

**DEVELOPMENT OF INTERFEROMETRIC OPTICAL FIBER  
BIOSENSOR FOR HCC1806 BREAST CANCER CELL  
DETECTION**

**Timur Meirambekuly, BSc in Biological Sciences**

**Submitted in fulfillment of the requirements  
for the degree of Master of Science  
in Biomedical Engineering**



**School of Engineering and Digital Sciences  
Department of Chemical & Materials Engineering  
Nazarbayev University**

53 Kabanbay Batyr Avenue,  
Astana, Kazakhstan, 010000

**Supervisors: Daniele Tosi, Cevat Eriskan**

**April 2024**

## DECLARATION

I hereby, declare that this manuscript, entitled “Development of Interferometric Optical Fiber Biosensor for HCC1806 Breast Cancer Cell 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.

-----  
Name: Timur Meirambekuly

Date: April 5<sup>th</sup>, 2024

# Abstract

This thesis explores the use of semi-distributed interferometric (SDI) optical fiber biosensors for detecting breast cancer cells, with a focus on assessing the potential and limitations of this technology. Given the critical importance of early detection in improving breast cancer outcomes, this research aims to advance diagnostic methodologies by leveraging the sensitivity and specificity advantages of optical fiber technology, combined with targeted antibody functionalization.

The study commenced with the fabrication of optical fiber sensors, incorporating interferometric tips to single-mode optic fiber pigtails. Calibration was performed using specialized software and equipment, selecting sensors with optimal sensitivity based on their performance in standardized tests. These sensors were then functionalized with CD44 antibodies, targeting the detection of HCC1806 breast cancer cells across a range of concentrations. Experimental trials were conducted to evaluate the sensors' ability to detect cell concentrations from sterilized PBS up to  $10^6$  cells per ml, with data collection at regular intervals.

Despite the innovative approach and the meticulous fabrication and calibration processes, the research confronted significant challenges that impacted the results. While the biosensors demonstrated the capacity to detect breast cancer cells at higher concentrations ( $10^5$  and  $10^6$  cells/ml), their efficacy at lower concentrations was not observed. Factors contributing to this limitation included potential issues with cell viability, inaccuracies in cell handling, and the unintended functionalization of sensor surfaces beyond the designated tip area. These findings suggest that, although promising for high-concentration detection, the current iteration of the biosensors requires further refinement to improve their performance at lower concentrations.

This thesis underscores the promise of SDI sensors in breast cancer detection while acknowledging the hurdles faced in realizing their full diagnostic potential. The encountered challenges, from technical and logistical issues to limitations in sensor design and functionality, highlight the complexities of developing a new diagnostic tool. Despite these obstacles, the research contributes valuable insights into the feasibility of using optical fiber biosensors for cancer detection, laying a foundation for future improvements. By building on the lessons learned and continuing to refine the biosensors' design and application, there is

potential to enhance their sensitivity and specificity, moving closer to a viable tool for early-stage breast cancer diagnosis. This study advances our understanding of optical fiber biosensor technology and creates a foundation for further research on the involvement of SDI sensors in breast cancer cell detection.

# Acknowledgments

I express my gratitude towards my supervisors Daniele Tosi and Cevat Eriskan for guiding me through my studies in this program. Their wisdom and practical advice allowed me to channel my efforts most effectively.

Furthermore, I wish to express my appreciation to my peers and dedicated coursework professors whose collective expertise and insights have enriched my learning experience. Their contributions have been instrumental in broadening my understanding of the biomedical engineering field and fostering intellectual growth.

Lastly, I am deeply indebted to my beloved mother, Marina Maratovna, whose love and encouragement have been the cornerstone of my academic pursuits. Her selfless dedication and sacrifices have provided me with the foundation and motivation to pursue my studies with determination and resilience.

# Table of contents

<b>Abstract .....</b>	<b>2</b>
<b>Acknowledgements .....</b>	<b>4</b>
<b>List of Abbreviations .....</b>	<b>6</b>
Chapter 1 – Introduction.....	8
1.1 General.....	8
1.1.1 Background & Context.....	8
1.1.2 Research Problem & Rationale.....	8
1.1.3 Aims & Objectives.....	9
1.2 Techniques & Methodologies.....	10
1.3 Significance & Contribution.....	11
1.4 Scope & Limitations.....	13
1.5 Thesis Structure.....	13
Chapter 2 – Literature Review.....	15
2.1 Introduction .....	15
2.1.1 The Significance of Early Detection in Cancer.....	15
2.1.2 Advancements in Optical Sensing for Cancer Detection.....	15
2.2 Optical Fiber Biosensors and SDI Sensors.....	17
2.2.1 SDI sensor concept.....	17
2.2.2 Advantages & Applications of SDI Sensors.....	17
2.2.3 SDI Sensors for Whole Cancer Cell Detection.....	18
2.3 Methodological Rationale.....	19
Chapter 3 – Methods of Sensor Fabrication and Exploitation.....	21
3.1 Sensor Fabrication & Calibration.....	21

3.1.1 Fabrication.....	21
3.1.2 Calibration.....	23
3.2 Surface Functionalization.....	26
3.2.1 Preparation & Piranha solution treatment.....	26
3.2.2 Silanization.....	26
3.2.3 Cross-linking & Antibody Attachment.....	27
3.2.4 Antibody Binding & Blocking.....	27
3.2.5 Final Steps & Storage.....	27
3.3 Cell growth.....	28
3.4 Detection.....	28
Chapter 4 – Results and Discussions.....	31
4.1 Sensor Calibration & Sensitivity.....	31
4.2 HCC1806 Count.....	35
4.3 Detection.....	35
4.4 Challenges & Constraints .....	40
<b>Literature Cited.....</b>	<b>44</b>
<b>Further Reading .....</b>	<b>47</b>
<b>Appendix A.....</b>	<b>48</b>
<b>Appendix B.....</b>	<b>48</b>
<b>Appendix C.....</b>	<b>49</b>
<b>Appendix D.....</b>	<b>50</b>
<b>Appendix E.....</b>	<b>51</b>
<b>Appendix F.....</b>	<b>52</b>

# List of Abbreviations & Symbols

SDI	Semi-Distributed Interferometer
SMF	Single Mode Fiber
OFB	Optical Fiber Biosensor
SPR	Surface Plasmon Resonator
RI	Refractive Index
NP	Nanoparticle
dB/RIU	Decibels per Refractive Index Unit.
HCC1806	Breast cancer cell line
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
H <sub>2</sub> SO <sub>4</sub>	Sulfuric acid
GA	Glutaraldehyde
APTMS	(3-Aminopropyl)triethoxysilane
EDTA	Ethylenediaminetetraacetic acid
m-PEG	Methoxy Polyethylene Glycol
PBS	Phosphate-Buffered Saline
AFP	Alpha-Fetoprotein
CTC	Circulating Tumor Cells
LoD	Limit of Detection

# Chapter 1 - Introduction

## 1.1 General

### 1.1.1 Background & Context

Cancer cells of the type that form solid tissue akin to those formed during breast cancers are especially known to develop cancer stem cells that contribute to their malignancy (1). Characterized by their ability to self-renew and sustain tumor growth, these cells also generate the differentiated cells that comprise the majority of the tumor. The concept of cancer stem cells carries significant implications for treatment strategies, suggesting that targeting this specific cell population could be key to developing curative therapies, given their critical role in driving cancer growth (1).

Breast cancer ranks as the most common cancer diagnosed in women, aside from skin cancer, and stands as the second primary cause of death due to cancer among women in developed nations (2). 60% of breast cancer-related deaths are attributed to low-income and developing countries (3). Therefore, it is crucial to develop a cost-effective, accessible, and effective diagnostic tool to detect breast cancer cells in biological medium.

### 1.1.2 Research problem & Rationale

At the core of the global challenge posed by breast cancer lies a critical bottleneck in the current diagnostic paradigm—early and accurate detection. Despite advances in medical science, the ability to detect breast cancer at its nascent stages remains limited, particularly in populations without overt risk factors. This shortfall is not merely a technical issue but a pivotal barrier to improving survival rates, as early detection significantly increases treatment options and efficacy. The existing methodologies, while beneficial, often fall short in sensitivity and specificity, especially in the early stages of the disease where interventions could be most impactful.

This research is poised at the intersection of this pressing need and the potential for innovation in diagnostic technologies. The development of functionalized interferometric optical fiber biosensors represents a promising frontier in the quest for more effective detection mechanisms. These biosensors, by their design and functionalization, aim to offer a

higher degree of sensitivity and specificity in identifying breast cancer cells. The rationale behind focusing on this technology stems from the hypothesis that enhancing the precision and reliability of early-stage breast cancer detection can significantly alter clinical outcomes. This aligns with global health objectives, aiming not only to mitigate the mortality associated with breast cancer but also to address the disparities in detection and treatment outcomes across different regions and populations. The urgency of this issue and the potential impact of a breakthrough underscore the significance of this research in the broader context of cancer care and patient prognosis.

### 1.1.3 Aims & Objectives

The overarching goal of this thesis is to explore the capabilities and limitations of semi-distributed interferometric sensors in the context of breast cancer cell detection. This exploration is framed by a real-world acknowledgment of the challenges and complexities inherent in pioneering new diagnostic technologies. Recognizing the preliminary nature of this research, the study is designed with a dual focus: to investigate the theoretical potential of SDI sensors in detecting cancer cells and to pragmatically assess the feasibility of these sensors.

Specifically, this thesis aims to:

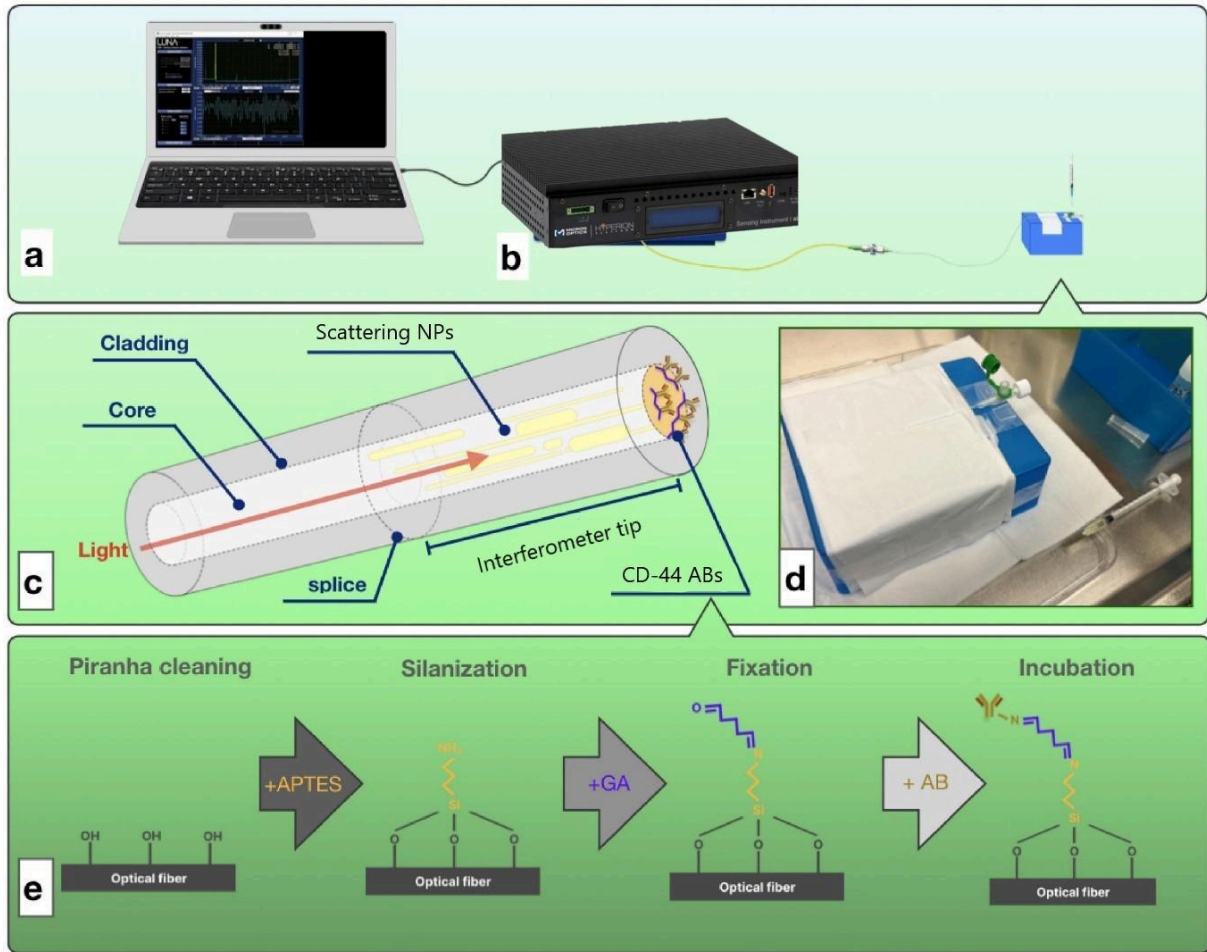
1. Evaluate the Detection Capabilities of SDI Sensors: To ascertain the effectiveness of SDI sensors in identifying breast cancer cells across varying concentrations, with a particular emphasis on understanding the highest and lowest detectable limits under experimental conditions.
2. Investigate the Impact of Fabrication Parameters: To explore how different aspects of sensor design, including the calibration process and sensor surface functionalization, influence the detection efficiency. This includes an examination of the interaction between CD44 antibodies and breast cancer cells as a case study in sensor specificity.

