and notch alternatives, the D-shaped geometry provided both lower LOD and more consistent responses in experiments. Protein detection by sensors, functionalized without antibody, demonstrated non-linear and unstable responses. In summary, this research results in an affordable POF sensing system which is portable and can detect biomarkers selectively from non-invasive fluids. The developed platform has a huge scope for home-clinic, telemedicine and IoT based healthcare in future.

Acknowledgements

I would like to express my sincere gratitude to my main supervisor Professor Daniele Tosi, as well as my co-supervisor Professor Gulsim Kulsharova for providing me with guidance and constructive feedback throughout this Master's program. I am particularly indebted to Professor Daniele Tosi for his lasting patience and keen advice. He was always open for research discussions, and easily helped make even the most challenging experiments achievable.

I owe a different but equally important debt to Professor Carlo Molardi and senior researcher Aliya Bekmurzayeva. They helped me to develop my ideas further and paid attention to details in my research which strengthened it.

I am also thankful to Kuanysh Seitkamal for his help with laboratory reagents. His technical expertise made experimental work smoother and led to reliable results.

A special thanks to my family and friends for being my moral support and creating an enabling environment. By working together, exchanging ideas and cheering each other, the thesis writing became so much more enjoyable.

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List of Abbreviations

AFM — Atomic force microscopy

API — Application programming interface

APTMS — Aminopropyltrimethoxysilane

BSA — Bovine serum albumin

COPD — Chronic obstructive pulmonary disease

GA — Glutaraldehyde

IL-8 — Interleukin-8

KIM-1 — Kidney injury molecule-1

KOH — Potassium hydroxide

LCN1 — Lipocalin-1

LOD — Limit of detection

mPEG — Methoxy polyethylene glycol

OFB / OFS — Optical fiber biosensor / Optical fiber sensor system

PBS — Phosphate-buffered saline

PMMA — Polymethyl methacrylate

POF / SOF — Plastic optical fiber / Silica optical fiber

RI / RIU — Refractive index / Refractive index unit

SPR-POF — Surface plasmon resonance plastic optical fiber

SERS — Surface-enhanced Raman scattering

VEGF — Vascular endothelial growth factor

aM, fM, pM, nM — Attomolar, Femtomolar, Picomolar, Nanomolar

pg/mL — Picograms per milliliter

fps / FPS — Frames per second

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Chapter 1 - Introduction

Biosensor technology is at the forefront of modern high-sensitivity biomarker detection. Since their invention in 1962 [1], a wide variety of biosensors have been developed. Optical fiber biosensors (OFBs) are a category of new-generation sensors combining a biological element with a physicochemical transducer for analyte recognition. The growth of digital healthcare suggests a need for integration of biosensing devices with the Internet of Things (IoT). This capability can decrease hospital admissions and regular outpatient visits [2]; thus, IoT-based biosensors may be used for remote, real-time, and reliable monitoring.

The ability to detect cancer biomarkers in an early stage is important for clinical aspects. However present biosensing technologies are constrained by cost as well as portability and accessibility. Usual optical biosensors are reliant on large instrumentation and controlled environments that prevent their use in point-of-care [3]. In contemporary healthcare, cloud data processing and live reports are indispensable [4], yet not all of the existing biosensing platforms offer these features. Even if optical fiber biosensors have promising sensitivity and specificity features, they are vulnerable to environmental noise and instability in dynamic conditions.

Recent research developed OFB technology through the use of polished fiber geometries. Geometries such as D-shaped, cylindrical, and notch structures increase the interaction between light and the analyte, improving detection sensitivity and offering diverse fabrication pathways [5]. However, a systematic comparison of these geometries for biomarker detection in non-invasive samples such as saliva and tears remains limited.

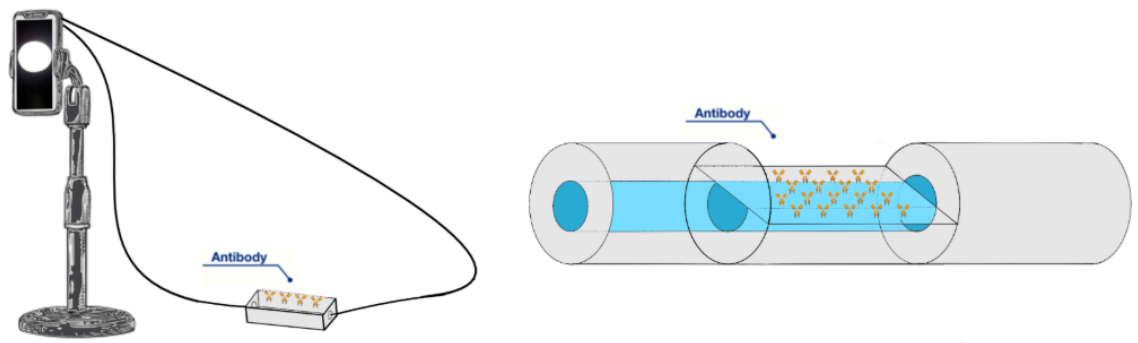


Figure 1-1: Smartphone-Integrated POF Biosensing Concept.

This research addresses the gap by designing and developing a portable IoT-integrated optical fiber biosensor that enables biomarker detection in non-invasive samples such as saliva and tears (Figure 1-1). The aim of this research is therefore fourfold: develop, calibrate, and compare plastic optical fiber with different geometries using a smartphone. Then, detect biomarkers in non-invasive fluids with functionalized biosensors. Thirdly, improve signal acquisition through optimized optical coupling and a dedicated app. And, lastly, analyze POF biosensor performance using signal processing. To implement these tasks four research questions were developed.

1. Which fiber geometry provides the best performance when compared using smartphones?
2. Can functionalized biosensors reliably detect biomarkers in non-invasive fluid samples?
3. How can signal acquisition be improved through optimized coupling and a custom app?
4. Is a POF biosensor sensitive enough to detect a low concentration biomarker in non-invasive fluid?

The structure of this thesis consists of four main chapters. In Chapter 2, the state of the art related to POF sensors is discussed including geometries, fabrication techniques and applications for detection of biological markers in non-invasive liquids like saliva and tears. It also reviews portable biosensing systems, such as those based on smartphones and surface functionalization methods for POF and PMMA materials. Chapter 3 presents the methodology followed for this study, including materials used, sensor preparation, calibration protocol development, surface functionalization, mobile application development and biomarker detection experiments along with data analysis methods. Chapter 4 discusses the experimental results including sensor performance, sensitivity and detection capabilities. Finally, Chapter 5 concludes the work by presenting the major findings of this thesis and suggesting future research lines.

The portable biosensor will be implemented in home clinics where traditional laboratory tests are not available. The suggested technology will eliminate the need for centralized laboratories, and people will be able to prevent illness in advance by detecting them on site. An easy-to-use biosensor has an edge in terms of affordability due to low-cost source-detectors.

Chapter 2 - Literature Review

2.1 Plastic optical fiber sensors

2.1.1 POF Geometries for biosensing

Optical fiber sensors were initially known as photonic technology-based instruments used in the telecommunications industry [1]. The working principle of biosensors lies in detecting changes in light parameters caused by interaction with external physical, chemical, or biological factors. The purpose of detection is to find the value of an external parameter by measuring and analyzing light modulations [6]. In case of biosensing applications, OFBs detect changes in the refractive index (RI) of the surrounding medium due to biomolecular interactions.

The material of OFB has a key role in measurements and efficiency tradeoff. Plastic (POF) and silica (SOF) optical fibers are the commonly used fiber types. The advantages of POFs are unique, such as flexibility, affordability, and biocompatibility, which makes it available to biomedical applications. In contrast, SOFs are brittle, rigid, and complex, restricting their application to portable and wearable biosensors [7]. As real-time applications require robustness, POFs are better interfacing with wearable and smartphone-based biosensors, than SOFs.

Different geometric configurations exist for plastic optic fiber. Some research investigated cut, bent and polished versions of this fiber [8]. For this research it is decided to try out polished modification of POF made by buffing machines, where D-shape, cylindrical, and notch structures are most popular. Such sensors are fabricated by etching a length of the fiber cladding away, thereby making the fiber core directly interact with the surrounding environment through a flat surface [9]. For D-shaped fiber semicore structure enables direct interaction with biomolecules in the ambient medium, improving detection efficiency. The main sensing principle of polished POFs relies on evanescent wave absorption and reflection,

resulting from binding of biomolecules on the exposed areas of the fiber core, and then modulates the effective RI, which in turn modulates the transmitted light intensity.

Similarly, cylindrical or unclad POFs expose the fiber core directly to the external medium, enhancing light–analyte coupling [5]. Here, Rayleigh scattering is the main source of attenuation, where light gets scattered backward and sideways along the fiber. Lastly, notched POF is made by periodic cuts and grooves along the length of cladding. It disturbs the guided modes and creates controlled light leakage with scattering. Thus, it leads to high response to refractive index or liquid-level changes [10]. Even if major studies evaluate different polished POF geometries for biomarker detection, there is still lack of research in portable and smartphone-integrated systems.

2.1.2 D-shaped POF sensors

For most POF oriented papers published, D-shaped optical fiber sensors are widely used because their geometry increases the interaction between guided light and the surrounding environment. In a conventional optical fiber the light is confined inside the core and cladding, which limits interaction with external materials [11]. When some of the cladding is removed to create a D-shape structure, thus decreasing the effective RI, which can lead broader guided optical mode. This phenomenon is known as mode broadening [12]. As a result, the evanescent field extends outside the fiber and interacts with materials placed on the polished flat surface. These materials may include sensing polymers or nanomaterials such as VO_2 , CNTs or rGO– TiO_2 , which allows the fiber to be used in chemical sensing and nonlinear optical applications [13, 14].

The penetration depth of the evanescent wave in plastic optical fibers is relatively small, typically ranging from several tens to a few hundred nanometers [14, 15]. However, the overall light–matter interaction can be improved by increasing the interaction length. In

D-shaped fibers the sensing region can extend from micrometer scale to centimeter scale, which enhances sensitivity [12]. The performance of the sensor strongly depends on geometric parameters such as polishing depth, coating thickness and surface roughness. Increasing the polishing depth increases the interaction area between the evanescent field and the analyte, which improves sensitivity [16]. However, excessive polishing may weaken the mechanical stability of the fiber.

2.1.3 Fabrication and Characteristics of D-Shaped POF

D-shaped fibers are often used for surface refractive index sensing. When molecules such as 4-chlorophenol are adsorbed on the sensing layer, the refractive index near the fiber surface changes and modifies the evanescent field [14, 16]. The polished sensing part of surface plasmon resonance sensors is coated with a thin gold layer - it causes resonance wavelength shifts that indicate refractive index changes.

Evanescent field absorption, surface roughness or particles, and fiber geometry influenced respectively on transmission losses, scattering, and intrinsic insertion loss in D-shaped fibers [14, 15]. The output intensity follows the Lambert–Beer relation [14],

$$I_{out} = I_{in} \exp(-\epsilon(n_x)L) \quad (2.1)$$

where $\epsilon(n_x)$ - attenuation, L - sensing length. The Lambert–Beer relation is used to calculate the sensor response. In the equation the transmitted intensity exponentially decays when attenuation and sensing length increase. Binding of molecules on the fiber surface occurs as the biomarker accumulates, it alters the local refractive index and leads to greater attenuation. Decrease in transmitted intensity is caused, which matches experimental trends observed. Larger interaction regions such as D-shaped fibers have better effective attenuation and thus higher sensitivity. Saturation effects happen at higher concentrations because of the

diminishing returns on attenuation, where more increases in attenuation will not result in much change to output intensity.

Fabrication of D-shaped fibers typically involves controlled removal of the cladding using grinding or polishing techniques [11]. The fiber coating is first stripped and the fiber is fixed in a V-grooved block [12, 15]. Afterwards the material is gradually removed using diamond grinding films of varying grit sizes. Specialized grinding machines can polish the fiber at controlled speeds around 80 rpm, while simple setups may use manual polishing with fine abrasive films [13-15]. During fabrication the polishing depth is carefully monitored, often by measuring insertion loss or using optical microscopy to determine the core-to-surface distance. After polishing the fiber surface is cleaned before deposition of sensing coatings such as gold films, nanoparticles or molecularly imprinted polymers [13].

