2017-09-01

Improved Single Molecule Detection Platform Using a Buried ARROW Design

Thomas Allen Wall
Brigham Young University

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Improved Single Molecule Detection Platform Using a Buried ARROW Design

Thomas Allen Wall

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Aaron Hawkins, Chair
Brian Mazzeo
Gregory Nordin
Stephen Schultz
Daniel Smalley

Department of Electrical and Computer Engineering
Brigham Young University

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ABSTRACT

Improved Single Molecule Detection Platform Using a Buried ARROW Design

Thomas Allen Wall
Department of Electrical and Computer Engineering, BYU
Doctor of Philosophy

As the microelectronics industry pushes microfabrication processes further, the lab-on-a-chip field has continued to piggy-back off the industry’s fabrication capabilities with the goal of producing total chemical and biological systems on small chip-size platforms. One important function of such systems is the ability to perform single molecule detection. There are currently many methods being researched for performing single molecule detection, both macro and micro in scale. This dissertation focuses on an optofluidic, lab-on-a-chip platform called the ARROW biosensor, which possesses several advantages over macro-scale single molecule detection platforms. These advantages include an amplification-free detection scheme, cheap parallel fabrication techniques, rapid single molecule detection results, and extremely low volume sample probing, which leads to ultra-sensitive detection.

The ARROW biosensor was conceived in the early 2000s; however, since then it has undergone many design changes to improve and add new functionality to the lab-on-a-chip; however, water absorption in the plasma enhanced chemical vapor deposited silicon dioxide has been a problem that has plagued the biosensor platform for some time. Moisture uptake in the oxide layer of the ARROWS leads to loss of waveguiding confinement and drastically decreases the overall sensitivity of the ARROW biosensors. New ARROW designs were investigated to alleviate the negative water absorption effects in the ARROWS. The new waveguide designs were tested for resiliency to water absorption and the buried ARROW (bARROW) design was determined to be the most successful at preventing negative water absorption effects from occurring in the PECVD oxide waveguides. The bARROWS were integrated into the full biosensor platforms and used to demonstrate high sensitivity single molecule detection without any signs of water absorption affecting the bARROWS’ waveguiding capabilities. The bARROW biosensors are not only water resistant, they also proved to be the most sensitive biosensors yet fabricated with average signal-to-noise ratios around 80% higher than any previously fabricated ARROW biosensors.

Keywords: Thomas A. Wall, Aaron Hawkins, optofluidics, single molecule detection, integrated optics, ARROW, fluorescence, biosensor, lab-on-a-chip, hollow waveguides, microfluidics, PECVD, water absorption
ACKNOWLEDGMENTS

I would like to acknowledge the large amount of support that I have received throughout my education here at BYU. First, I would like to thank my graduate advisor, Dr. Aaron Hawkins, who gave me my first taste of academic research, by hiring me into his undergraduate research program IMMERSE. I feel that this program truly “immersed” me into the world of academic research and helped me develop into the researcher I am today. I consider myself lucky to have been able to remain working alongside Dr. Hawkins as a graduate student and would like to thank him for his continued support and guidance.

I would also like to express my gratitude to the many colleagues and fellow researchers that I have had the opportunity to work with throughout my entire time here. I thank Dr. Matthieu Giraud-Carrier, my initial mentor, for all the training he offered me and I appreciate the lasting friendship that developed. I am also grateful to Cameron Hill, Matthew Stott, and many others that have assisted me on my project and for the friendships they have afforded me.

As a graduate student, I received funding through the Utah NASA Space Grant Fellowship and I would like to acknowledge their support and thank them for the opportunities that they afforded me. I also acknowledge that I have been supported with funds from NIH and NSF grants.

I also want to acknowledge Joshua Parks, Damla Ozcelik, Dr. Holger Schmidt and others, my collaborators on this project working at UCSC. They performed much of the required testing and biological handling required to demonstrate the functionality of our lab-on-a-chip biosensors.
Finally, I want to thank all my family members for their support over the years. I am especially appreciative of my wife, Holly. She has been very patient and remained supportive throughout the entire, long process, for which I will always be grateful.
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1 INTRODUCTION

1.1 Project Background

The end goal of the work in this dissertation is the creation of a rapid, cheap, portable, and highly sensitive, single molecule detection (SMD) platform. This work involves research in the fields of integrated optics and optofluidics in order to design, fabricate, and test a lab-on-a-chip platform that is truly capable of high sensitivity, single molecule detection.

There already exist many techniques and methods for performing single molecule detection; however, many of the most prominent techniques used today involve an amplification method to increase the detection signal to measure levels on the macro scale. Amplification brings with it some major disadvantages, which include a much longer detection process (the amplification step is typically quite long when compared to the actual detection mechanism) and adds in the possibility of erroneous amplification that can lead to false positives and even false negatives.

The focus of this dissertation is the anti-resonant reflecting optical waveguide (ARROW) biosensor, an optofluidic, lab-on-a-chip biosensor that makes use of integrated solid-core and liquid-core ARROWS to perform SMD directly on individual molecules, without the need of an amplification step. This method of detection allows for more confident SMD, because the molecules are detected directly, and it also represents a much faster detection method, significantly decreasing the time until results are available. The ARROW biosensor began to be
researched and investigated in the early 2000s and has been used to demonstrate SMD on a wide variety of biological objects, including several different viruses, bacteria, and cancer cells.