3. Lay Groundwork for Future Research: Despite the initial challenges and the recognition of the sensors' current limitations in sensitivity and specificity, to outline a roadmap for future investigations that could enhance the practicality and effectiveness of SDI sensors in cancer detection. This involves proposing areas for improvement, optimization, and further study based on the insights gained from the research.

## **1.2 Techniques & Methodologies**

This study employs a series of steps combining optical engineering with biochemical processes to create and test biosensors for detecting breast cancer cells early on. The process begins with the precise assembly of optical fibers, which are carefully modified to include a specific section designed for high sensitivity to biological markers. These modified fibers are then calibrated using a method that involves both air and solutions of varying densities to establish a baseline for their sensitivity. This calibration is essential for identifying which sensors are sensitive enough for further use.

Figure 1.2.1 illustrates the main steps of calibration and functionalization of the sensor with antibodies (4). Once selected for their high sensitivity, these fibers undergo a cleaning and preparation process. This involves removing any contaminants that could interfere with the sensor's ability to detect specific biological markers. Following this, the fibers are treated to allow for the attachment of molecules that can specifically recognize and bind to markers found on breast cancer cells. This selective binding is crucial for the sensor's specificity, ensuring it responds primarily to the presence of cancer cells. After preparation, the sensors are exposed to cell samples at various concentrations to test their ability to detect the presence of cancer cells. This step is critical in determining the sensors' practical sensitivity and their potential usefulness in a real-world diagnostic context. The response of the sensors to these samples is meticulously recorded and analyzed, looking for changes that indicate the presence of cancer cells.



**Figure 1.2.1: Experimental setup**

The methodology integrates careful sensor preparation, precise calibration, and targeted detection techniques to create a tool that can potentially identify breast cancer cells at early stages. This approach not only showcases the intersection of engineering and biology but also highlights the potential for such technologies to significantly impact early cancer detection. The specifics of each step, while streamlined here, involve detailed procedures that are crucial for the success of the biosensors' development and their eventual application in diagnosing breast cancer effectively.

### 1.3 Significance & Contribution

This study marks a step forward in the early detection of breast cancer, driven by its innovative approach that marries the simplicity of optical technology with the complexity of

cancer diagnostics. The application of semi-distributed interferometric single-mode fiber (SMF) optic sensors in this context is not just a scientific endeavor but a beacon of hope for improving global health outcomes against a backdrop of existing challenges.

The accessibility of the proposed detection method is a game-changer. Unlike conventional cancer biosensing techniques that demand sophisticated infrastructure and specialized skills, SDI fiber optic sensors simplify the detection process without sacrificing effectiveness. This ease of use potentially brings advanced diagnostic capabilities to regions and settings where such technology was previously unthinkable. In doing so, it democratizes access to crucial health technology, making early detection of breast cancer a more attainable goal across diverse healthcare landscapes.

Cost-effectiveness is another hallmark of this research. The adoption of optic fibers, a staple in telecommunications for their affordability and availability, as the backbone of this biosensing method addresses a critical barrier in healthcare: cost. By capitalizing on an inexpensive material, the project facilitates broader implementation, especially in low- and middle-income countries where economic constraints often limit access to life-saving diagnostics. This approach not only contributes to the scientific knowledge about optical sensors in cancer detection but may also open up new avenues for resource allocation in the fight against breast cancer.

The novelty of this research lies in its direct application of SDI optic sensors for cancer cell detection—a stark departure from traditional methods that rely on biomarkers or label-dependent techniques. This innovative step is not merely an academic exercise but a potential paradigm shift in how cancer is detected, offering a faster, label-free method that could significantly enhance diagnostic accuracy and timeliness. By charting a new course in biosensing technology, the study not only contributes valuable insights to the scientific community but also paves the way for future advancements in cancer detection. Beyond the technical achievements, the scalability and integration potential of SDI technology stands out. Its adaptability means it could be seamlessly incorporated into existing health systems, contributing to more comprehensive cancer care strategies. From screening programs to recurrence monitoring, the technology's versatility enhances its value, suggesting that its impact could extend well beyond initial diagnostic applications.

In sum, this research might not be ground-breaking, but it adds to what we know about cancer biosensing and also promises real-world implications that could transform the

landscape of breast cancer detection. By aligning optical fiber technology with practical, accessible, and cost-effective solutions, it embodies the potential of innovation to make a tangible difference in cancer detection.

#### **1.4 Scope & Limitations**

This thesis investigates the use of functionalized interferometric optical fiber biosensors for detecting breast cancer cells, focusing on a cost-effective and accessible method. It covers the entire process, from the biosensors' conceptual design to their application in targeting specific cancer cells, emphasizing the CD44 antibody's role in identifying breast cancer cells. However, this journey encountered several practical challenges.

One major hurdle is maintaining the precision of the splicing and detection equipment. Handling the cells and antibodies adds complexity, potentially affecting the biosensors' accuracy and reliability. The detailed methodology, involving extensive mixing and pipetting, was prone to errors, complicating the achievement of accurate results. The intended detection at the sensor tip sometimes picks up unexpected background signals, introducing noise and making data analysis more challenging.

Recognizing these challenges is essential, not as a concession of failure but as an acknowledgment of the obstacles faced in advancing scientific inquiry. These issues, from technical and logistical to methodological, highlight the complexities involved in creating a new diagnostic tool. Despite these obstacles, the experience has laid the groundwork for future research, offering valuable lessons for refining this diagnostic approach and enhancing its effectiveness.

#### **1.5 Thesis structure**

This thesis is structured into five comprehensive chapters, systematically presenting the research from its inception to its conclusions. Initially, it sets the stage for an in-depth exploration of breast cancer detection using innovative optical fiber biosensors:

1. Introduction: This opening chapter establishes the groundwork for the research, highlighting the motivation behind the study, its scope, and defined objectives. It offers

an overview of breast cancer and introduces the promising capabilities of optical fiber biosensors in detecting this pervasive disease.

2. **Literature Review:** An extensive review of current research follows, where the focus is on the methodologies employed in breast cancer detection and the burgeoning significance of optical fiber biosensors within this context. This section critically assesses existing techniques and identifies gaps that this research aims to address. It will showcase prior knowledge in the field of breast cancer detection to justify the effort put into this project.
3. **Methods Development:** Detailed within this chapter are the procedural aspects of creating and calibrating the optical fiber biosensors. It sheds light on the intricate technicalities involved in the project, from the fabrication of sensors to their calibration, functionalization, detection and to growing cancer cells, providing a clear understanding of the methodology.
4. **Results and Discussions:** Here, the core findings of the study are unveiled, along with a comprehensive discussion on their relevance and potential impact on breast cancer detection. This chapter not only presents the effectiveness of the developed SDI biosensors but also considers the broader implications for future research in the field. It candidly addresses challenges encountered during the project, reflecting on aspects that were curtailed or significantly affected by unforeseen circumstances.
5. **Conclusion:** This final chapter summarizes the key contributions of the study to the broader scientific community and outlines potential directions for further research. It encapsulates the significance of the findings and the prospective advancements they herald for the early detection of breast cancer, offering closing reflections on the research journey.

# Chapter 2 – Literature Review

## 2.1 Introduction

### 2.1.1 The Significance of Early Detection in Cancer

The significance of early detection in cancer treatment and prognosis is underscored by a multitude of studies, each contributing to our understanding of how timely identification can drastically improve patient outcomes. One particularly noteworthy example is found in Ref. 5, which explores the prognostic implications of detecting whole cancer cells in early-stage lung cancers. This study posits that identifying metastatic whole cells early can be a harbinger for relapse prediction, thereby underscoring the prognostic potential of these cellular markers in assessing patient outcomes. Similarly, recent findings highlight the importance of cancer detection before and after adjuvant chemotherapy in early-stage breast cancer patients (6).

Existing studies underline the importance of early cancer detection in improving treatment outcomes and survival rates. The critical role of recognizing early symptoms as a fundamental component of comprehensive cancer control is thoroughly examined in Reference 7. This review advocates for interventions aimed at 'down-staging' cancer by increasing awareness of detectable signs and symptoms, positing that such strategies could markedly enhance cancer survival rates. It calls for a nuanced approach to categorizing cancer types based on early symptoms to bolster the efficacy of early diagnosis, especially in primary care settings within low-income regions. The evolution and potential future of cancer detection technologies offer a retrospective and prospective analysis of screening and surveillance methodologies (8). The current project addresses this issue and strives to present a reliable way to detect breast cancers in the early stage.

### 2.1.2 Advancements in Optical Sensing for Cancer Detection

The concept of utilizing optical fiber technology for the effective detection of various cancer cells and biomarkers is increasingly being supported by evidence (9). OFBs are becoming essential tools in cancer diagnostics. These sensors stand out for their precise detection of biomarkers crucial for early diagnosis and ongoing monitoring of cancers. Biosensors have transformed diagnostics, offering specific and sensitive detection

capabilities. OFBs, by converting biological responses into optical signals, have shown significant promise in medical diagnostics, especially for early disease detection, where they can make a considerable difference in treatment outcomes (10). The detection of breast cancer biomarkers using OFBs highlights the role of these sensors in identifying early-stage cancers.

Recent advancements in OFB technology, including the development of plasmonic OFBs and the detection of biomarkers like CD44 and AFP, further underline the potential of these sensors in breast cancer diagnostics. These advancements point towards OFBs' capacity for non-invasive, early detection and monitoring, aligning with the goals of improving diagnostic methods and patient care (11, 12). The evolution towards simpler, scalable OFB designs has opened up the possibility for their broader clinical application, particularly in early cancer detection. These designs have balanced the need for high biocompatibility, sensitivity, and specificity with the practicalities of manufacturing and potential disposability, illustrating the direction of recent advancements in OFB technology (13).

Furthermore, OFBs' ability to detect circulating tumor cells (CTCs) and tumor stem cells (CTSCs) showcases their clinical relevance. This capability suggests that SDI sensors could offer more sensitive and specific tools for breast cancer detection, potentially enhancing diagnostic accuracy and enabling more personalized treatment strategies (14). Contrasting optical methods with electrochemical cytosensors provides a broader perspective on cancer detection technologies. The authors of Ref. 15 were able to develop an optical cytosensor that achieved a stable range of cancer cell detection of 1-200 cells/mL, which proves that whole cancer cells can be detected with great levels of sensitivity. OFBs, through developments in optical biosensor design and application, represent a significant advancement in cancer diagnostics. Their high sensitivity and specificity in detecting biomarkers and cancer cells promise to improve breast cancer outcomes, indicating a shift towards more efficient, accessible, and personalized diagnostic methods.