2.2 Biomarker Detection in Non-Invasive Fluids

2.2.1 Salivary and tear cancer biomarkers

Saliva is a clinically relevant body fluid used for liquid biopsy and developed as a non-invasive diagnostic fluid [17]. Saliva collection is easy, safe and does not cause discomfort to the patients as compared to blood sampling [18]. Blood-borne biomolecules may leak into the saliva by passive diffusion, active transport and ultrafiltration of saliva glands [19]. Due to this permeability, almost 40% of plasma-circulating cancer biomarkers are detectable in saliva. However, analyte concentrations in saliva are generally much lower, on the order of 100–1000 times smaller than those for blood. Saliva contains over 2000 proteins, such as cytokines, enzymes and microRNAs. However, despite the relatively small amount of saliva available for testing it provides a useful diagnostic medium in disease diagnosis [17].

Inflammatory cytokines, including interleukin IL-6, IL-8 and tumor necrosis factor alpha (TNF- α), represent well-studied biomarkers among these proteins [17-19]. Interleukin-8 (IL-8) is a pro-inflammatory cytokine, which recruits inflammatory cells to sites of inflammation. In particular, because of stress or inflammation IL-8 biomarker undergoes massive upregulation, even if it is expressed at low baseline levels. IL-8 is reported to be increased in patients with oral squamous cell carcinoma, and also upregulated in periodontal inflammation and chronic obstructive pulmonary disease (COPD).

Tear fluid is another non-invasive diagnostic medium whose composition varies in disease [20]. It carries proteins like lipocalin-1 and vascular endothelial growth factor (VEGF). In healthy patients, VEGF biomarker is found at levels of 40–60 pg/mL in plasma, but this value rises significantly in pathological conditions [20, 21]. Diseases such as age-related macular degeneration and diabetic retinopathy involve angiogenesis, where VEGF is the main biomarker. Traditional ophthalmic diagnostics usually are based on expensive tools such as optical coherence tomography and fluorescence angiography.

2.2.3 Optical Fiber Biosensors for Biofluid Analysis

Blood is the main gold standard for biomarker diagnostics, but it is not suitable for low-cost optical interrogation using smartphones, microcontrollers, and basic optical spectrum analyzers. The reasons are in blood's opacity, strong light absorption, and complex composition. Non-invasive biofluids such as sweat, urine, tears, and saliva offer practical advantages. They are easier to collect, simple in composition, and optically more transparent. These prove their suitability for biosensing with POF. In couple years, researchers could make remarkable progress in salivary biomarkers detection using POF sensors functionalized with antibodies or aptamers. One of such papers demonstrated an SPR-POF sensor that was used to detect MMP-8 in saliva and had a limit of detection of 225 pM [22]. Similarly, a cortisol

biosensor built on SPR-POF technology underscored the promise of optical fiber platforms for monitoring stress-related biomarkers [9].

Tears have also received significant interest as a target biofluid for optical fiber biosensing. Discerning LCN1 in tear fluid was reported on a laser spliced ball resonator biosensor which displayed an impressive limit of detection (LOD) of 240 ag/mL and even incorporated into wearable goggle systems for free-end, real-time monitoring [23]. Another strategy utilized a semi-distributed interferometric fiber sensor for simultaneous detection of LCN1 and VEGF, with the corresponding limits of detection being 5.98 ng/mL and 26.6 fg/mL respectively — evidencing that label-free multiple sensing of biomarkers is possible directly in artificial tear flow [24]. In the case of metabolic monitoring, a self-powered optical fiber photoelectrochemical biosensor coated with glucose oxidase specifically identified tear glucose at a LOD of 4.1 nM and sub-second response times, signifying potential for mobile, energy-autonomous operation [25]. In addition to these, other biofluids are also gaining research interest. For example, a wearable Janus textile in conjunction with microstructured optical fiber achieved SERS-based detection of lactate and urea in sweat below physiological concentration levels [26], and a semi-distributed interferometer biosensor measured the kidney injury biomarker KIM-1 in urine with an ultralow LOD of 13.6 aM, exhibiting high sensitivity and specificity in clinical samples [27]. All of these investigations support the non-invasive fluids' great potential for portable optical fiber biosensing.

Besides these benefits, there are still a lot of difficulties. Sensors with higher sensitivity and calibration stability are required due to non-invasive fluids. Usually they have lower biomarker concentrations than blood. Environmental factors in the form of evaporation or contamination may also affect reproducibility of sensors, whereas matrix variation such as pH, ionic strength, and enzyme levels in saliva or tears may weaken signal stability [28]. Moreover, the immediate practicality of proof-of-concept demonstrations either at home or in

the field is limited by the fact that most remain laboratory-based optical schemes and not completely portable IoT-integrated platforms. This disconnect calls for comparative studies that fill the gap between the non-invasive biomarker detection, functionalization, and geometrical design.

2.3 Portable biosensing systems

2.3.1 Smartphone integrated sensors

By coupling optical fiber sensors into smartphones, portable sensing technologies have opened a new horizon, leading to on-site and real-time control in biomedicine. OFB technologies have benefited greatly from smartphone-based sensing that exploits built-in cameras and flashlights [7], [29]. The suggested IoT structure will always combine the latest computational capabilities, interconnectedness and user-friendly nature of smartphones with the advantages of optical fiber sensors as we see on Figure 2-1(A). An average OFS system integrated with a smartphone consists of four components including LED, optical fiber, sensing structure, and a photodetector.

In modern IT medicine gadget-based sensing systems have been employed in many fields due to their flexibility. In chemical biosensing, IoT sensors have been used in the detection of chemical analytes such as pH, heavy metals and glucose concentrations. To illustrate, a phone-integrated sensor device proved high capability in detecting mercury ions in river water samples, which may be a solution in polluting industries [30]. Another case with IoT sensors demonstrates the importance of temperature and shape measurement. One example was the formation of an IoT-based shape sensor for in situ monitoring of infrastructural deformations, which can solve engineering problems [31]. In addition, smartphone-assisted OFBs encourage remote monitoring with cloud- based IoT frameworks [4]. Gadgets make it possible to perform data analysis in real time via image-processing and

AI-based detection algorithms. The fusion of biosensors with mobile applications can enable patients to periodically transmit health data to medical workers, benefiting telemedicine applications.

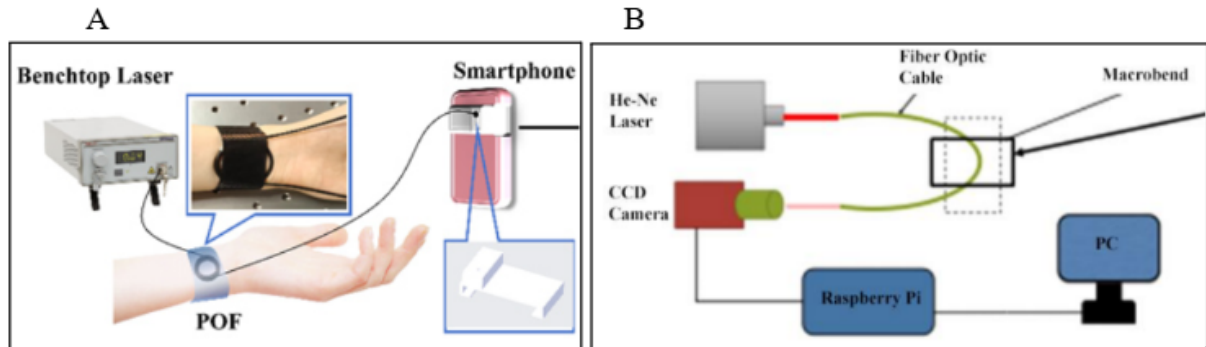


Figure 2-1: A. Smartphone-Integrated POF Speckle Sensor [29]. B. Microcontroller-Integrated POF Sensor Setup [32].

2.3.2 Microcontroller integrated sensors

Recent work demonstrated that microcontrollers such as the Raspberry Pi can be directly interfaced with plastic optical fiber sensors to build compact and affordable interrogation systems. Marak et al. developed an intensity-based macro-bend POF sensor where the light transmitted through a bent fiber was captured by a CCD and analyzed using Python scripts on a Raspberry Pi, based on Figure 2-1(B), proving effective for real-time refractive index monitoring of liquids with a simple and reproducible setup [32]. One research demonstrated a Raspberry Pi-based miniaturized spectrometer through which complete spectral data could be extracted from fiber sensors, enabling more advanced analysis such as wavelength shift monitoring and fluorescence signals [33]. These examples illustrate that the Raspberry Pi can be utilized not only as a cheap data logger but also as a versatile signal processor, with the freedom in measurement methods. As a result, the creation of an integrated compact biosensing platform using optimised POF geometries, mobile signal

acquisition and IoT connectivity specifically is still a bottleneck towards their application in practical scenarios.

2.3.3 RGB-based Image Analysis

RGB measurements are widely used as a simple method to convert visual color changes into digital quantitative information [34]. In many sensing systems RGB data acts as a link between physical or chemical response and numeric analysis. Generally, imaging is done with smartphone cameras incorporating CMOS sensors [35]. Some commonly used equipment among studies were Redmi K50, iPhone 7 Plus and iPhone 16 Pro Max [34-36]. The acquired images are analyzed in software such as ImageJ to extract Red (R), Green (G) and Blue (B) intensity values. Experiments are typically conducted inside controlled imaging boxes equipped with stationary LED lighting where chromatic noise and environmental interference are decoupled from the measurement to improve measurement reproducibility [36].

RGB values are often expressed as a single parameter for total intensity of color. One study, for example, took a weighted intensity value as $I = 0.3\text{Red} + 0.59\text{Green} + 0.11\text{Blue}$ [34]. This converts three-channel RGB data into a single-coordinate for linear regression analysis in terms of one channel. For some sensing applications one channel appears to be more dominant in the response. The green channel gave the best linearity and sensitivity in detecting vitamin B6 compared to red and blue channels [36].

The RGB sensing technique is mostly used in colorimetric assays, when visible color change occurs after chemical reaction. Such color transitions can be green-to-colorless types for uric acid detection or orange-to-yellow changes for vitamin B6 analysis [36]. These variations in color are detected with the smartphone camera and distinct RGB values extracted

from them are regressed to obtain concentration of the analyte of interest. Cheers in environmental monitoring (where multiple bands are used as input or processed the images) has been undertaken through similar RGB analysis that targets spectral, as well as texture features based on R, G and B bands.

More advanced research directly applies machine learning models like Random Forest or Gradient Boosted Decision Trees to RGB features for prediction tasks [35]. Yet, these methods are primarily applicable for complex datasets while simpler sensing systems use direct RGB intensity analysis.

2.4 Surface Functionalization of POF and PMMA surfaces

2.4.1 Chemical Activation

Surface functionalization represents a crucial step in optical fiber biosensors since it allows their surface to specifically interact with biological elements [37]. Most biosensing systems usually employed chemical modification of PMMA or plastic optical fiber to introduce reactive groups that could bind specific biological recognition elements [38, 39]. After biomolecules attach to the surface the refractive index near the fiber changes, which alters the optical signal of the sensor. Therefore surface chemistry strongly affects sensitivity, stability and repeatability of measurements.

Different chemical strategies are used to activate and modify POF or PMMA surfaces [37, 40]. In many cases the first step is surface hydrolysis to create reactive functional groups. Treatments with acids or bases can form carboxyl groups on the PMMA surface [37, 38]. These groups can then be converted into other functional groups or use for further linkage. Carboxyl groups, for example, can be converted into amine groups through the use of hexamethylene diamine (HMDA) after which glutaraldehyde can serve as a cross-linker to

provide immobilization of antibodies via Schiff's base bonding [37]. This modification allows antibody–antigen recognition for biosensing applications.

2.4.2 Silanization, Polymer scaffolds, and Metal coatings

Other approaches rely on silanization chemistry to introduce reactive molecules on the fiber surface. APTES and GPTMS silane compounds in order to increase number of reactions form amine groups and attach to active polymer surfaces [38, 39]. Afterwards attachment of nanoparticles and bioimmobilization may use these groups. According to the paper [38], after EDC/NHS activation using electrostatic interaction gold nanoparticles can be attached to silanized surfaces. In some designs found that sensitivity to specific analytes can be improved by chemically reactive coating. In such paper the silica sol-gel was added as this coating.

Polymer scaffolds are also used to increase the surface area for biomolecule attachment. One example is the grafting of a carboxymethyl dextran (CMD) layer on the modified PMMA surface [39]. This polymer matrix creates a 3D environment which supports a lot of immobilized antibodies. In such systems EDC/NHS chemistry is often used to form stable amide bonds between the polymer scaffold and the antibodies [39]. This approach can improve binding capacity and also sensor response. Because of this, it is used quite often.