## **2.2 Optical Fiber Biosensors and SDI Sensors**

### **2.2.1 SDI sensor concept**

The SDI sensors developed within this project bear a resemblance to the traditional Fabry-Perot structures, albeit in a more simplified form (16). These sensors consist of a single-mode fiber (SMF) that is joined with an approximately 1mm section of an interferometer. In this instance, the interferometer is comprised of an optical fiber imbued with stretches of Mg-silicone nanomaterial. This particular material enhances the scattering of light, thereby inducing an additional phase shift in the signal. Such an increased scattering rate leads to a randomization effect, significantly amplifying certain peaks in the recorded signal compared to those without the interferometer (17).

What sets the SDI sensors apart from similar devices is the unique modification of only the horizontal tip of the fiber core. This tip is specifically treated to facilitate functionalization with antibodies or analogous agents, thereby promoting interaction and altering the refractive index (18). The experimental setup incorporates the use of SDI in conjunction with fiber Bragg gratings (FBGs), which serve to accentuate the signal and establish a stable reference point within the light scattering spectrum. The incorporation of FBGs into the study of refractive indices, particularly within the realm of optical biosensors, has consistently yielded enhanced sensitivities and elevated refractive index values, thereby improving the outcomes of such research (19, 20, 21).

### **2.2.2 Advantages & Applications of SDI Sensors**

Optical fiber sensors, renowned for their high sensitivity in detecting breast cancer biomarkers, serve as a cornerstone in the early diagnosis and monitoring of cancer. Studies have consistently shown these sensors' capacity to detect minute changes associated with cancerous cells and their biomarkers, establishing a solid foundation for their use in oncological research (15, 19, 22, 23). Within this context, SDI sensors emerge as a noteworthy development, combining the proven sensitivity of optical fiber technology with innovations that enhance detection capabilities. Specifically, the research highlighted in Ref. 4 demonstrates SDI sensors' ability to detect the CCL5 cancer biomarker with a notably low limit of detection (LoD). This breakthrough suggests the potential for broadening the scope of detectable cancers, paving the way for the application of SDI sensors in researching various cancer types that may not have been previously associated with such low LoDs.

In addition to their sensitivity, SDI sensors exhibit high refractive index (RI) sensitivity and increased scattering rates. This enhancement is attributed to the strategic incorporation of Mg-silicone nanoparticles (NPs) within the sensors, a design choice that amplifies light scattering and, consequently, the clarity and strength of the signal received (24). The principle underlying this technology—whereby increasing the involvement of Mg-silicone NPs leads to improved signal outcomes—underscores the role of nanotechnology in refining biosensor performance. Indeed, the field of nanotechnology has been pivotal in advancing optical biosensors, offering promising avenues for achieving rapid response times, heightened sensitivity, and specificity in detection (25, 26, 27). The integration of nanoparticles into optical fibers significantly elevates light scattering levels, a critical factor in enhancing biological detection across various media.

Beyond their technical and operational advantages, SDI sensors stand out for their production efficiency and cost-effectiveness. Unlike other high-tech diagnostic tools that may be hindered by high costs and complex manufacturing processes, SDI sensors can be produced swiftly and economically (13, 18, 27). This ease of production, coupled with their effectiveness in detecting cancer biomarkers, positions SDI sensors as an accessible option for both research and clinical applications (13, 26, 28). Furthermore, the capability of SDI sensors to be employed in situ adds a layer of practicality to their use, facilitating real-time studies and experiments that can yield immediate, relevant data (18). This aspect is particularly beneficial for in-field applications and enhances the potential for SDI sensors to contribute significantly to ongoing and future cancer research.

In essence, the introduction and development of SDI sensors within the field of cancer detection represent a confluence of sensitivity, innovation, and practicality. By leveraging the advancements in nanotechnology and optical sensing, SDI sensors offer a promising pathway toward more effective, efficient, and accessible cancer diagnostics.

### **2.2.3 SDI Sensors for Whole Cancer Cell Detection**

The exploration into the capabilities of SDI sensors for the detection of whole cancer cells signifies a pivotal evolution in cancer diagnostic techniques, building upon the foundational applications of these sensors in biomarker identification. Intriguingly, Ref. 26 showcases the successful application of Surface Plasmon Resonance (SPR) biosensors in detecting whole lung cancer cells, prompting the question of whether SDI sensors could be

similarly employed for the direct detection of whole breast cancer cells. This potential extension of SDI sensor applications aims to address the gaps identified in the reliance on biomarkers for cancer detection, offering a more direct and potentially clearer diagnostic avenue (5,6).

The merit of detecting whole cells lies in its direct approach to diagnosis, potentially circumventing the ambiguities associated with the presence of biomarkers in both cancerous and non-cancerous tissues. Specifically, the direct capture and analysis of Circulating Tumor Cells (CTCs) could yield valuable insights into disease progression and treatment responsiveness. This is particularly relevant in breast cancer diagnostics, where different types and subtypes of cancer might share common biomarkers, such as CD44, thus complicating diagnosis and treatment strategies (15, 31, 32). The feasibility of utilizing optical fiber sensors, including those modified with gold coatings and aptamers for enhanced cell capture, has been demonstrated, with successful attachment of breast cancer cells to sensor surfaces highlighting the potential for these sensors to isolate and detect cancer cells directly from biological samples (22). While the application of SDI sensors specifically for whole cell detection has not been extensively explored, the integration of signal amplification techniques, such as the use of gold nanoparticles alongside SDI sensors, promises to improve detection sensitivity for whole cancer cells at lower concentrations, aiding early diagnosis and the monitoring of minimal residual disease (19). This novel application of SDI sensors represents a significant step forward in the direct detection of cancer cells, marking a new frontier in the field of oncological diagnostics that warrants further research and development.

### **2.3. Methodological Rationale**

The experimental approach adopted in this study, while not revolutionary, draws on a rich history of research into optical fiber biosensors (OFBs), leveraging insights from several notable studies. Previous work has explored a variety of OFB configurations, such as ball resonators and reflectorless fibers that do not incorporate scattering materials (20, 21, 23, 28). These studies have consistently demonstrated the potential for achieving remarkable sensitivity in the detection of biomarkers, with some even reporting detection limits at the attomolar level for CD44, a biomarker critical in breast cancer diagnostics (23). Such findings underscore the efficacy of existing optical fiber technologies in sensitive and specific

biomolecular detection, providing a solid foundation for the current study's experimental design.

Significantly, the use of SMFs has been highlighted for their exceptional specificity and sensitivity in CD44 detection across various studies (16, 28). This specificity is particularly crucial in the context of breast cancer, where accurate identification of cancer cells via CD44 interactions can greatly enhance diagnostic precision. Among the techniques explored, the functionalization of fibers through silanization stands out, offering heightened sensitivity in detecting CD44. This methodological choice is informed by past research that demonstrated the enhanced performance of silanized fibers in biosensing applications, pointing towards their potential for improved breast cancer cell detection through CD44 interactions (23).

Furthermore, the methodology surrounding the fabrication of SDI sensors, inspired by similar studies, holds significant promise for the advancement of biosensing technology (17, 18, 22). These studies, which share a methodological affinity with our approach, have illustrated the utility of interferometric materials in the detection of a wide array of biological molecules of interest. By building on these precedents, the current study aims to further explore the capabilities of SDI sensors, not just as a tool for detecting known biomarkers but as a versatile platform for the identification of various biological targets, potentially broadening the scope of applications in biosensing.

## **Chapter 3 – Methods of Sensor Fabrication and Exploitation**

### 3.1 Sensor Fabrication & Calibration

This segment of the thesis delineates the processes of sensor fabrication and calibration, which are foundational to the functionality and reliability of the optical fiber sensors used in the experiment. These steps are meticulously documented to illustrate the precision and attention to detail required in developing sensors capable of detecting breast cancer cells with high specificity.

#### 3.1.1 Fabrication

The fabrication of the sensor represents a methodical process, beginning with a pigtail of a single-mode optical fiber. This fiber is initially coated in two layers of protective material, designed to safeguard the integrity of the fiber during handling. The removal of these protective layers is executed with specialized pliers, preparing the fiber for precise cutting. Utilizing the Fujikura CT08 cleaver (Figure 3.1.1.1), horizontal cuts are made in both the interferometer (Figure 3.1.1.2) and the pigtail, facilitating the subsequent splicing process.



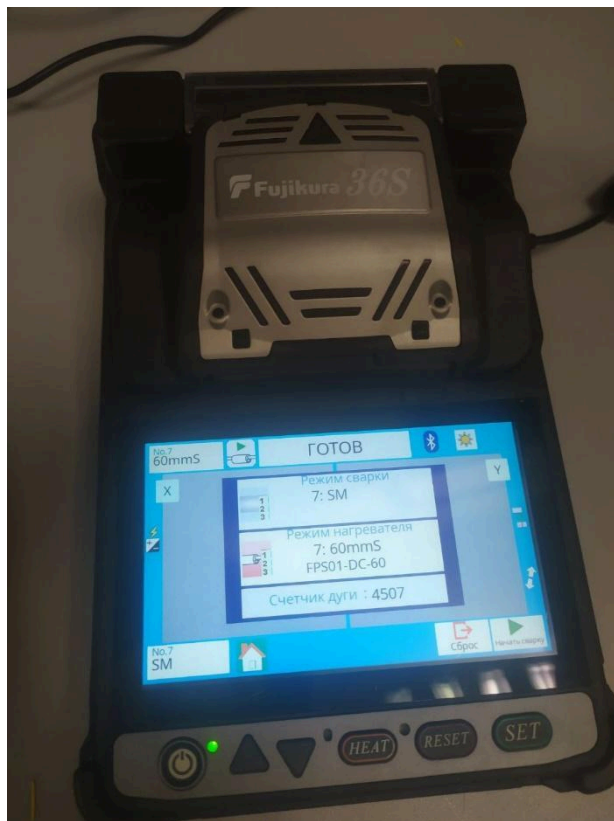
*Figure 3.1.1.1: Fujikura CT08 cleaver*

The splicing, performed with the Fujikura 36S splicer (Figure 3.1.1.3), is a critical step where the cut ends of the fiber are fused. This device also assesses the splicing efficiency by measuring the power loss in decibels.



*Figure 3.1.1.2: Interferometer stock*

In the pursuit of enhancing sensor quality, any splicing resulting in losses greater than  $\sim 0.08$  dB was redone, setting a higher standard than the 0.2 dB loss warning threshold of the device.



***Figure 3.1.1.3: Fujikura 36S splicer***

Following a successful splice, a precise marking is made 1-2 mm from the side of the interferometer fiber, where it is then cut using the Fujikura CT08 cleaver. Interferometers trimmed to this specific length have demonstrated optimal performance in SDI sensors.