Another way of functionalization is metal-coated fibers. In many surface plasmon resonance plastic optical fiber (SPR-POF) sensors, a thin gold layer is deposited on the fiber surface [40, 41]. This metallic coating helps for further modification. On the gold surface, self-assembled monolayer (SAM) can be formed, for example alpha-lipoic acid. This layer gives terminal carboxyl groups on surface. After that, EDC/NHS chemistry is usually used to activate them for antibody immobilization [40]. Then, after binding step, blocking agents like

ethanolamine or bovine serum albumin (BSA) are added. This is important to reduce nonspecific adsorption and to make sensing layer more stable [37, 40].

Even if many methods try to fix recognition elements on fiber surface, they are not same - structure is different. Also complexity is different and sometimes confusing. Some works just use glutaraldehyde, it is simple cross-linker, it is easy way but not always good result. Other protocols go more complicated, like polymer scaffold or silane chemistry, to make binding more efficient [37–39]. EDC/NHS methods are usually better, at least in many cases. They give stronger attachment and are more controlled, also more stable on surface [38, 39]. But it still depends on conditions. However, when more layers are added, fabrication is harder and reproducibility goes down, which is not very practical sometimes.

As a result, surface chemistry is still a critical factor in OFB performance. Even if many protocols improve sensitivity and specificity, reproducibility and long-term stability of biosensors still remain as a question. It is important to optimize functionalization strategies, especially protocols for portable and smartphone-based biosensing systems.

Chapter 3 - Methodology

The methodology is divided into five key stages: materials and experimental setup, sensor fabrication, calibration, surface functionalization, biomarker detection, and data analysis. I will also mention the development of the mobile application.

3.1 Materials and experimental setup

The materials were provided from the Laboratory of biosensors and bioinstruments. For fabrication stage plastic optical fiber with a core/cladding diameter of 980/1000 μm and an abrasive polishing tool are used (Figure 3-1(a, b)). For smartphone positional stabilization and perfect coupling the holder and the connector were modeled in Autodesk Fusion 360 (Figure 3-2) and printed on a 3D Bambolab printer using black PLA plastic (Figure 3-1(d)). Measurements of image capturing were conducted with a Samsung A16 smartphone, which worked as light source and detector (Figure 3-1(c)).



Figure 3-1: Materials used for fabrication and calibration stages.

To reduce the use of protein solutions in sensing experiments, a customized cuvette was fabricated through 3D printing (Figure 3-4(B, C)). Given that biomarker antibodies and protein solutions are quite costly, the design sought to reduce the amount of sample volume while still allowing complete immersion of the sensing region of the plastic optical fiber. POF are considerably thicker than silica optical fibers, which means the sensing chamber needed for them is slightly wider. The cuvette in which the experiment was performed had internal dimensions of $2\text{ cm} \times 1\text{ cm} \times 0.5$, giving it a total volume 1 cm^3 ($\approx 1\text{ mL}$). Second, this volume allows the entire sensing region of the fiber to be completely in contact with the analyte solution but still limited reagent usage.

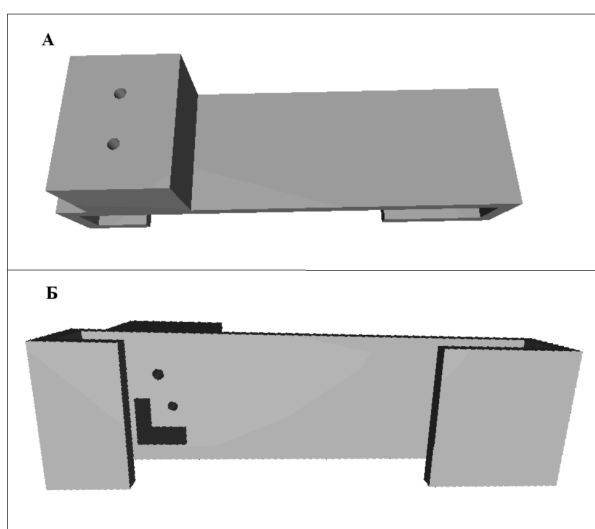


Figure 3-2: 3D-Printed Smartphone Case Design

The optical fiber sensors were surface functionalized with laboratory equipment consisting of a Qsonica digital ultrasonic cleaner used for sonication, a BioForce Nano UV-Ozone cleaner to activate surfaces, and the Thermo Scientific Lab Rotator 2314 employed in incubation and mixing steps (Figure 3-3).

The reagents used for the functionalization of fibers were:

- Potassium hydroxide solution (KOH)
- Deionized (DI) water
- APTMS solution prepared in isopropanol
- Glutaraldehyde (GA) solution
- IL-8 or VEGF antibodies
- Methoxy polyethylene glycol (mPEG) or bovine serum albumin (BSA) solution
- Phosphate-buffered saline (PBS) solution



a) Qsonica digital ultrasonic cleaner



b) BioForce Nano UV-Ozone cleaner



c) Thermo Scientific Lab Rotator 2314

Figure 3-3: *Equipment used for functionalization stage.*

The fabrication, calibration, sensing measurements and surface functionalization of the POF biosensors were performed using these individual materials and reagents.

To integrate the sensor into an accessible and mobile setup, a smartphone-based experimental configuration is used (Figure 3-4). One end of the fiber is aligned with the flashlight of a Samsung A16 smartphone, while the other end is positioned over the smartphone camera. To maintain stable coupling, to block ambient light interference, and to fix image capture a black holder and case were 3D printed (Figure 3-4(A)). This design simplifies the experimental setup and promotes portability and cost-effectiveness of the system.

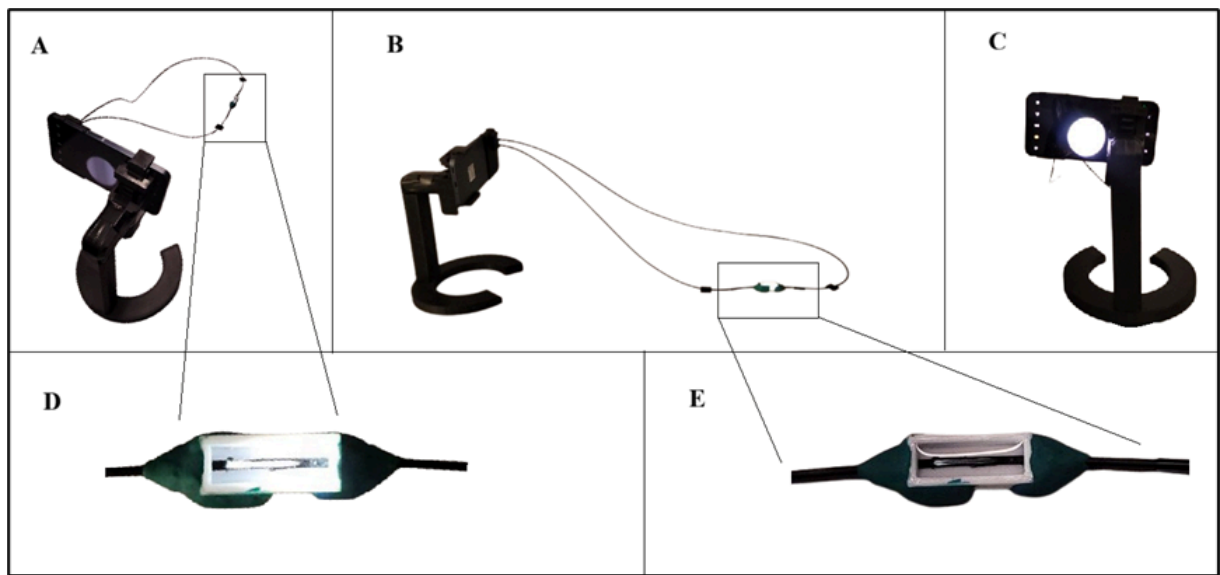


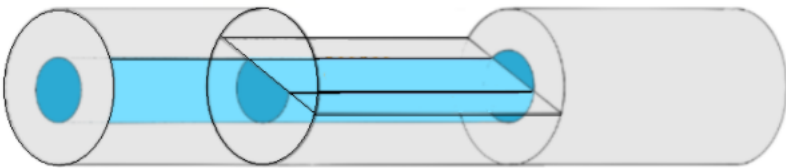
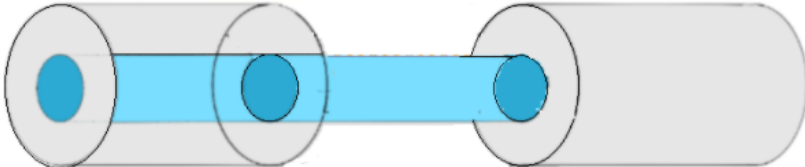
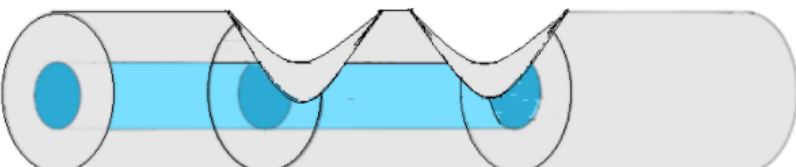
Figure 3-4: Experimental Setup.

3.2 Sensor fabrication

Plastic optical fiber with a core/cladding diameter of 980/1000 μm is used for sensor fabrication. The main pros of POFs are inherent mechanical flexibility, chemical compatibility, and potential for surface modification. The sensing section of the fiber is modified to enhance its interaction with the surrounding environment. D-shaping, Cylindrical shape, and Notch is performed on a 1–3 cm middle segment using a polishing engraving machine. The possible structures of different geometrical configurations are shown on **Table 3-1**. A D-shaped plastic optical fiber is fabricated by mechanical polishing or grinding of one

side of the fiber using a rotary polishing tool until 45% to 60% of the core is removed (Figure 3-1(A)). Preserving the mechanical stability of the fiber is essential. The resulting planar region generates a guided optical mode as well as a sensing interface that allows binding of biomolecules or sensing coatings.

Table 3-1: *Different surface geometrical modifications of POF.*

Geometry	Structure
D-shape sensor	
Cylindrical sensor	
Notch sensor	

A fully cladding-removal cylindrical POF sensor is created by polishing the protective cladding with an abrasive along the sensing length. This technique allows the circular core surface to be directly exposed to the surrounding medium throughout the full circumferential length of fiber over sensing length of 1-2 cm. The core is fully exposed to light-analyte interaction, as both the analyte and light travel around the entire cylindrical surface, improving evanescent-field coupling.

The notched plastic optical fibers are fabricated by introducing localized grooves or cuts along the fiber cladding using micro-milling tools. Each notch encroaches on the cladding and disrupts guided optical modes, leading to controlled leakage of light at the notch positions. The width and depth of each notch as well as the spacings between adjacent notches determine sensor sensitivity and scattering behaviour.

3.3 Calibration Procedures

Calibration of the fabricated sensors is a crucial process to ensure their proper operation and sensitivity to changes in refractive index (RI). Choosing only those sensors with clear linear behaviour in response to changing refractive index over sucrose concentrations, these were subsequently used throughout the study. Sensors with high performance and reproducible linear calibration curves were selected for subsequent biological detection experiments. This selection process ensures only reliable sensors will be used in biomarker detection and possible cancer diagnosis. All measurements were made with a camera controlled through a custom app in Android Studio (Figure 3-4(D)). The flashlight and camera act as the main light source and detector of signals. In assembly, the fiber was glued into a 3D-printed connector to hold at the focal point in the cellphone camera frame. In vivo conditions the connector avoided micro-shift which contributed to $\sim 1\text{--}1.6\%$ baseline noise reduction in blank tests for air, water and tear. The experiments took place in dark environments to avoid optical noise or external light that could have interfered with the result. The sensor in air was recorded first, and this served as the calibration baseline for sensor exposures.

Measurements under each of the refractive index conditions (air and sucrose solutions) were taken over 5 minutes at intervals of 10 seconds. Images were taken at a burst rate of 3 frames/s, for about a total of 90 images per value of RI. To calibrate all sensors we used

10-18% sucrose solutions. The solutions' refractive indices are 1.35 to 1.39 respectively, it is necessary to evaluate the sensor's response under lab RI change conditions.

In order to check the correctness of experimental setup, its stability and reliability, control experiments with air, tear and water were done for 2 hours with bare POF. DI water was used as control to provide a signal baseline. Liquid medium media with different refractive index and chemical properties were further examined by the sensor response in ethanol solutions at 10%-18% concentration. We show here how the signals from these sensors are stable and can be used as fingerprint to distinguish between solutions by their optical behaviors.

3.4 Surface Functionalization

Multi-step functionalization on the fiber was done to achieve specificity in biomarker detection [13]. The principle in functionalization is called "key-lock". In order to bind specific bioreceptors such as antibodies or aptamers, the sensor surface is chemically modified. The "locks" are these bioreceptors - they selectively join to their "key" biomarkers. The reason is to make only the target molecules produce a detectable optical signal.

Firstly, a pre-cleaning stage is carried out: the fiber is placed in a KOH ultrasonic bath for 20 min to remove organic contaminants and increase surface reactivity in the sensing region (Figure 3-3(a)). This is followed by UV-ozone treatment for 30 min, introducing hydroxyl groups that activate the surface for subsequent chemical modifications (Figure 3-3(b)). Next, silanization is performed using APTMS in isopropanol for 20 min, followed by oven heating at 70 °C for 1 hour, to introduce reactive amine groups for further biochemical tethering. The surface is then activated using glutaraldehyde to create aldehyde groups, enabling covalent attachment of antibodies. Target-specific antibodies (IL8 or VEGF) are then immobilized via natural adsorption and allowed to stand overnight to be stably attached onto

the sensor surface (Figure 3-3(c)). Such a multi-step process is necessary in order to enable detection of target biomarkers. In order to block unreacted sites and reduce non-specific binding BSA or mPEG is added. It produces a specific and accurate biosensor. The functionalization process lasts for 8 hours.

Each step of the functionalization process was characterized by atomic force microscopy (AFM) to examine the surface morphology of the POF sensor. As shown on Figure 3-5, the topographic surface morphology of PMMA was significantly altered following KOH treatment. It transformed from a relatively smooth and featureless surface into an extremely rugged nano-textured surface with irregular protrusions driven by hydrolysis to produce reactive functional groups. Further processing via UV–ozone treatment resulted in a more homogeneous morphology at the expense of nanoscale features, indicating enhanced oxidation and an increase in oxygen-containing surface groups. After the deposition of APTMS, the surface displays granular nanostructures and small clusters (tens of nm) in size, which indicates that a silane layer with amine functional groups has been developed as anchoring points for further chemical reactions.

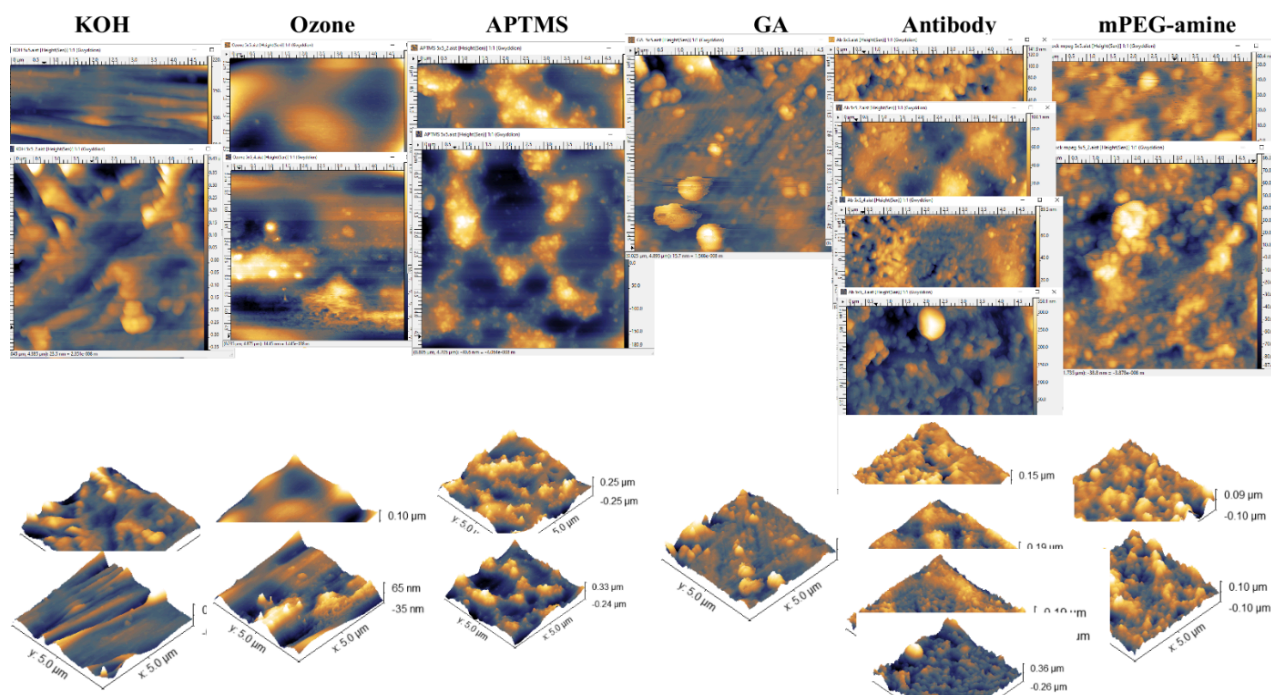


Figure 3-5: AFM Analysis of Functionalization Stages

Morphological results show that after glutaraldehyde crosslinking, elongated and comb-like structures are formed on the surface, indicating the localized formation of aldehyde-functionalized linkers on the silanized surface. Antibodies immobilization leads to more heterogeneous topography with several nanoscale protrusions due to the protein molecules mechanically adsorbed at the surface. Ultimately, as the mPEG-amine blocking step yields a more homogenous and flatter morphology, this indicates that the PEG chains cover remaining free active sites which decrease nonspecific adsorption. The stepwise formation of functionalizing sensing surfaces is also evidenced by progressive changes in AFM images for such stacked structures confirming the emergence of each layer with respect to height.

3.5 Mobile app development.

A stable smartphone–fiber setup was used where the smartphone flash acted as the light source and the rear camera recorded the transmitted intensity. A custom Android Studio application based on the Camera2 API controlled all imaging parameters (Figure 3-6). Full manual mode was applied:

- `SENSOR_SENSITIVITY = 800`
- `SENSOR_EXPOSURE_TIME = 20,000  $\mu$ s`
- `FLASH_MODE = TORCH`
- `CONTROL_AE_MODE = OFF`
- `CONTROL_AWB_MODE = OFF`
- `NOISE_REDUCTION_MODE = OFF`
- `EDGE_MODE = OFF`

Calibration followed a consistent routine: air baseline followed by six RI steps, each recorded for ~5 minutes. Measurements were performed in both ambient light and inside a dark enclosure; dark-box measurements reduced fluctuation by 35–50% and improved linearity.

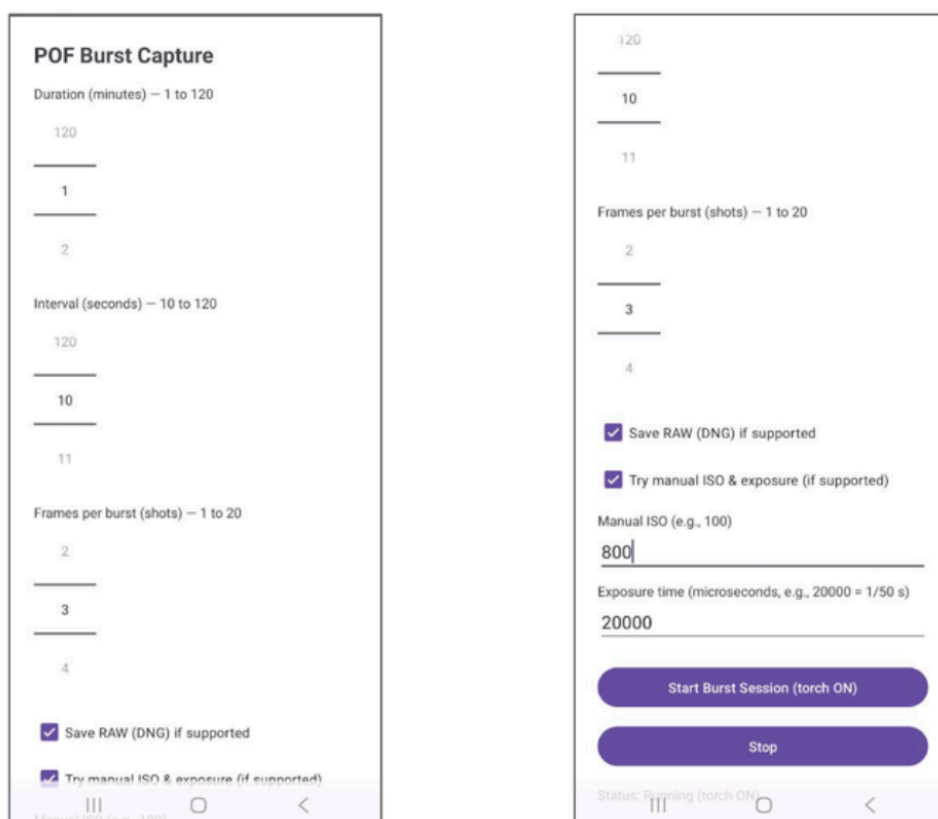


Figure 3-6: Mobile application for capturing images.

3.6 Biomarker Detection Experiments

The prelast step is detection of biomarkers in fluid by functionalized biosensor. Artificial tear or saliva solutions containing different concentrations of biomarkers such as VEGF or IL-8 proteins are prepared. Artificial tear fluid is prepared from SYSTANE eye drops with added glucose, nitrites, and bovine serum albumin in distilled water. To adjust pH level to 8, Trisma base and Trisma hydrochloride were added to the solution. To evaluate the sensitivity and detection limits of the sensor, solutions with a wide dynamic range, from 100 nM to 1 mM, are typically prepared for measuring biomarkers. A total of twelve analyte concentrations were tested for each three sensors. The concentrations, prepared in artificial saliva, covered the range from 100 nM to 1 aM; artificial saliva without protein was included as negative control. The concentrations were arranged into three measurement sets:

1. Artificial saliva, 1 aM, 1 fM, 1 pM, 1 nM
2. Artificial saliva, 10 aM, 10 fM, 10 pM, 10 nM
3. Artificial saliva, 100 aM, 100 fM, 100 pM, 100 nM.

Twelve concentrations (C12–C1) were measured, where C12 corresponds to the highest concentration (100 nM), and C1 indicates artificial saliva mixed with IL-8 protein. All solutions were made in a working volume of 1 mL. The necessary volumes were determined using the standard dilution equation:

$$C_1 V_1 = C_2 V_2 \quad (3.1)$$

where C1 and V1 indicate concentration and volume of stock solution respectively; C2 and V2 refer to the concentration and volume of diluted solution.

The highest concentration (100 nM) of IL-8 was subsequently prepared by diluting 1.2 μ L of the work solution in 998.8 μ L of artificial saliva. Then, serial dilutions were performed and to obtain subsequent concentrations, 100 μ L of the previous solution was added with 900 μ L of artificial saliva. After preparation, each solution was mixed thoroughly for at least 30 s to ensure proper homogenization prior to measurements.

It was maintained under lab conditions the sensor performance was the best while detection of different concentrations of biomarker [11]. We immerse the functionalized fiber in each biomarker solution for at least 1 hour to ensure sufficient time for antigen-antibody binding. The measurements were made in a fashion analogous to calibration, except longer acquisition. Burst photos were taken with 3 FPS for each concentration in IL-8 experiments resulting in 1080 images. Earlier, videos (30 FPS, ISO 800, 1 hr) were employed for VEGF quantification producing 324 000 frames/concentration.

3.7 Data Analysis

Image processing and signal extraction were done in MATLAB. First, each image was cropped to a fixed region of interest, and then the RGB channels were separated. The main parameter extracted was the mean intensity of the red, green and blue channels. For all experiments, an intensity-integration method was also used, where pixel values above certain thresholds (135 and 250) were summed to create an “outer” and “inner” integral, and the difference between them was taken as the signal. Two processing methods were compared. The equations used to calculate the intensity of the signal:

$$I_{outer} = \sum_{(x,y) \in ROI} I(x,y) 1_{\{I(x,y) > T_1\}} \quad (3.2)$$

$$I_{outer} = \sum_{(x,y) \in ROI} I(x,y) 1_{\{I(x,y) > T_2\}} \quad (3.3)$$

$$SI = I_{outer} - I_{inner} \quad (3.4)$$

Where, SI is intensity of the signal, $I(x,y)$ is pixel intensity at position (x,y) in the selected ROI, T_1 and T_2 are thresholds. In our case $T_1 = 135$ and $T_2 = 250$, where always $T_2 > T_1$.