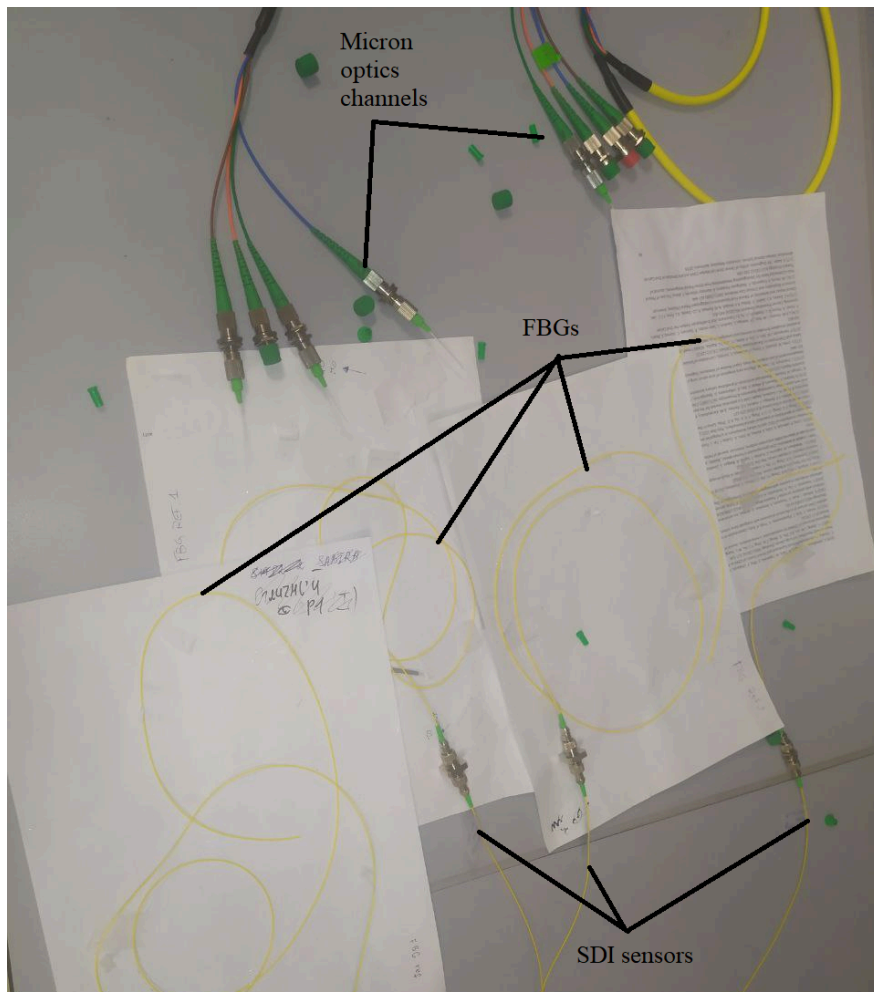
**3.1.2 Calibration**

The calibration of the fabricated sensors is a detailed process, crucial for ensuring their accuracy and sensitivity in detecting specific biological targets. Initially, the interferometric tips of the sensors are exposed to different calibration environments, including air and a series of sucrose solutions. The calibration regimen involves placing the sensor tips in air, followed by immersion in 6ml of a 10% sucrose solution and five subsequent 400-microliter increments of a 40% sucrose solution, totaling seven distinct calibration environments. This procedure employs the “Enlight” software in conjunction with the Micron Optics “Hyperion” device (Figure 3.1.2.1), which facilitates the sensing and recording of signals through a Fiber Bragg Grating (Figure 3.1.2.2).



***Figure 3.1.2.1: “Hyperion” micron optics device***

FBGs serve as crucial reference points within the fiber optic system. By reflecting a specific wavelength of light while allowing others to pass, they offer a consistent marker for calibration or measurement adjustments between devices. This is particularly important as the HYPERION device may not always detect peaks in the SPR sensors, potentially complicating data interpretation. The inclusion of an FBG ensures a prominent and consistent peak, aiding in the calibration process.



**Figure 3.1.2.2: Calibration setup**

The calibration data collected is then analyzed using a MATLAB script specifically developed for this project. This script processes the signal data, assessing sensor sensitivity in decibels per RIU. By processing the signal data from the Micron Optics “Hyperion” device, the script identifies changes in light interaction within the sensors, directly correlating to refractive index variations. This analysis facilitates the calculation of sensor sensitivity,

producing concise graphical representations of sensor performance against the calibration substances.

Calibration is performed to ensure that each sensor's response to refractive index changes can be accurately measured and compared. The use of air and sucrose solutions with known refractive indices creates a controlled set of conditions that allows for the consistent assessment of sensor performance. By calibrating sensors against these known standards, it is possible to generate reproducible and reliable graphs of sensor sensitivity, crucial for subsequent detection tasks. This calibration process ensures that the sensors are precisely tuned for the specific detection of breast cancer cells, utilizing the CD44 antibodies for targeted sensing.

### **3.2 Surface Functionalization**

The process of surface functionalization is pivotal in preparing the optical fiber sensors for specific detection of breast cancer cells through CD44 antibody binding. This section details the meticulous protocol followed to achieve the desired sensor functionality, divided into several subsections to elaborate on the significance and methodology of each step.

#### **3.2.1 Preparation & Piranha solution treatment**

The process begins in a carefully prepared controlled environment, where sensors, once separated from their pigtailed, are affixed to glass rods to facilitate subsequent chemical treatments. The pigtailed, integral for future detection stages, are stored for later reattachment. The cleaning phase involves the application of a piranha solution, meticulously prepared by mixing 12ml of sulfuric acid ( $H_2SO_4$ ) with 3ml of hydrogen peroxide ( $H_2O_2$ ), to rid the sensor surfaces of organic residues. This step ensures the sensors are primed for Silanization, followed by a comprehensive rinse with deionized water to eliminate any remaining cleaning agent.

#### **3.2.2 Silanization**

Silanization introduces functional groups to the sensor surface via the application of (3-Aminopropyl)trimethoxysilane (APTMS) in methanol, utilizing a solution comprised of 14.85ml of methanol and 0.150 $\mu$ l of APTMS. APTMS serves as a saline coupling agent that binds to the hydroxyl groups present on the cleaned sensor surface, introducing amine groups that are essential for the subsequent cross-linking step. These amine groups are reactive sites for the attachment of glutaraldehyde, enabling a stable linkage to the CD44 antibodies. Methanol's low surface tension ensures the uniform coverage of the sensor surface. After Silanization, sensors are washed with methanol to prevent hydrolysis of the silane bonds, preserving the integrity of the functionalized layer.

### **3.2.3 Cross-linking & Antibody Attachment**

The subsequent heating at 110°C for 60 minutes aims to remove solvents and promote cross-linking of silane molecules, enhancing the durability of the functionalized surface. The application of glutaraldehyde, in a 50% solution mixed in equal parts with phosphate-buffered saline (PBS) to achieve a 50% glutaraldehyde solution, facilitates the formation of stable covalent bonds between the amine groups on the sensor surface and the CD44 antibodies, crucial for antibody immobilization. 500  $\mu$ l GA and 500  $\mu$ l PBS were used each time.

### **3.2.4 Antibody Binding & Blocking**

The sensors are then incubated with CD44 antibodies in a solution of 10  $\mu$ g/mL at 4°C, prepared by diluting 10 $\mu$ l of antibody stock in 990 $\mu$ l of PBS. This specific binding is fundamental for the sensor's detection capabilities. To minimize non-specific binding, the sensors are treated with a blocking solution of 10% m-PEG in PBS, formulated by mixing 30 $\mu$ l of m-PEG with 270 $\mu$ l of PBS, which effectively occupies any unbound sites on the sensor surface.

### **3.2.5 Final Steps & Storage**

Following the blocking step, sensors are rinsed with PBS to remove unbound m-PEG and stored in PBS within a laboratory refrigerator. This storage method is temporary, as prolonged storage could potentially diminish the functional integrity of the antibodies.

Consequently, detection experiments are ideally conducted within one to two days post-functionalization, ensuring the optimal performance of the sensors.

This comprehensive functionalization protocol equips the optical fiber sensors with the necessary biochemical interface for specific and efficient detection of breast cancer cells through CD44 antibody binding. There were insignificant departures in terms of volumes of reagents for convenience of saving said reagents and accommodating for large numbers of sensors and sticks/rods in one vial/beaker, however the ratios always remained the same as in steps of the section 3.2.

### **3.3 Cell growth**

The growth and maintenance of HCC1806 breast cancer cells in the laboratory were conducted following a standardized protocol, ensuring optimal conditions for cell viability and proliferation. Initially, the culture medium was discarded from the 75 cm<sup>2</sup> flasks, which were then rinsed with a 0.25% (w/v) Trypsin-0.53 mM EDTA solution to eliminate serum traces containing trypsin inhibitors. Subsequently, 2.0 to 3.0 mL of Trypsin-EDTA solution was applied to each flask. The cells were observed under an inverted microscope until the cell layer dispersed, a process taking approximately 5 to 15 minutes. It was critical to avoid agitating the cells during this period to prevent clumping. For cells that exhibited difficulty detaching, a brief incubation at 37°C was employed to aid their dispersal. Once detached, 6.0 to 8.0 mL of complete growth medium was added, and the cells were gently aspirated by pipetting to ensure a uniform suspension. This suspension was then allocated into new culture vessels at a subculture ratio ranging from 1:2 to 1:4, and incubated at 37°C to promote growth.

To accurately quantify the cell density for subsequent experiments, a standard calculation using trypan blue dye exclusion was employed. This technique allowed for the differentiation between viable and non-viable cells, providing an accurate cell count per mL. The dye exclusion method was pivotal for adjusting the cell concentration to the desired level for detection experiments. Ensuring the cell viability at the point of detection, cells were utilized within one hour after being sub-cultured. This practice was important in maintaining high cell viability, thereby it is aimed at increasing the reliability of the detection outcomes.

### 3.4 Detection

The detection phase commenced with the precise calculation of the number of HCC1806 breast cancer cells present in the stock solution, employing ratio mathematics for accurate dilution. Initially, a specific volume of cell stock was diluted in Phosphate-Buffered Saline (PBS) to achieve a concentration of 1 million cells per 1 ml of PBS. This concentration served as the basis for subsequent serial dilutions, wherein 100 microliters of the solution were diluted in 900 microliters of PBS, creating a range from  $10^6$  to  $10^0$  cells per ml with single power increments (Figure 3.4.1). This methodical dilution process resulted in a total of eight measurements, starting with PBS alone (as a negative control) and incrementing through to the highest concentration of  $10^6$  cells per ml.



**Figure 3.4.1: 7 concentrations of serial dilution**

In addition to this standard dilution series, an alternative set of concentrations was tested to include both PBS and RPMI1640 media as solvents, aiming to compare the detection efficiency in different media environments. This set comprised eight distinct concentrations, ranging from PBS alone and RPMI1640 media alone (both serving as negative controls) to

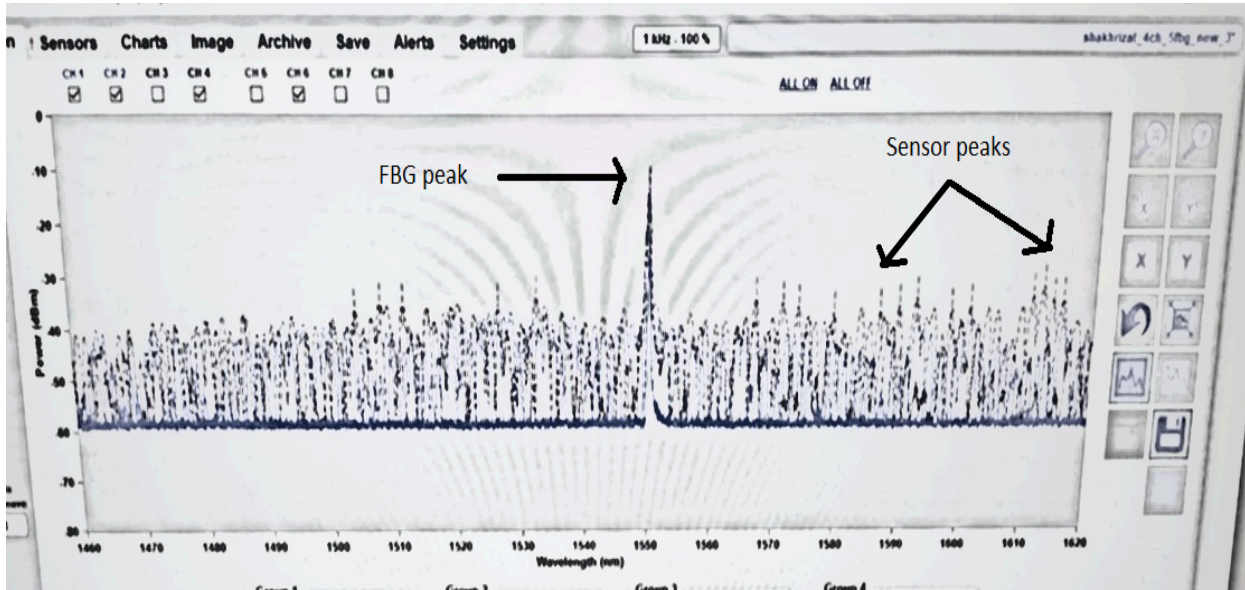
various cell concentrations in PBS and RPMI1640 media, peaking with 1,000,000 cells in PBS and descending to 10,000 cells in RPMI1640 media. The inclusion of RPMI1640 media was strategic, serving as a comparative measure against the PBS measurements, to assess the sensors' sensitivity across different media types.

Following the preparation and measurement of these cell concentrations, MATLAB scripts were employed to analyze the data, generating graphs that illustrate the sensitivity of the SDI sensors to the presence of HCC1806 breast cancer cells. These graphs were pivotal in visualizing the sensors' performance, highlighting their capability to detect varying concentrations of breast cancer cells with precision. The combination of serial dilutions and computational analysis provided a comprehensive view of the sensor's sensitivity, enabling the preliminary understanding of their potential application in cancer detection.

# Chapter 4 – Results and Discussions

## 4.1 Sensor Calibration & Sensitivity

In the calibration and sensitivity evaluation phase of the biosensors, the research focused on assessing the initial performance metrics of the sensors fabricated for the purpose of breast cancer cell detection. This phase was constrained by the timeline of the thesis, which will be further discussed in section 4.5, limiting the number of sensors produced and the breadth of detections performed. Despite these limitations, the preliminary results, documented between March 21st, 2024, and April 5th, 2024, across three sets of experiments, provided valuable insights. The date and timelines of fabrication and detection events are mentioned in this chapter because in the context of this experiment antibody and cell viability can be affected by the amount of time they are kept in PBS post-functionalization and post-sub-culturing. Figure 4.1 depicts a desirable calibration pattern during calibration. 2 sensors are attached to 2 out of 4 active channels in Enlight. Both sensors show distinct peaks and the grating piece through which they are attached is also clearly visible is the highest peak (FBG peak).



**Figure 4.1.1: Desirable calibration pattern**

The calibration results for sensors manufactured on March 8th, 2024, are presented in Table 4.1. The sensitivity of these sensors, a critical parameter for the efficacy of the detection system, ranged from 144.56 dB/RIU to 267.68 dB/RIU. This sensitivity spectrum not only exceeds the 100 dB/RIU threshold deemed necessary for proceeding with further experiments but also signifies an above-average sensitivity for sensors employing this specific type of interferometer. Consequently, the entire batch was deemed suitable for subsequent experimental stages.

**Table 4.1.1: First batch sensor sensitivity (08.03.2024)**

SP4 #	Sensitivity (dB/RIU)
1	152.17
2	164.72
3	194.52
4	180.98
5	144.56

6	267.68
---	--------

A more extensive batch of 24 sensors was produced on March 25th, comprising an equal distribution of SP3 and SP4 sensors (Table 4.2). Notably, the sensitivity of these sensors, which could be influenced by the quality of the single-mode optic fiber and the handling of the pigtails, did not exhibit significant variation between the SP3 and SP4 models. This observation suggests a consistency in manufacturing quality, with 15 sensors exceeding the 100 dB/RIU sensitivity benchmark.

**Table 4.1.2: Second batch sensor sensitivity (25.03.2024)**

SP3 #	Sensitivity (dB/RIU)	SP4 #	Sensitivity (dB/RIU)
1	140.73 (+)	1	77.22 (-)
2	12.06 (-)	2	576.02 (+)
3	38.26 (-)	3	144.50 (+)
4	129.03 (+)	4	109.15 (+)
5	134.57 (+)	5	72.14 (-)
6	81.77 (-)	6	39.21 (-)
7	128.84 (+)	7	339.90 (+)
8	58.73 (-)	8	108.12 (+)
9	127.48 (+)	9	136.07 (+)
10	84.44 (-)	10	115.39 (+)
11	49.73 (-)	11	73.97 (-)
12	207.86 (+)	12	116.72 (+)

Further, Table 4.1.3 outlines the sensitivities of SDI sensors functionalized on March 20th and tested the following day. Sensors 1-5, as listed in Table 4.1.1, were included in this set, displaying sensitivities between 144.56 dB/RIU and 194.52 dB/RIU. Subsequent tables

(Tables 4.1.4 and 4.1.5) document the sensitivities of sensors functionalized and tested on later dates, with the range extending from 108.12 dB/RIU to 576.02 dB/RIU on the third detection set. This wide disparity in sensitivity readings underscores the variability inherent in the sensor functionalization process and presents an opportunity to explore the correlation between calibration sensitivity and detection efficacy.

***Table 4.1.3: Sensitivities of the first detection set***

Detection date - 21.03.24		
Sensor name	Sensitivity (dB/RIU)	Production date
SP4 – Sensor 1	152.17	08.03.24
SP4 – Sensor 2	164.72	08.03.24
SP4 – Sensor 3	194.52	08.03.24
SP4 – Sensor 4	180.98	08.03.24
SP4 – Sensor 5	144.56	08.03.24

***Table 4.1.4: Sensitivities of the second detection set***

Detection date - 31.03.24		
Sensor name	Sensitivity (dB/RIU)	Production date
SP4-sensor 12	116.72	25.03.24
SP3-sensor 9	127.48	25.03.24
SP4-sensor 10	115.39	25.03.24
SP4-sensor 7	339.9	25.03.24
SP3-sensor 12	207.86	25.03.24

**Table 4.1.5: Sensitivities of the third detection set**

Detection date - 05.04.24		
Sensor name	Sensitivity (dB/RIU)	Production date
SP4-sensor 2	576.02	25.03.24
SP3-sensor 5	134.57	25.03.24
SP4-sensor 8	108.12	25.03.24
SP4-sensor 9	136.07	25.03.24
SP3-sensor 7	128.84	25.03.24

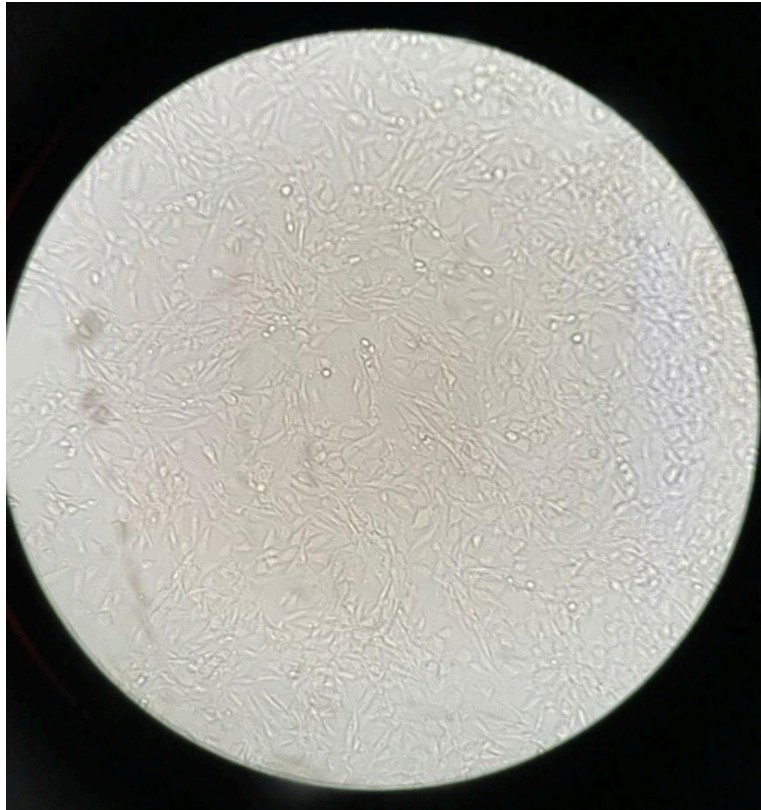
Overall, the calibration phase yielded a comprehensive range of sensor sensitivities from 108.12 dB/RIU to 576.02 dB/RIU with a mean value of maximum sensitivity 188.53 dB/RIU. This variability not only highlights the potential for optimization in the sensor manufacturing process but also sets the stage for in-depth analysis of how sensor sensitivity impacts the accuracy and reliability of breast cancer cell detection in the broader scope of the experiment.

## 4.2 HCC1806 Count

The experiment heavily relies on viability of the HCC1806 cell culture, where the number of live cells directly impacts the perceived level of detection. Handling cells should always be performed with care during growth and storage as to keep the actual number of cells in stock close to the calculated number. It was noted after serial dilution that higher concentrations  $10^5$  and  $10^4$  (cells/ml) did not have sufficient muddiness associated with the abundant presence of cells, which hints at the presence of errors either during cell growth, storage or serial dilution. Such trend was observed during all 3 preliminary detection sessions. It might have caused a significant departure from the expected results. Elimination of this error requires a higher number of detections with a higher number of SDI sensors to establish whether this error is there and if it is, what is its origin.

## 4.3 Detection

To start with, the HCC1806 cells were checked under a light microscope for viability. Figure 4.3.1 shows the achieved abundancy of cancer cells in the stock prepared for detection.

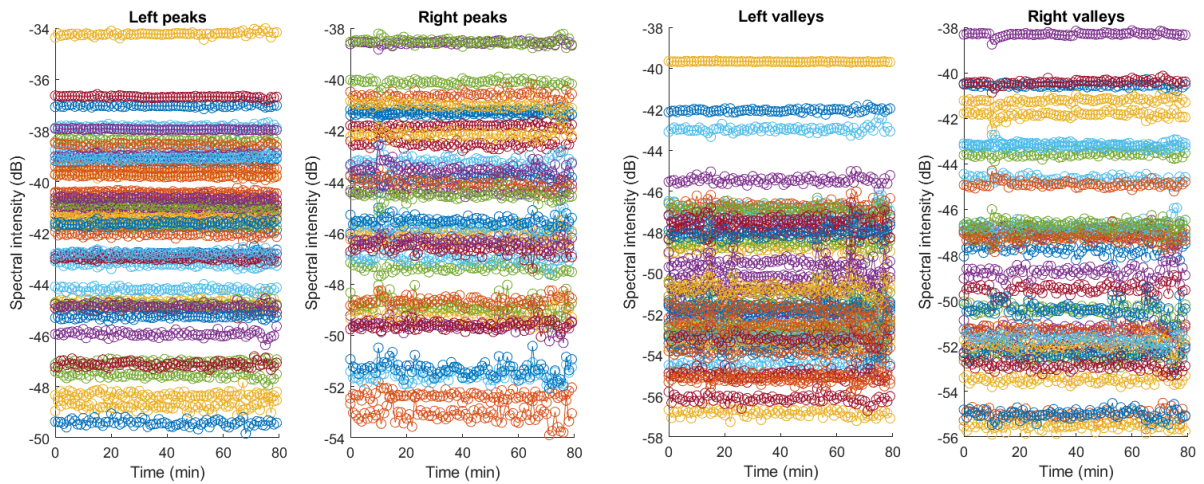


*Figure 4.3.1: HCC1806 cells under light microscope*

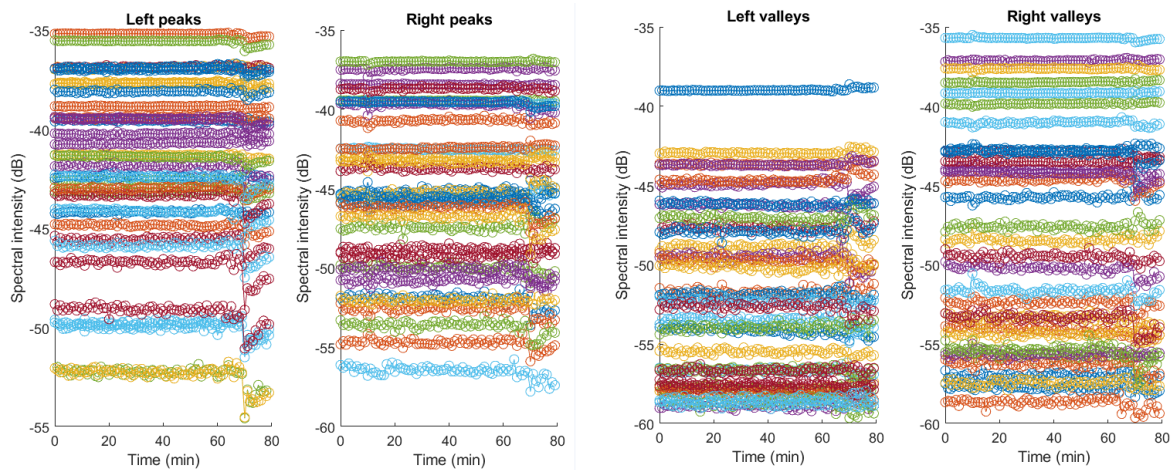
Two MATLAB codes were used to first load the measurement files and then analyze and create graphs with custom parameters. The graphs show signal intensity against time. Peaks and valleys to the left and the right of the FBG signal (see Figure 4.1.1) are represented.