Butterworth low-pass filtering (4th order, cutoff relative to sampling frequency) was used mainly for videos. It removed high-frequency noise but slightly shifted the signal and made transitions less sharp. Resampling was used for burst photos to correct the non-uniform timing between images. The equations for resampling and windowing are respectively:

$$\hat{y}(t) = \text{interp}(y(t)) \quad (3.5)$$

$$\bar{y}(t) = \frac{1}{W} \sum_{m=1}^W \hat{y}(t_m) \quad (3.6)$$

Where, $y(t)$ is the extracted signal value from image at time, interp is the MATLAB function for interpolation, $\bar{y}(t)$ is the smoothed resampled signal, W is the window size.

MATLAB's interpolation function and a moving-average window (around 20 points) produced smoother curves while keeping the original step shape between concentrations.

The limit of detection (LOD) will be determined to measure the lowest concentration of analyte that is distinguishable from baseline noise of the sensor. LOD equation used in this work:

$$ax^2 + bx + c = y_{blank} + 3\sigma_{max} \quad (3.7)$$

$$x = \log_{10}(LOD) \quad (3.8)$$

$$LOD = 10^x \quad (3.9)$$

where σ is the standard deviation of baseline signal and S is sensitivity.

The σ is calculated from the normalized intensity fluctuations of control samples such as an artificial saliva or tear. S is defined as the slope of the calibration curve, which can be obtained from the linear regression analysis correlating normalized intensity vs. concentration. y_{blank} is the average signal of the blank. This technique provides a quantitative assessment of the sensor performance on detecting low concentrations of a target biomolecule.

To normalize the RI sensing and biosensing results these respective equations were implemented:

$$Response (\%) = \frac{I_{blank} - I_i}{I_{blank}} \times 100\% \quad (3.10)$$

$$Response (\%) = \frac{I_i - I_{air}}{I_{air}} \times 100\% \quad (3.11)$$

Then, the average intensity at each refractive index point was fitted with a linear model via polyfit function in MATLAB for calibration and this slope of this fitting line was considered as the sensitivity. Finally, measurements performed in light and dark conditions were compared with each other showing lower variability and drift in the absence of light.

Chapter 4 - Results and Discussion

Twenty sensors were fabricated and tested for detection of IL-8 and VEGF, but four sensors with distinct geometries were selected based on their superior performance in terms of sensitivity and LOD. These sensors, after functionalization, presented the most reliable and consistent responses, avoiding outliers as representative examples for comparison.

The experiment was carried out using different sensing lengths of the polished POF fiber, ranging from 0.5 cm to 2 cm. From the calibration result of the D-shaped sensor, the 1.2 cm sensing length showed more linear and higher sensitive characteristics. Conversely, the short and long sensing lengths (0.5 cm and 2 cm) acted exceptionally, exhibiting poor linearity as outliers. This may be explained by insufficient or excessive evanescent-field interaction, or other reasons such as optical losses caused by light leakage into air or incomplete reflection within the surrounding medium.

Figure 4-1 demonstrates the RI changes in the form of images of the D-shape sensor after calibration in sucrose solutions. The RGB images exhibit a uniform and circular bright illumination area for the different refractive index values. Dimensionality changes in intensity distribution observed along with this increase. The global shape of the light spot is stable, small changes in brightness and edge sharpness can be seen, especially in the surrounding halo region — a signature of how the light propagates through the fiber. These differences are confirmed by the corresponding blue-channel intensity maps. Also we can observe a well-defined high-intensity core and smooth gradients towards the edges of the 0.25 mm footprint. In result, structural deformation plays little role in sensor response, it rather dominates as an intensity redistribution due to hot-spot localization.

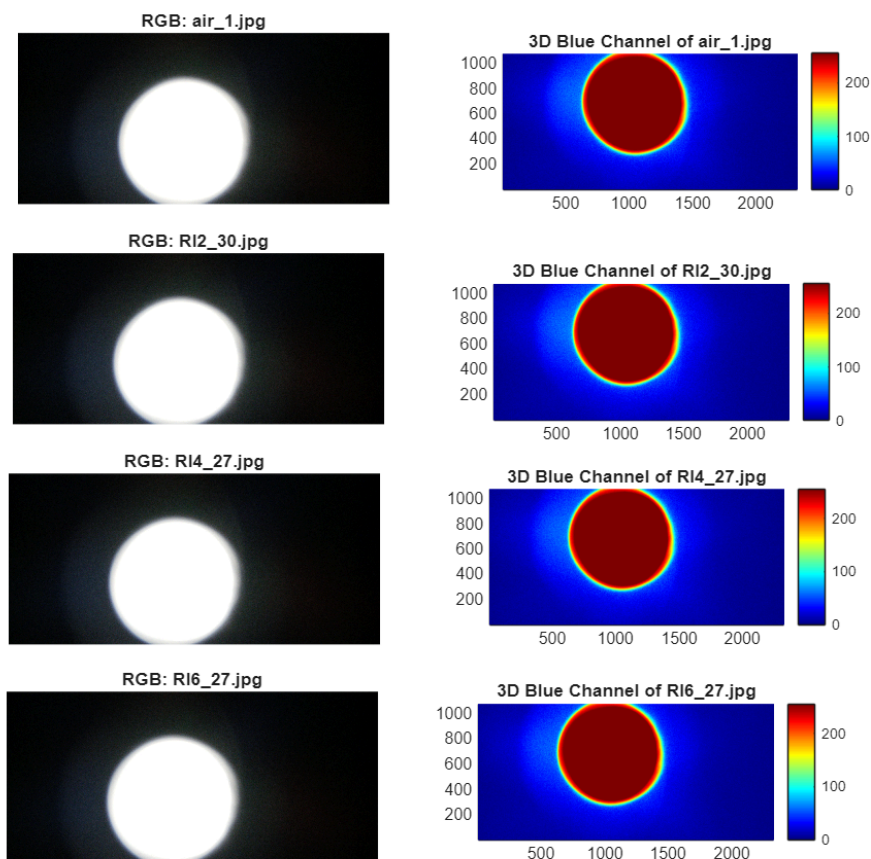


Figure 4-1: *RI response images in usual and RGB form.*

The calibration curves obtained for the D-shaped, cylindrical and notch sensors clearly distinguish them before functionalisation during the detection of IL-8 in artificial saliva (**Figure 4-2**). The D-shaped sensor shows the most linear and consistent response with a negative slope indicating high sensitivity and very little deviation between points—high stability (**Figure 4-2(A)**).

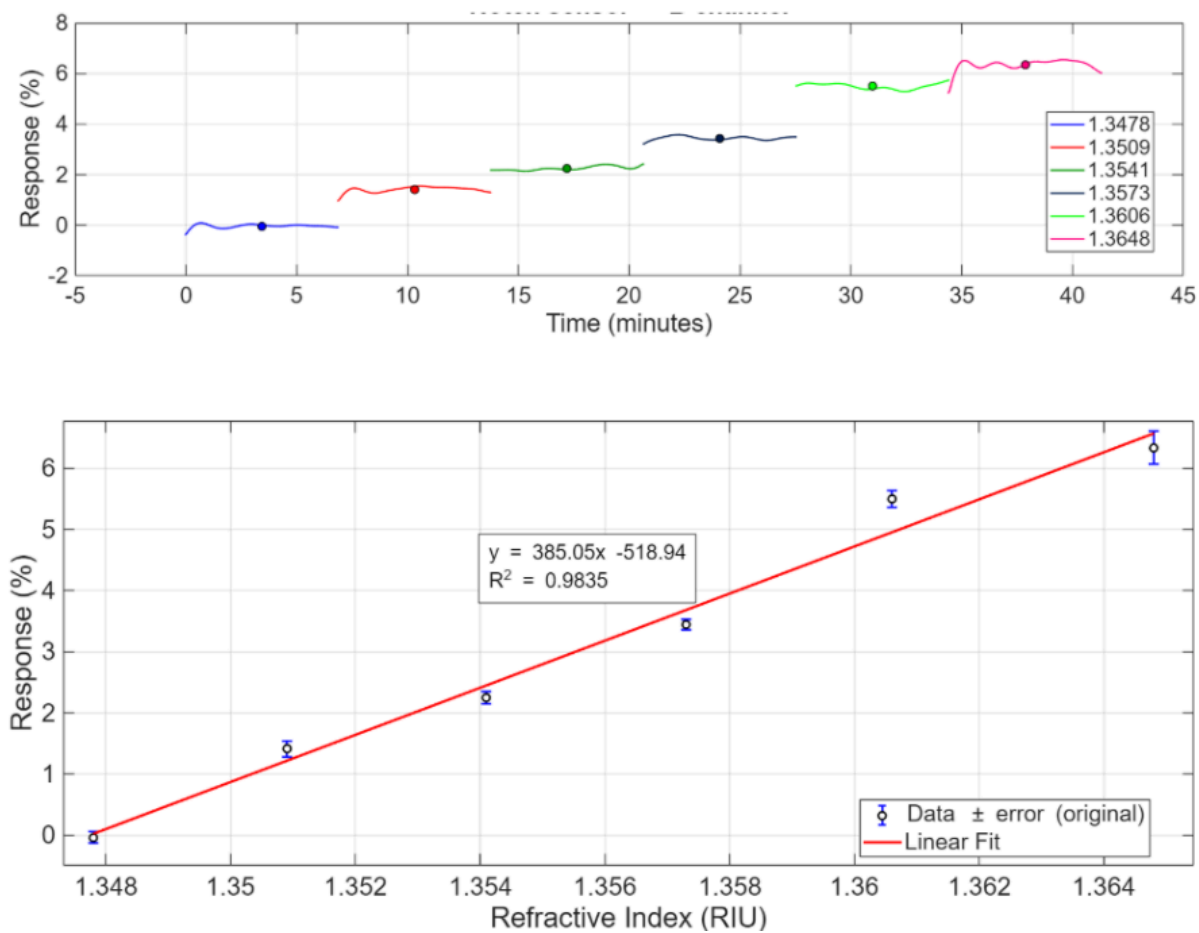


Figure 4-2(A): RI sensing: Time response plot and calibration curve of D-shape Sensor.

On the other hand, the cylindrical sensor displays a relatively steep slope, but with larger variability and lower reproducibility—an effect likely attributable to more advanced fabrication constraints in addition to the unstable functionalization process (**Figure 4-2(B)**). The notch sensor displays moderate sensitivity but not as consistent behavior when compared with the D-shaped configuration (**Figure 4-2(C)**). Among the different possible sensor geometries, these results overall confirm that D-shaped sensors can be the most reliable and sensitive platform for biomarker detection while cylindrical sensors are harder to fabricate and generally exhibit instability after surface functionalization.

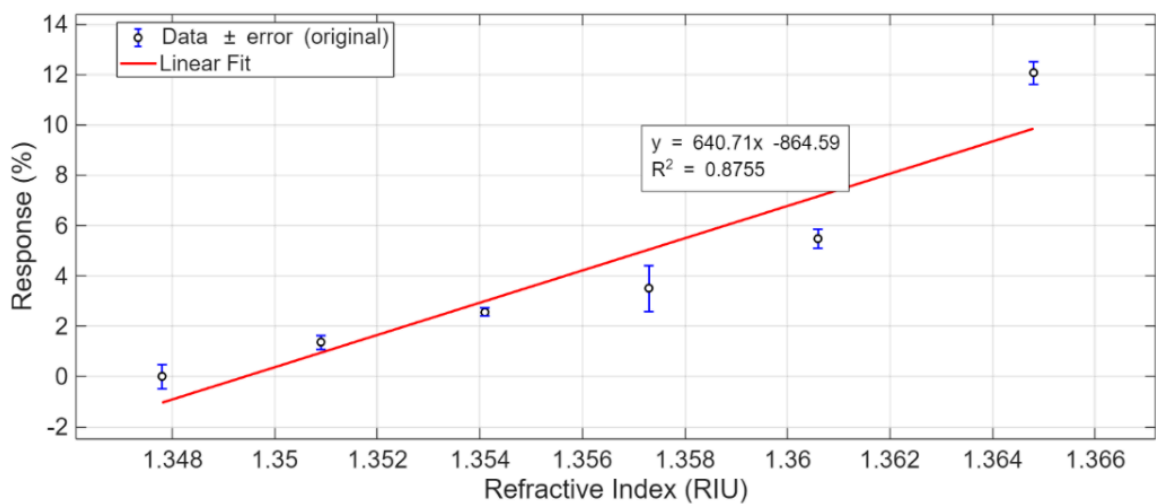
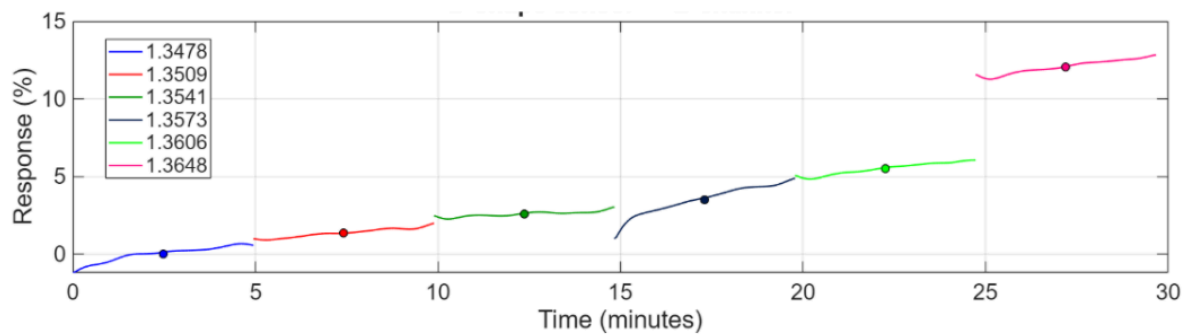


Figure 4-2(B): RI sensing: Time response plot and calibration curve of Cylindrical Sensor.