To compare and show the difference, the lowest and highest sensitivity sensors were compared from the first set of detection (Table 4.1.3). In Figures 4.3.2 and 4.3.3 it is visible that there is no detectable difference in terms of intensity of the signal. Since 8 concentrations were recorded every minute for 10 minutes each, the timeline is  $10 \times 8 = 80$  minutes for each separate graph. Notably, SP4-Sensor3 only shows detection at the highest concentration (70-80-minute intervals) with a high level of phase shift only at  $10^6$  cells/ml. On the other hand, Lower sensitivity SP4-Sensor 5 shows detection at 60-80-minute intervals, indicating a better low limit of detection that starts from  $10^5$  cells/ml. This shows that there is no correlation between higher or lower sensitivity within the batch and the level of HCC1806 cell detection. All 5 sensors from the first set showed consistent detection at the last

concentration of  $10^6$  cells/ml with only two of them showing spectral activity at  $10^5$  cells/ml concentration. Although the presence of intensity fluctuation shows that breast cancer cell attachment is successful, the lower LoD is very poor compared to similar studies



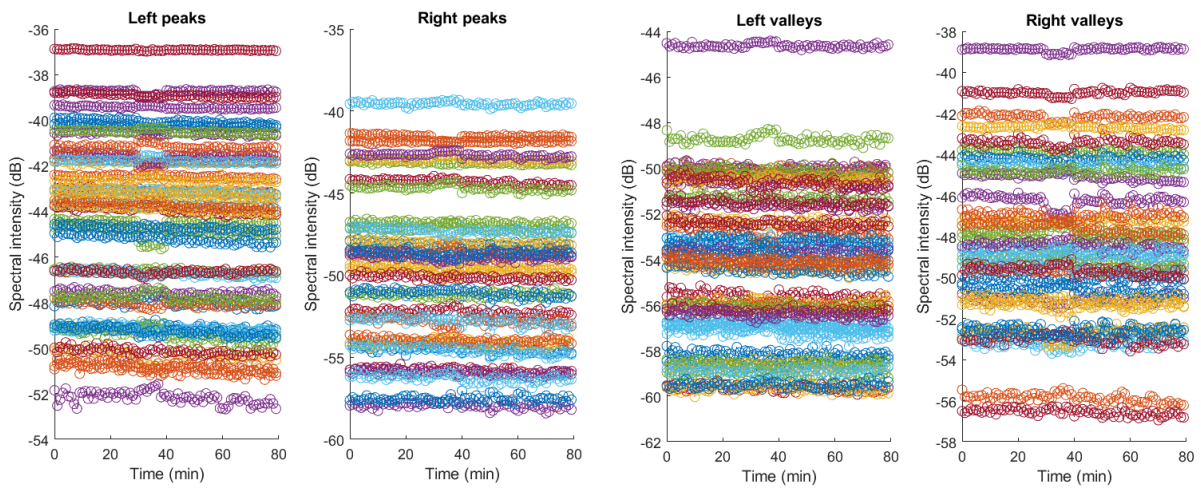
**Figure 4.3.2: Highest sensitivity SP4-Sensor 3 detection (Set 1)**



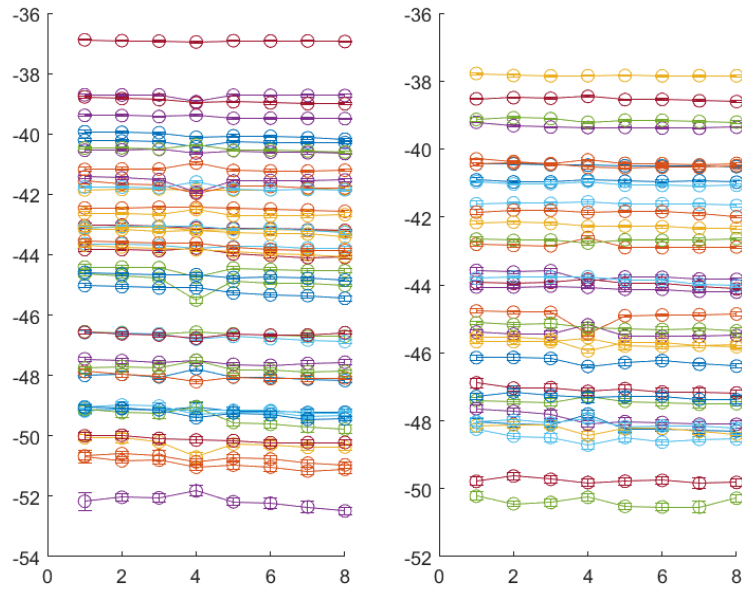
**Figure 4.3.3: Lowest sensitivity SP4-Sensor 5 detection (Set 1)**

Next, the second set was characterized by the involvement of RPMI media and the elimination of low concentrations. Instead  $10^4$ – $10^6$  cells/mL were tested in both PBS and RPMI. In this set, the cells were detected each minute for 10 minutes as well. 0-40-minute intervals reflect the lowest-to-highest concentrations of cells in PBS and 40-80-minute

intervals reflect the lowest-to-highest concentrations of cells in RPMI media. As an example, SP4-Sensor 7 from the second set (Figure 4.3.4) was selected. Spectral activity at 30-40 minutes is the only 10-minute interval showing deviation from the rest of the signal detection timeline. This means that only the highest concentration  $10^6$  cells/mL of cells in PBS is detected, while RPMI growth media shows no detection at all. It might suggest that RPMI prevents effective light scattering at the tip of the interferometer. Figure 4.3.5 shows average and standard deviation of each value averaged over the measurement time for SP4-Sensor 7 and it is seen that only measurement 4 ( $10^6$  cells/mL HCC1806 concentration in PBS) shows significant deviation..

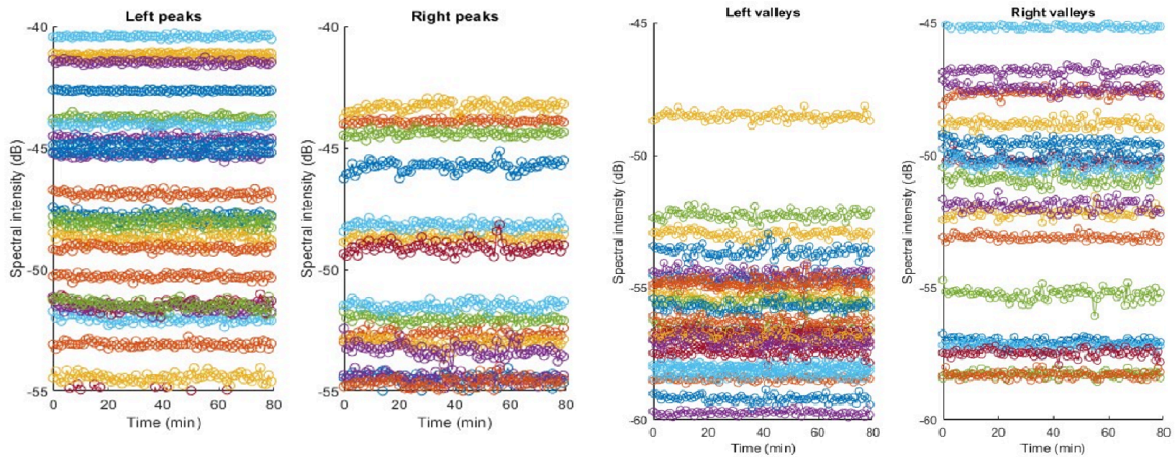


**Figure 4.3.4: SP4-Sensor 7 detection (Set 2)**



**Figure 4.3.5: SP4-Sensor 7 averaged values (Set 2)**

Lastly, the third set showed the worst performance, where two out of 5 sensors showed no detection at all, and one other showed a grift-type response that did not depend on concentrations as well. Notably, SP4-sensor 2 which has the highest concentration among all 3 sets was one of the sensors with no detection. Only two out of 5 sensors showed small spectral intensity deviation at the last concentration. Figure 4.3.6 shows one of those two sensors. Here, measurements were conducted for 20 minutes and the first four concentrations were removed from the graph. 60-80-minute interval shows a small spectral intensity shift, which is consistent with the  $10^6$  cells/mL concentration.



**Figure 4.3.6: SP4-Sensor 8 detection (Set 3)**

#### 4.4 Challenges & Constraints

Throughout the course of this project, several significant challenges were encountered that influenced both the methodology and the results obtained. These challenges, ranging from equipment malfunctions to logistical constraints, had profound implications on the overall execution and outcomes of the research.

Initially, the project was set back by the malfunction of the advanced Fujikura Laser Splicing System, a critical component in the fabrication of the optical fiber sensors. The complexity of repairing this system exceeded the available resources in terms of time, effort, and funding, leading to an unavoidable delay. Compounding this issue was the breakdown of the LUNA Optical Backscatter Reflectometer (OBR) machine, necessitating a switch to the Hyperion Micron Optics system for signal recording. This substitution, while pragmatic, likely impacted the project's efficacy, as the OBR system offers superior capabilities for refractive index profiling, crucial for studies centered on Semi-Distributed Interferometry (SDI). The shift in equipment is believed to have affected the sensitivity and quality of the sensors, as well as the efficiency of their production and analysis. The constrained timeline further limited the quantity of detection sets that could be analyzed, falling short of the intended 20-30 sets necessary for robust conclusions regarding the detection of breast cancer cells using SDI sensors. Additionally, the planned comparison of detection efficiency through flow cytometry was deferred due to these timing constraints.

Access to a biological laboratory posed another significant challenge, particularly because the nature of this project required cell passaging, diverging from the typical

requirements for biomarker-based studies. Laboratory access was secured only a month prior to the thesis submission, severely limiting the scope of experimental work that could be conducted. Additionally, sourcing the specialized interferometric fiber, not readily available in the market, presented another difficulty. Even when basic components like optical fiber pigtailed and cancer cells were obtainable and affordable, delivery delays occasionally disrupted the project timeline.

Regarding the experimental results, the anticipated detection of low-concentration cancer cells did not materialize in the observed data. This outcome may partly stem from the shift to the Hyperion Micron Optics system and potential conceptual flaws, such as unintended functionalization along the sensor sides, introducing noise into the signal detection process. It was previously shown that sensors can be functionalized on the cladding of the sensor (27). However, cell viability is deemed an unlikely factor, given the immediate use of cells post-passaging. Despite these setbacks, it's recognized that standard experimental errors, including those associated with pipetting and measurement, likely influenced the results to some extent, though their impact is considered to be minimal.

These challenges underscore the complexities and unforeseen hurdles inherent in experimental research, particularly when reliant on sophisticated equipment and specific laboratory conditions. The experiences gained from navigating these obstacles are invaluable, contributing to a deeper understanding of the practical aspects of conducting high-stakes research and the importance of flexibility and resilience in the face of adversity.

## Chapter 5 - Conclusion

In this thesis, we embarked on an exploratory journey to assess the feasibility of employing SDI sensors for the detection of whole breast cancer cells. Despite the ambitious nature of this endeavor, the investigation confronted a spectrum of challenges that inevitably shaped the trajectory and outcomes of the research. Notably, the lowest limit of detection (LoD) achieved was at  $10^5$  cells/mL, with the most reliable detection observed at a concentration of  $10^6$  cells/mL. While these findings affirm the potential of SDI sensors in capturing and identifying breast cancer cells, the sensitivity levels attained fall short of practical application, as concentrations of cancer cells at such elevated levels are unlikely in biological specimens. It is obvious that at this stage the results do not support the idea of SDI cancer cell biosensing

The examination revealed an insight: the variability in sensor sensitivity observed during calibration exercises did not significantly impact the efficacy of cancer cell detection. This suggests that a lower sensitivity threshold of 100dB/RIU may serve as an adequate benchmark for future research utilizing SDI sensors in the domain of whole cancer cell detection. However, it is imperative to acknowledge the array of both anticipated and unforeseen hurdles encountered throughout this study—from equipment malfunctions to logistical constraints—which have, to some extent, compromised the depth and breadth of data presented.

Nevertheless, it is within these challenges that opportunities for growth and improvement emerge. The experiences and insights garnered from navigating these obstacles provide a valuable foundation for future endeavors in this field. The pursuit of refining and optimizing SDI sensor technology for the early detection of breast cancer remains a worthy and compelling objective. While the results of this initial exploration may not have met all expectations, the groundwork laid by this research illuminates a path forward, highlighting areas for development and pointing towards the potential for significant advancements in non-invasive cancer diagnostics.

In closing, the journey of this thesis, though marked by setbacks, stands as a testament to the iterative nature of scientific inquiry—a process defined not by its pitfalls, but by its potential to drive progress through perseverance and innovation. The promise shown by SDI

sensors in detecting breast cancer cells, albeit at higher concentrations than ideal, lays the foundation for continued exploration and improvement. As we forge ahead, the lessons learned and the knowledge accrued from this study will undoubtedly contribute to the evolution of more sensitive, accurate, and practical tools for cancer detection, underscoring the enduring optimism that defines the spirit of scientific research.

# Literature Cited

1. **Garcion, E., et al.**, "Cancer stem cells: Beyond Koch's postulates," *Cancer Letters*, 278, pp. 3–8 (2009).
2. **Siegel, R.L., et al.**, "Cancer statistics, 2017," *CA: A Cancer Journal for Clinicians*, 67, pp. 7–30 (2017).
3. **Molyneux, E., et al.**, "Cancer treatment in low- and middle-income countries," in *Oxford Textbook of Cancer in Children*, pp. 90–96 (2020).
4. **Rakhimbekova, A., et al.**, "Fiber-optic semi-distributed Fabry-Perot interferometer for low-limit label-free detection of CCL5 cancer biomarker," *Optics & Laser Technology*, 168 (2024).
5. **Murlidhar, V., et al.**, "Poor prognosis indicated by venous circulating tumor cell clusters in early-stage lung cancers," *Cancer Research*, 77, pp. 5194–5206 (2017).
6. **Hepp, P., et al.**, "Association of ca27.29 and circulating tumor cells before and at different times after adjuvant chemotherapy in patients with early-stage breast cancer – the success trial," *Anticancer Research*, 36, pp. 4771–4776 (2016).
7. **Ott, J.J., Ullrich, A., Miller, A.B.**, "The importance of early symptom recognition in the context of early detection and cancer survival," *European Journal of Cancer*, 45, pp. 2743–2748 (2009).
8. **Schiffman, J.D., Fisher, P.G., Gibbs, P.**, "Early detection of cancer: Past, present, and future," *American Society of Clinical Oncology Educational Book*, pp. 57–65 (2015).
9. **Leitão, C., et al.**, "Cost-effective fiber optic solutions for Biosensing," *Biosensors*, 12 (2022) 575.
10. **Mehrotra, P.**, "Biosensors and their applications – A Review," *Journal of Oral Biology and Craniofacial Research*, 6, pp. 153–159 (2016).
11. **Kumar, S., et al.**, "Optical fiber-based plasmonic biosensors: Trends, techniques and applications," CRC Press, Boca Raton, Florida, FL (2023).
12. **Kal-Koshvandi, A.**, "Recent advances in optical biosensors for the detection of cancer biomarker  $\alpha$ -fetoprotein (AFP)," *TrAC Trends in Analytical Chemistry*, 128 (2020).

13. **Tosi, D., et al.**, "Minimalistic design and rapid-fabrication single-mode fiber biosensors: Review and Perspectives," *Optical Fiber Technology*, 72 (2022).
14. **Hu, Y., et al.**, "Detection of circulating tumor cells and circulating tumor stem cells in breast cancer by using flow cytometry," *Tumor Metastasis*, 33, pp. 561–569 (2016).
15. **Vajhadin, F., et al.**, "Electrochemical cytosensors for detection of breast cancer cells," *Biosensors and Bioelectronics*, 151 (2020).
16. **Yun-Jiang, R., et al.**, "Fiber-optic Fabry-Perot sensors: An introduction," 1st ed., CRC Press, Boca Raton, Florida, FL (2025).
17. **Kazhiyev, S., et al.**, "Semi-distributed interferometers fiber-optic sensors for high-sensitivity refractive index detection: Design and Sensitivity Analysis," *Measurement*, 220 (2023) 113327.
18. **Rakhimbekova, A., et al.**, "Semi-distributed fiber-optic interferometer as a simple and rapid sensor for detection of cancer biomarkers." (2023).
19. **Chen, X., et al.**, "Label-free detection of breast cancer cells using a functionalized tilted fiber grating," *Biomedical Optics Express*, 13 (2022).
20. **Yang, H., et al.**, "Intensity-modulated refractive index sensor based on the side modes of fiber Bragg grating," *Optics Communications*, 505 (2022).
21. **Karipbayeva, K., et al.**, "Optical fiber semi-distributed interferometer assisted by an FBG for thermorefraction and sweat sensing," *IEEE Sensors Journal*, 23 (2023).
22. **Loyez, M., et al.**, "Rapid detection of circulating breast cancer cells using a multiresonant optical fiber aptasensor with plasmonic amplification," *ACS Sensors*, 5, pp. 454–463 (2020)
23. **Liu, Z., et al.**, "Microstructured optical fiber-enhanced light–matter interaction enables highly sensitive exosome-based liquid biopsy of breast cancer," *Analytical Chemistry*, 95, pp. 1095–1105 (2023).
24. **Adilkhanova, A., et al.**, "Fiber optic refractive index sensing using an inline dual semi-distributed interferometer," *Optik*, 302 (2024).
25. **Ramesh, M., et al.**, "Nanotechnology-enabled biosensors: A Review of Fundamentals, design principles, materials, and applications," *Biosensors*, 13 (2022).

26. **Li, M., *et al.***, "Advances in novel nanomaterial-based optical fiber biosensors—a review," *Biosensors*, 12 (2022).
27. **Shaimerdenova, M., *et al.***, "Reflector-less shallow-tapered optical fiber biosensors for rapid detection of cancer biomarkers," *Journal of Lightwave Technology*, 41 (2023) 4114–4122.
28. **Blanc, W., *et al.***, "Nanoparticles in optical fiber, issue and opportunity of light scattering [invited]," *Optical Materials Express*, 12 (2022) 2635.
29. **Yang, Q., *et al.***, "A computerized global MR image feature analysis scheme to assist diagnosis of breast cancer: A preliminary assessment," *European Journal of Radiology*, 83, pp. 1086–1091 (2014).
30. **Sajan, S.C., *et al.***, "Silicon Photonics biosensors for cancer cells detection—a review," *IEEE Sensors Journal*, 23, pp. 3366–3377 (2023).
31. **Addanki, S., *et al.***, "Applications of circulating tumor cells and circulating tumor DNA in precision oncology for breast cancers," *International Journal of Molecular Sciences*, 23 (2022).
32. **Tellez-Gabriel, M., *et al.***, "Current status of circulating tumor cells, circulating tumor DNA, and exosomes in breast cancer liquid biopsies," *International Journal of Molecular Sciences*, 21 (2020).

## Further Reading

1. **Chen, L., *et al.***, "Ultrahigh-sensitivity label-free optical fiber biosensor based on a tapered singlemode- no core-singlemode coupler for *Staphylococcus aureus* detection," *Sensors and Actuators B: Chemical*, 320 (2020) 128283.
2. **Hu, J., *et al.***, "Fiber laser-based lasso-shaped biosensor for high precision detection of cancer biomarker-CEACAM5 in serum," *Biosensors*, 13 (2023) 674
3. **Loyez, M., *et al.***, "In situ cancer diagnosis through online plasmonics," *Biosensors and Bioelectronics*, 131 (2019) 104–112.
4. **Sharma, A.K., *et al.***, "Fiber-optic sensors based on surface plasmon resonance: A comprehensive review," *IEEE Sensors Journal*, 7 (2007) 1118–1129.