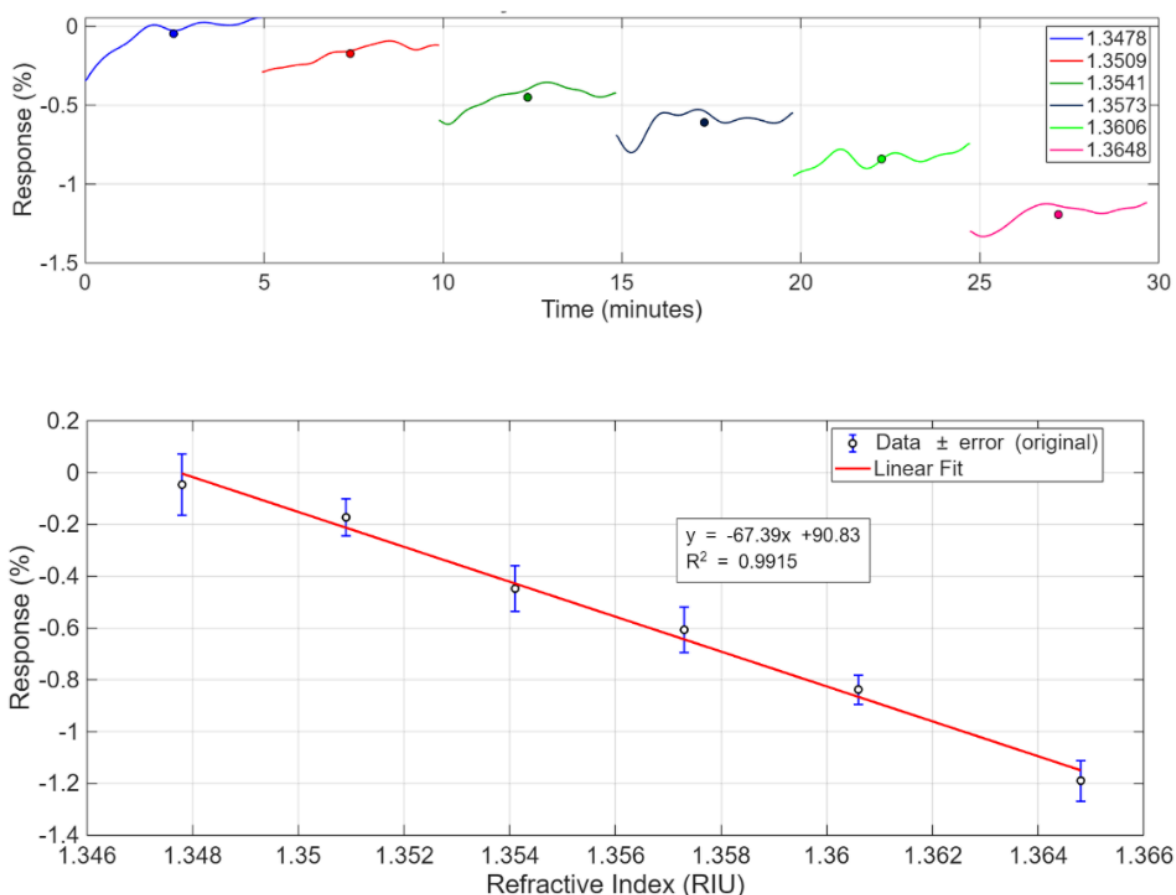


Figure 4-2(C): RI sensing: Time response plot and calibration curve of Notch Sensor.

Data shown in **Figure 4-3** demonstrates the D-shaped sensor's response to a range of VEGF protein concentrations (femtomolar to nanomolar). As shown in the intensity plot for analysis, the stepwise decline in signal with increasing concentration highlights the ability of this sensor to respond reliably at different concentrations of a target biomolecule. At low concentrations, including 1 fM to 10 pM, the signal is stable and fluctuates slowly, suggesting that the binding interactions were controlled and the sensor was well stabilized. At higher concentrations (100 pM and 1 nM), however, overall noise increases alongside a large signal drop that indicates stable binding may be challenging at high analyte levels due to saturation effects. The most significant shift is from 10 pM to 100 pM, where we see a rapid drop in signal intensity that highlights a region of high sensitivity.

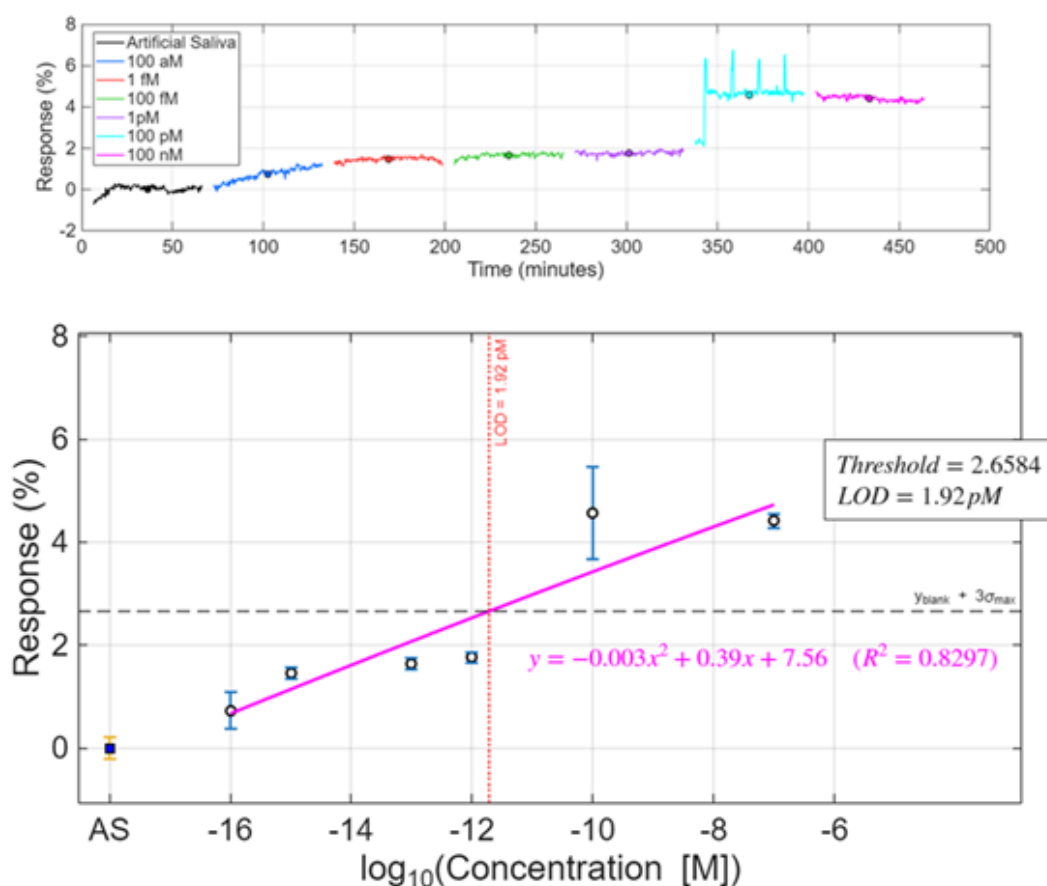


Figure 4-3: VEGF Detection Using D-Shaped Sensor.

The error bars are fairly low at lower concentrations and lack homogeneity at higher concentrations, suggesting less measurement stability or increased environment effects (**Figure 4-3**). Indeed, the slope of the linear regression indicates a negative relation, which further proves that as analyte concentration increases, sensor response decreases; this trend correlates with expected changes in refractive index around the D-shaped sensing region. The sensor showed a sensitivity of 0.46 % per log₁₀(M). It is important to have low LOD to show the sensor's strong features - detect small changes in the lowest concentration range. The sensor's LOD is 1.92 pM, which is positive for our research.

The normalized RGB spectrum shows three distinct intensity peaks corresponding to blue (450 nm), green (540–550 nm) and red (650 nm) wavelengths (**Figure 4-4**). The intensity increases uniformly over all three channels as the concentration of IL-8 increases from artificial saliva to 100 nM. Relative changes in the intensity are largest at 450 nm (blue region) where we see an increase of 35% for artificial saliva over 100 nM (100%) with a relative change of 65%. This suggests that the blue channel is most responsive to variation in analyte concentration and predominates response of the sensor. In the green region (540–550 nm) intensity increases from 25% to 55%, though moderate change (30%) corresponds. It indicates that the green channel contributes to the signal but with a lower sensitivity than the blue channel. At the 650 nm (red) region, the intensity varies from 10–15% to ~60%, and the change is around 45–50%. The red channel on the other hand has a much more spread out signal, while having a lower average baseline than the blue channel means that it is overall less stable.

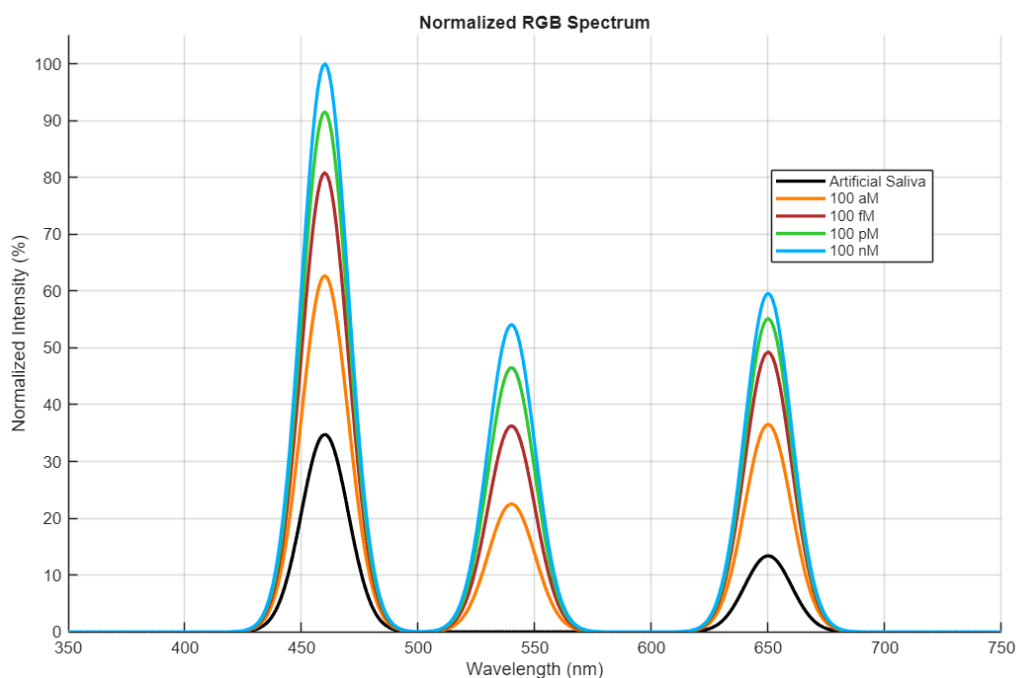


Figure 4-4: Normalized RGB Spectrum of D-shape sensor in IL-8 protein detection.

Due to a high sensitivity and significant signal contrast, the blue channel appears to be the most appropriate parameter for quantitative analysis (**Figure 4-4**). To confirm the analysis and calculate moderate sensitivity the green channel can be taken as the main one. The red one usually provides less stable response suggesting possible low intensity on the start and higher noisy errors in the signal. Meanwhile, we can see that results depict consistency in intensity increase in all RGB channels. Because of RI alteration of the surrounding medium as biomarkers bind, this shows the changes in light propagation and scattering features of the fiber. The overall conclusions of the RGB spectral analysis are that the blue channel has the best sensitivity and signal-to-noise ratio among all channels, thus it was selected as the primary parameter in this study for biomarker detection.