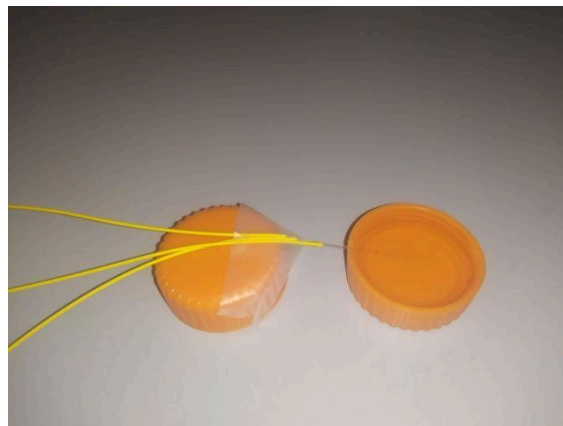
# Appendices

## Appendix A



*Figure A.1: Supplementary tools for sensor fabrication*

## **Appendix B**

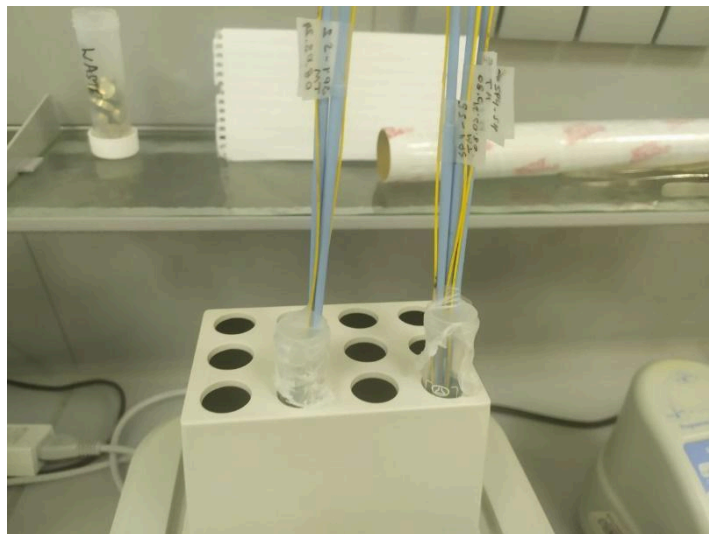


*Figure B.1: Sensors in sucrose during calibration*

## **Appendix C**

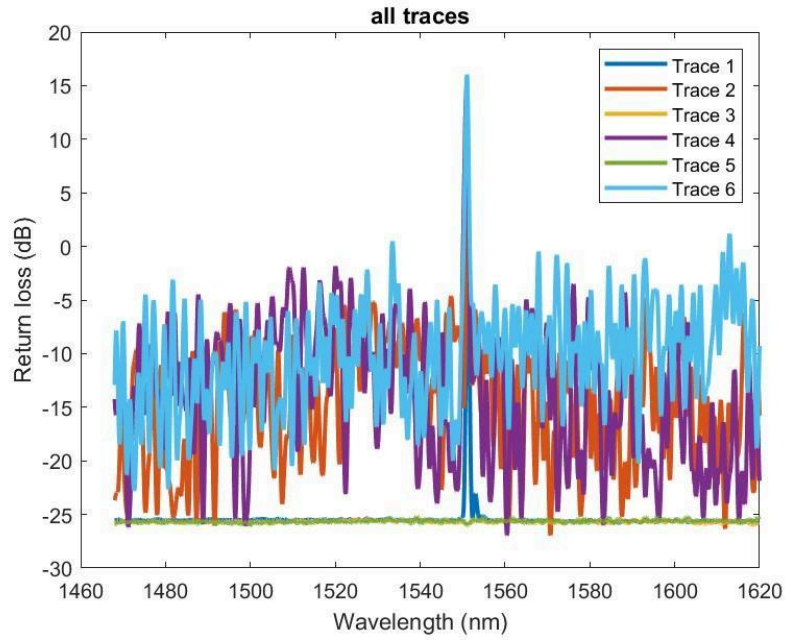


*Figure C.1: Sensors in oven*

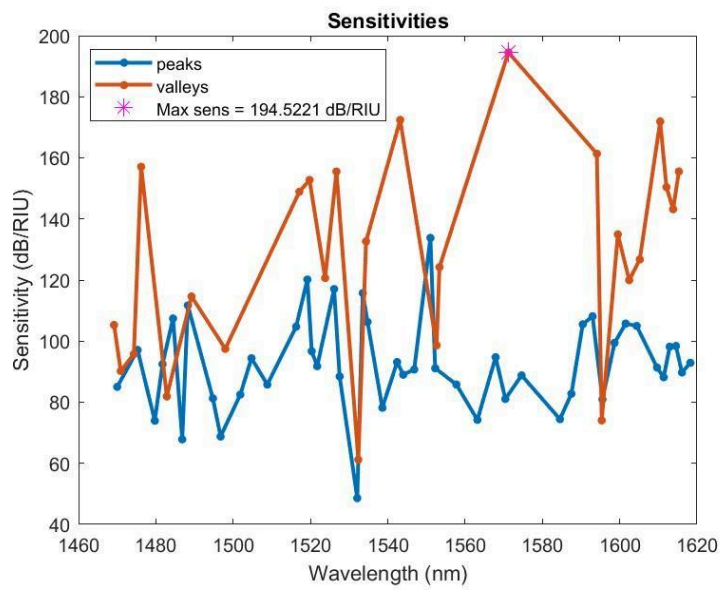


*Figure C.2: Ten Sensors in 1:1 GA+PBS in the shaker*

## **Appendix D**



**Figure D.1: Three sensors + reference FBG calibration signal**



**Figure D.2: High sensitivity sensor**

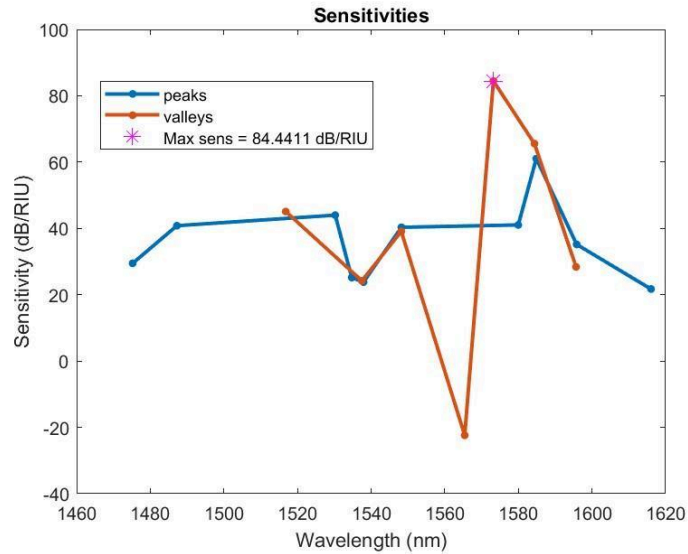


Figure D.3: Low sensitivity sensor

## Appendix E

```

7      %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
8
9      N_Conc = 3; % Number of Conc. values
10     N_Val = 20; % Number of times each Conc. was sampled
11
12     % Low-pass filter
13     [b,a] = butter(5,0.01);
14
15
16     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
17     %%%%%%%%% Load data from files %%%%%%%%%
18     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
19
20     kk = 1;
21     for ii = 6:8
22         for jj = 1:N_Val
23
24             Fname = strcat('concentration', num2str(ii), '_measurement', num2str(jj), '.txt');
25             disp(Fname);
26             Data = importdata(Fname);
27             Mat = Data.data;

```

Current Folder

- concentration5\_measurement10.txt
- concentration5\_measurement11.txt
- concentration5\_measurement12.txt
- concentration5\_measurement13.txt
- concentration5\_measurement14.txt
- concentration5\_measurement15.txt
- concentration5\_measurement16.txt
- concentration5\_measurement17.txt
- concentration5\_measurement18.txt
- concentration5\_measurement19.txt
- concentration5\_measurement20.txt

ScriptDataLoading.m (Script)

Command Window

New to MATLAB? See resources for [Getting Started](#).

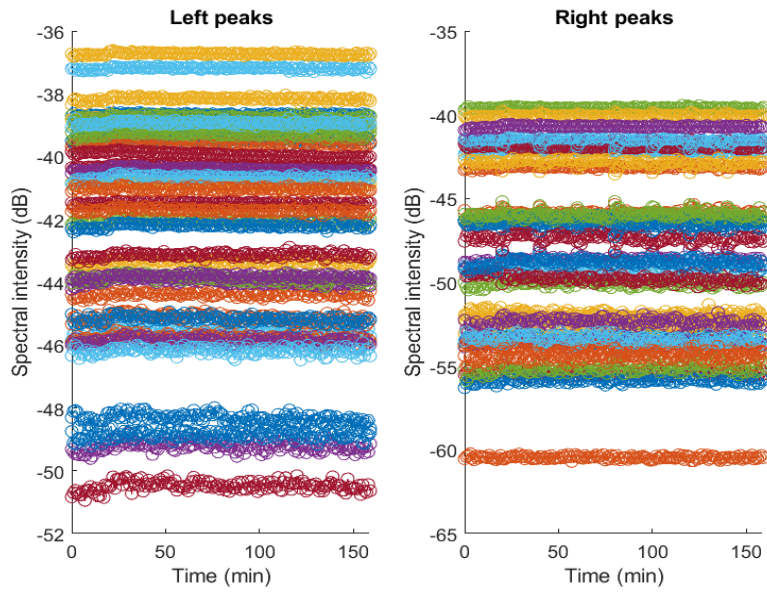
```

concentration8_measurement2.txt
concentration8_measurement3.txt
concentration8_measurement4.txt
concentration8_measurement5.txt
concentration8_measurement6.txt
concentration8_measurement7.txt
concentration8_measurement8.txt
concentration8_measurement9.txt
concentration8_measurement10.txt
concentration8_measurement11.txt
concentration8_measurement12.txt
concentration8_measurement13.txt
concentration8_measurement14.txt
concentration8_measurement15.txt
concentration8_measurement16.txt
concentration8_measurement17.txt
concentration8_measurement18.txt
concentration8_measurement19.txt
concentration8_measurement20.txt
>> save Experiment1.mat

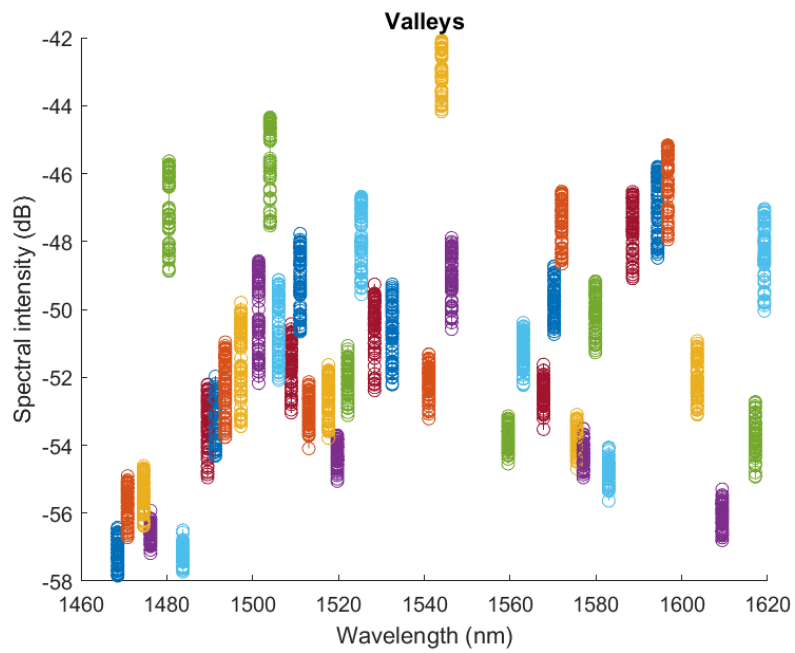
```

Figure E.1: MATLAB sensitivity analysis code excerpt

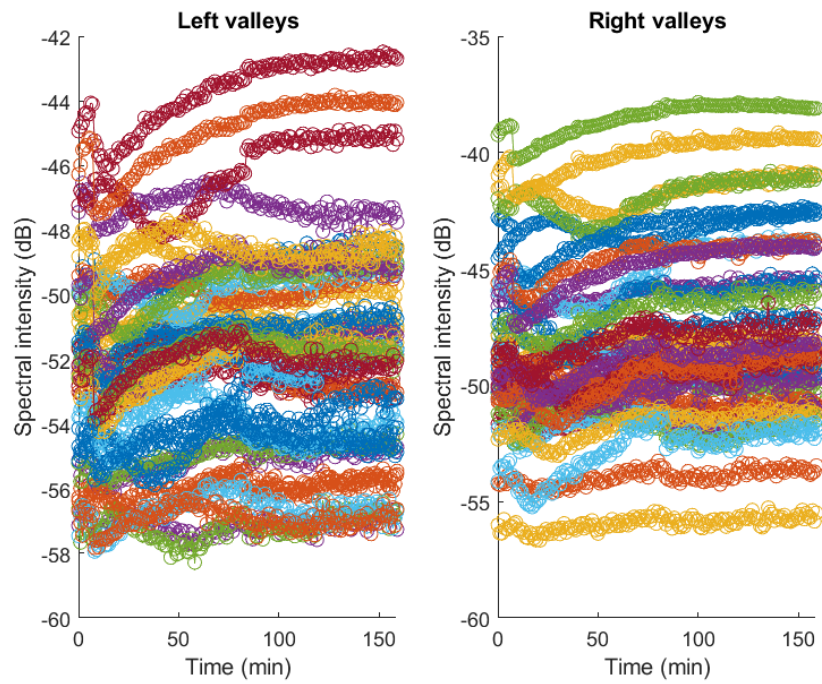
## Appendix F



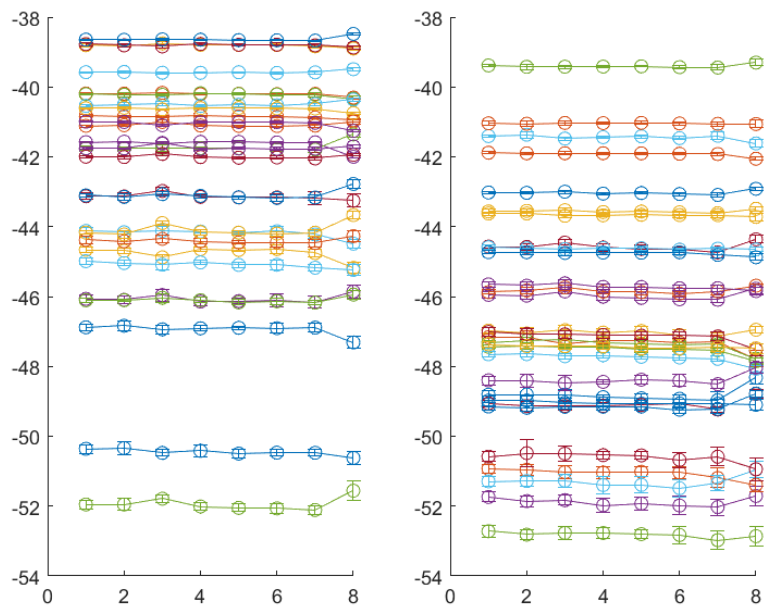
*Figure F.1: No detection spectral intensity pattern*



*Figure F.2: 3D view of a spectral intensity graph*



*Figure F.3: Grift-type spectral intensity pattern (faulty)*



*Figure F.4: Averaged spectral intensity values in the normal detection*