Figure 4-5 shows the sensor response of three sensors with different geometries after IL-8 protein detection. The response behavior, over the range of concentrations tested, is shown to be significantly different by the previous sensor configurations. The neat and monotone increase of the normalized intensity by concentration suggests a stable performance of the D-shaped sensor (**Figure 4-5(A)**). It has a sensitivity of 19 %/log₁₀(M) and lower LOD (162 aM), which allows for better detection capability at low concentrations when compared to other configurations.

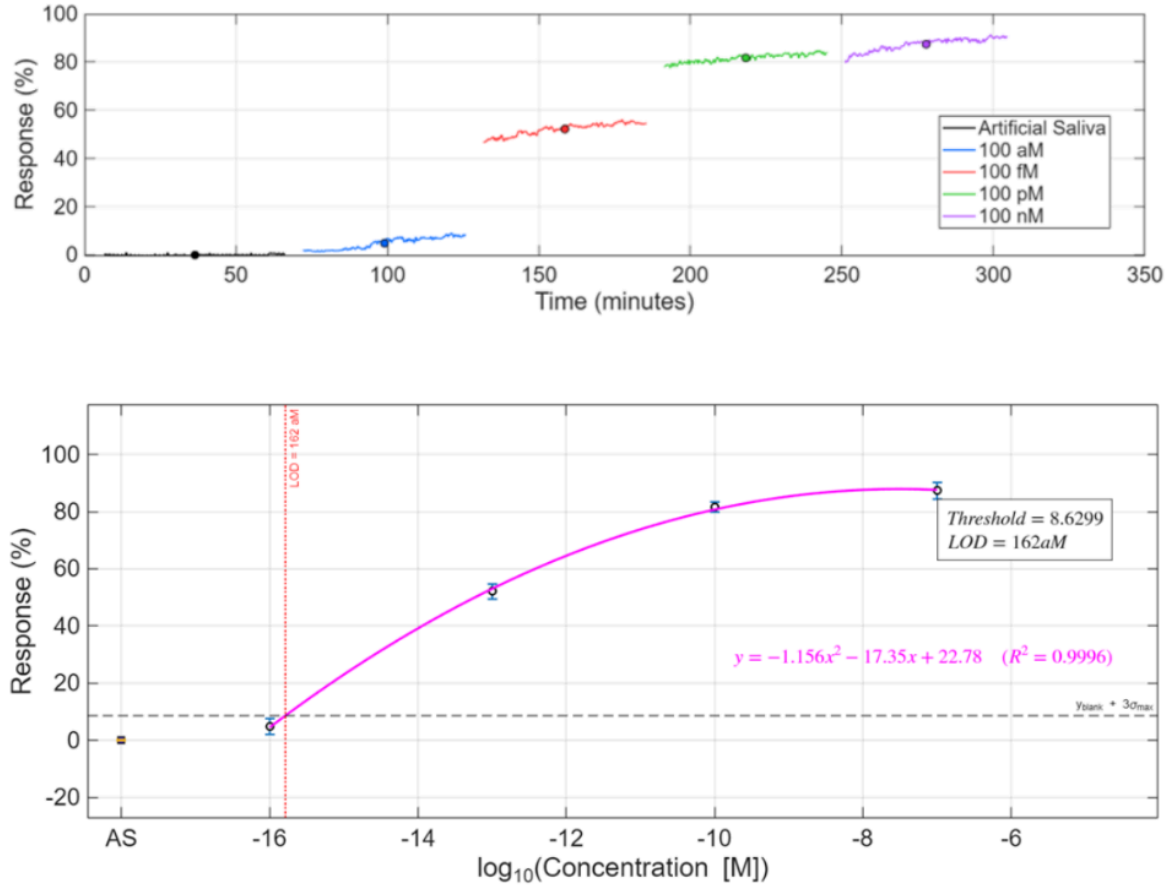


Figure 4-5(A): IL-8 protein biosensing: time response plot and calibration curve of D-Shaped Sensor.

The cylindrical sensor shows a similar pattern, but less steep and more random values between points (**Figure 4-5(B)**). With a lower sensitivity ($2.4 \text{ } \%/ \log_{10}(\text{M})$), the larger LOD of 4.62 fM correlates with lower detection resolution and reduced reliability, which matches the instability observed after functionalization. It is to be noted that the notch sensor behaves erratic and non-monotonically, with signal values tending towards saturation at larger concentrations (**Figure 4-5(C)**). That indicates the sensor response is possibly saturated by the biomarker, which lacks a dynamic range and leads to weak quantitative detection when it comes to the sensors. Overall, these results further confirm that the D-shaped sensor is the

best compromise between sensitivity and stability at similar limits of detection across all geometries tested.

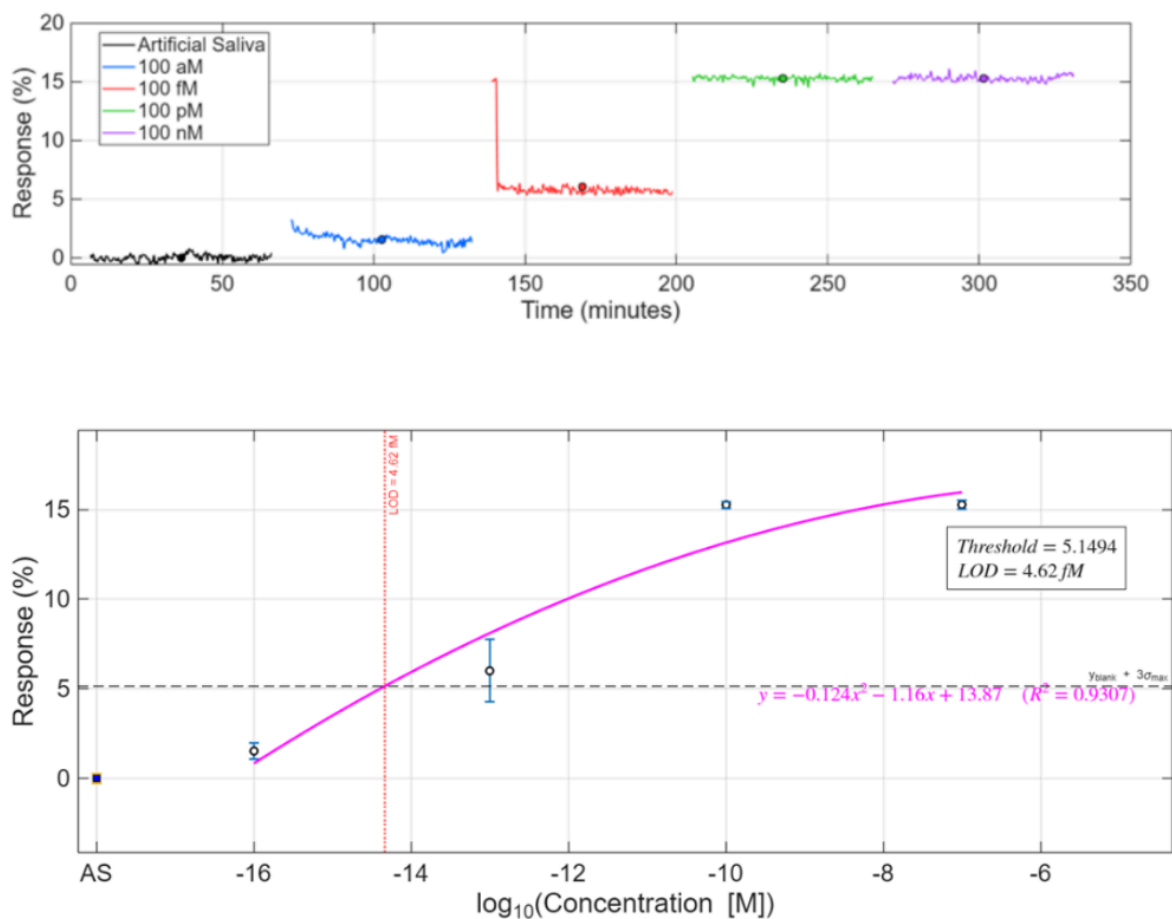


Figure 4-5(B): IL-8 protein biosensing: time response plot and calibration curve of Cylindrical Sensor.

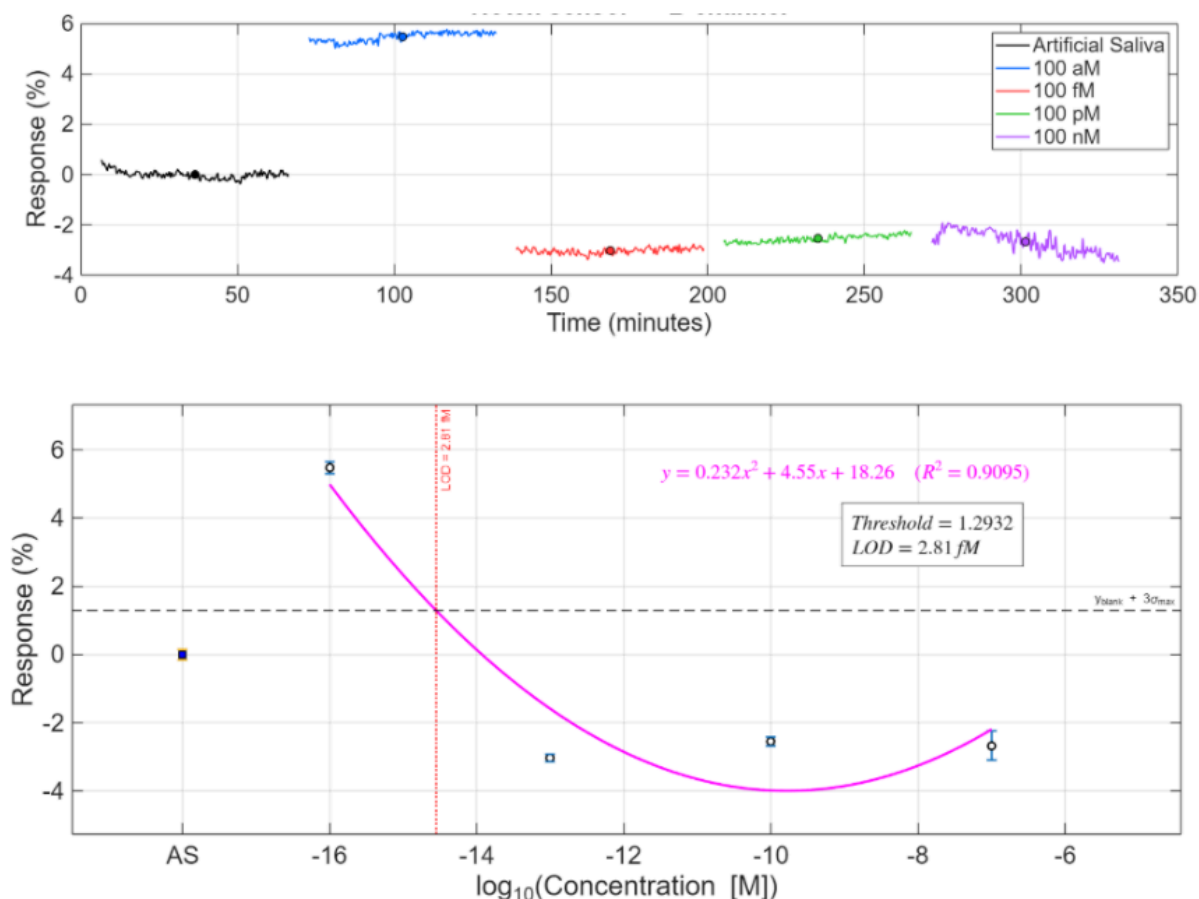


Figure 4-5(C): IL-8 protein biosensing: time response plot and calibration curve of Notch Sensor.

In **Figure 4-6** the detection responses of D-shaped and cylindrical sensors when IL-8 antibodies are not immobilized were shown. In either of the plots, the intensity does not show a clear or monotonic trend with increasing levels of IL-8 concentration which maintain low sensitivity and lack of specificity. For the D-shaped sensor (**Figure 4-6(A)**), all RGB channels show irregular fluctuations across concentrations. The blue channel, although having the highest absolute intensity (~70–100%), displays a non-monotonic behavior with a sharp drop at 100 fM then rises at higher concentrations. Similarly, the green and red channels exhibit inconsistent variations, including abrupt decreases and increases that do not correlate with concentration changes.

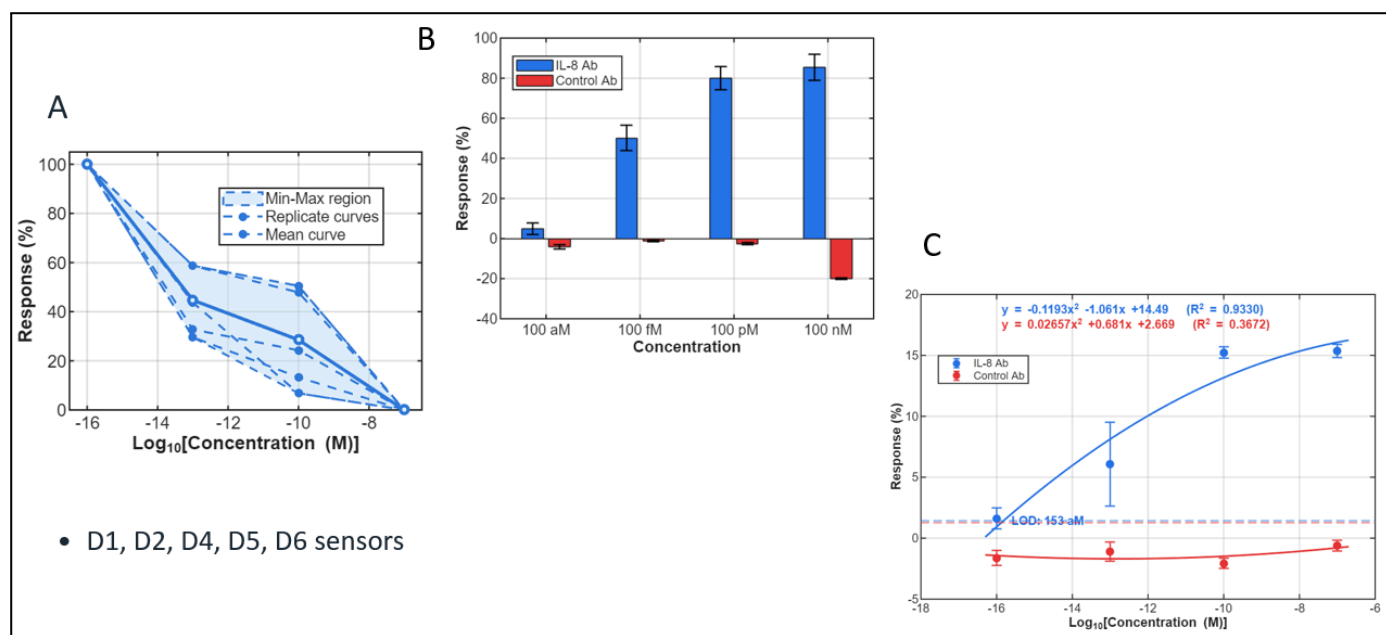


Figure 4-6: Repeatability of D-shaped sensors. Detection results of D-shape Sensor functionalized without IL-8 Antibody.

The same is true for the cylindrical sensor (**Figure 4-6(B)**). Similarly, the green and blue channels remain at high signals (~60–100% intensity) with their responses varying without clear concentration dependence. The weak and most unstable channel is the red one, in which large error bars further confirm noise dominated behavior. No trend can be seen on both sensor geometries, which indicates that without being immobilized by aptamer the sensors cannot specifically bind IL-8 molecules and thus are prone to non-specific interactions, leading to unstable optical responses. They are most likely random surface interactions, environmental interference or refractive index fluctuations that can only appear as droplets in the signal.

Based on the given results, **Table 4-1** is filled. According to the table, both sensors show significantly enhanced performance with clear trends and improved sensitivity after IL-8 antibodies functionalization. The results summarized herein indicate that while the D-shaped sensor has decreased in sensitivity (385 %/RIU → 101 %/RIU), it remains much

more stable and consistent, having a final response of 19.15 %/log₁₀(M). The cylindrical sensor exhibited the highest RI sensing performance - 641 %/RIU, the lower sensitivity after functionalization (2.39 %/log₁₀(M)), and an increase was also seen in comparison to its calibration post-functionalization (97 %/RIU).

The comparison **Table 4-1** shows that although a cylindrical sensor can reach higher values of sensitivity, it is usually less stable and highly dependent on the process in which they are functionalized. While the D-shaped sensor has lower sensitivity, it makes up for that with potentially more reliable and steady performance. In comparison, the notch sensor has the least sensitive response after (-2.2 %/log₁₀(M)) as well as a lowest calibration slope (-67 %/RIU → -12 %/RIU), confirming that it performs worse under functionalization conditions.

In terms of detection limits, D-shaped presented a low LOD (162 aM) which is to be the best ability for detecting IL-8 at low concentrations. Cylindrical sensor depicted LOD of 4.62 fM which shows lower detection resolution. The notch structured sensor demonstrated a non-linear result which is proved by LOD of 2.81 fM. This limits its applicability for reliable quantitative detection. The main cause can be attributed to the fast protein saturation of sensing regions in the notch sensor.

All in all, functionalization with antibodies is predominant for improving specificity in IL-8 detection based on our results. Amid all suggested geometries, we found out that the best trade-off between sensitivity, stability and practicality was given by D-shaped sensor. Cylindrical sensors, however, showed highest sensitive response but with worst strength.

Table 4-1: Sensitivity and LOD results of different sensors at calibration and IL-8 protein detection stages.

Sensors	Sensitivity				LOD
	Calibration and R2 value	calibration after functionalization	Detection (without IL-8 antibody)	Detection with IL-8 Ab	
D-shape	S = 385 %/RIU R2 = 0.9835	101 %/RIU	Not linear	S[LOD] = 19.15 %/log10(M)	162 aM
Cylindrical	S = 641 %/RIU R2 = 0.8755	97 %/RIU	Not linear	S[LOD] = 2.39 %/log10(M)	4.62 fM
Notch	S = -67 %/RIU R2 = 0.9915	-12 %/RIU	-	S[LOD] = -2.2 %/log10(M)	2.81 fM

To evaluate the sensor response distribution at each concentration, we plotted histograms of repeated measurements and fitted them to a Gaussian normal distribution (Figure 4-7). The average response progressively rises with increasing concentration, the spread of the data expressed by the standard deviation σ shown as $\pm 2\sigma$ bounds. It enables the evaluation of sensitivity (via peak position shifts) and noise (by the width of distribution). The distributions at the various concentrations were well separated (capturing good detectability),

and there was minimal overlap, thus supporting a reliable discrimination and therefore the calculated limit of detection.

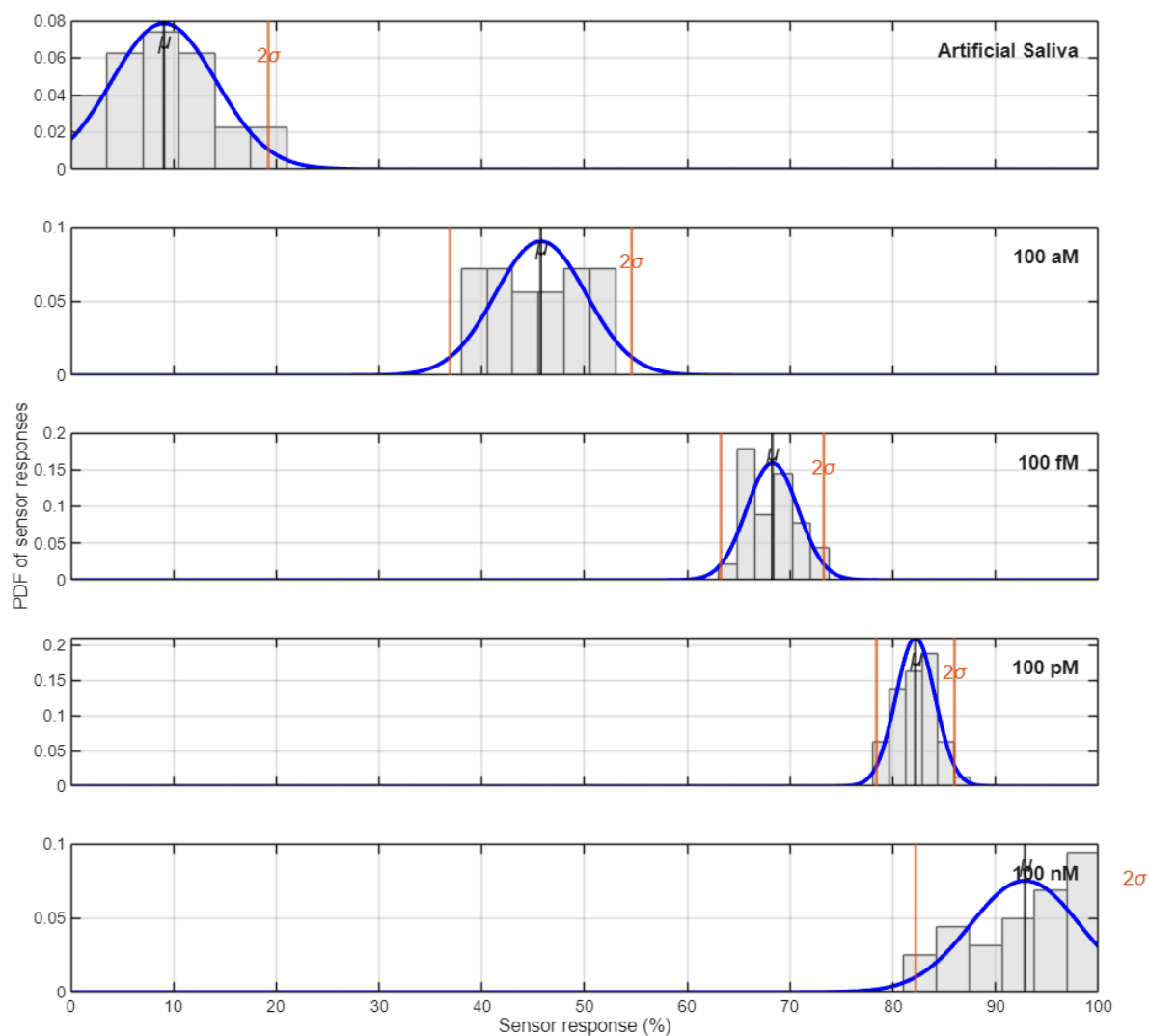


Figure 4-7: Gaussian Distribution of Sensor Responses Across Concentrations

Chapter 5 - Conclusion

The presented work included the design and development of a portable smartphone-based plastic optical fiber biomarker detection platform. The study integrated all modules of sensor fabrication, calibration, surface functionalization, and biomarker sensing into a single experiment. To fabricate our portable biosensors, three main geometrical structures (D-shaped, cylindrical, and notch) were taken into account. We found out that the sensor geometry in a big manner affects on optical signal response, stability of the signal and results of functionalization.

After testing all polishing configurations, it was observed that a D-shaped structure claimed to be most linear and stable in calibration stage, and even overall performance of the sensor after functionalization was positive. The next configuration - cylindrical sensor - was outstanding because of high sensitivity, however, the fabrication and reproducibility required hard work and preciseness. Without it, cylindrical sensor could show chaotic response and unstability. The last geometry, notch sensor, was not successful for biological experiment. Non-monotonic signal trend due to biomarker saturation affected on the quantitativity of the notch structure. Finally, sensitivity, durability and usefulness can be seen from D-shape structure. Functionalized fibers of such geometry could detect biomarkers VEGF and IL-8 in synthetic tear as well as saliva with concentration-dependent responses. This resulted in a LOD of 1.92 pM for VEGF with the D-shaped sensor, and had sensitivity of 0.46 %/log₁₀(M). For IL-8 detection, D-shaped sensors once again exhibited superior repeatability and reduced limits of detection compared to cylindrical and notch geometries. Furthermore, the validation of specificity confirmed the necessity of antibody immobilization for selective and stable detection as the sensors without antibodies exhibited non-linearity in responses.

In conclusion, the work presented here shows that POF biosensor integrated with a smartphone, can provide a low-cost and portable means to detect biomarkers in non-invasive

fluids. The system that has been developed shows promise for future point-of-care and home-based diagnostics. This work lays the groundwork for future research to ensure long-term stability, refine non-specific blocking strategies and create opportunities for IoT connectivity before using real clinical saliva and tear samples to validate this platform. As a future perspective, it is recommended to further develop the mobile application to enable real-time data analysis with computing sensitivity and LOD directly on the smartphone for remote monitoring.

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