However, in the past, a major disadvantage of the ARROW biosensor has been the fact that, due to water absorption in the waveguides, it required post-fabrication annealing at a temperature of 300°C to drive moisture out of the waveguides and allow for successful SMD. The waveguides on the biosensor absorbed water because they were made from oxides that were deposited using plasma enhanced chemical vapor deposition (PECVD) and their design exposed them to the moisture always present in the atmosphere. It is well known that the lower deposition temperature (~250°C) afforded by PECVD leads to lower quality oxide films and causes them to be hygroscopic, or water absorbent. This extra annealing step made the ARROW biosensor a poor fit for the commercial world because not only did the annealing cause irreversible damage to the fragile ARROWs on the biosensor, it did not drive out the moisture permanently, meaning that the ARROW biosensor would have to be annealed again at a future date to drive the moisture out before an actual test.

This problem was fixed by changing the ARROWs on the biosensor from standard single-oxide ARROWs to what it called a buried ARROW (bARROW). The bARROW adds an additional, protective cladding layer to the waveguide to prevent water absorption from reaching the guiding core and negatively affecting the waveguide. Biosensors that incorporated the new bARROWs were verified through experimentation to be completely immune to the negative effects of water absorption previously seen in the single-oxide ARROWs. The bARROW biosensors required no post-fabrication annealing to function properly and proved to possess the highest signal-to-noise ratios of any ARROW biosensor to date.
1.2 Organization

Chapters 2 and 3 are a literature review of both single molecule detection and lab-on-a-chip technologies, respectively. These chapters give important background information on single molecule detection and how it is being achieved using various lab-on-a-chip platforms and techniques. The end of chapter 3 introduces the optofluidic ARROW biosensor platform, which is the focus of this dissertation. Chapter 4 describes the ARROW biosensor platform in more detail: specifically, how it works optically and how it is used for single molecule detection. Chapter 5 discusses ARROW biosensor fabrication details. This chapter includes a brief description of each microfabrication process that was used in the fabrication process of the ARROW biosensors. Chapter 6 discusses important details about PECVD oxides. PECVD oxides are important because all the waveguides integrated into the ARROW biosensor are made using this material. Chapter 6 also goes into detail about water absorption in PECVD oxides, and how this water absorption can negatively affect an ARROW. Chapter 7 discusses new ARROW designs that were tested in order to mitigate the water absorption problem in PECVD ARROWs and introduces the bARROW design, which was most successful. Chapter 8 presents a new biosensor that is fully integrated with bARROWs, details any fabrication changes necessary, and provides SMD results using the new bARROW platform. Chapter 9 summarizes the objectives, results, and my contributions to the ARROW biosensor project.

This dissertation includes appendices that contain other useful information not included in its main body. Appendix A is a list of all my relevant journal publications and conference articles that have come as a result of my work on this project. Appendix B is the most up to date process recipe for fabricating the new bARROW biosensor available at the time this dissertation
was written. Appendix C includes various Matlab scripts that I wrote throughout the course of my work to help with waveguide characterization.

1.3 Contributions

Research into ARROWs at BYU began as early as 2004 [1] and has been going on continuously since, with important contributions coming from many different students and collaborators throughout the years. When I started working on this project in the summer semesters of 2013, I began by fabricating ARROW biosensors and I discovered that, over time, the combination of many past design changes in the biosensor platform had created an unexpected and, at the time, unexplained problem in the ARROWs: instead of possessing clean, confined mode profiles, light was scattering out of the ARROWs, leading to high optical loss values in the waveguides and significantly degrading biosensing results [2, 3]. As part of my research, I helped identify this problem as undesired water absorption in the PECVD oxide thin films making up the ARROWs [4]. As a solution, I introduced a new buried ARROW (bARROW) design for mitigating these negative effects in the waveguides [5]. The bARROW design was then integrated into fully-functional biosensor platforms and tested. These bARROW biosensors not only showed zero signs of negative water absorption effects in the bARROWS, but also yielded, by far, the highest signal-to-noise ratios (SNRs) ever measured in an ARROW biosensor.

The contributions listed above are the main focus of this dissertation; however, they only represent what I consider to be my primary intellectual contributions to this project. I have also contributed in many other ways the liquid-core ARROW project. As mentioned above, towards the beginning of my time as a graduate researcher I became heavily involved in the fabrication of a wide variety of ARROW biosensors. As part of this work, I helped update and clean up the
fabrication process recipe that had become outdated and was producing a low yield of somewhat crude ARROW devices. Many of the biosensors that I fabricated were subsequently shipped to UCSC and used in a variety of published biosensing applications, which are included in my publications list in Appendix A.

I was also the tool owner for the PECVD1 and PECVD2 machines in the cleanroom for the majority of my time as a graduate researcher. This work involved training new users, assisting with process development, tool maintenance and repair, and the installation of two new PECVD machines. I have also mentored several graduate and undergraduate students that have since began working on the liquid-core ARROW project alongside me.
2 SINGLE MOLECULE DETECTION

Single molecule detection (SMD), which requires sensitivities high enough to detect and analyze molecules on an individual level, is the ultimate goal in chemical and biochemical analysis [6]. SMD is a current hot topic in academic and commercial research and includes a wide range of applications that are being investigated within biology, chemistry, and medicine [7]. One of the primary applications of SMD is the sensing and identification of microorganisms involved in infectious diseases to improve upon our current clinical diagnostic techniques and subsequently improve our health care. The focus of this dissertation is the optofluidic, lab-on-a-chip ARROW biosensor, a biosensor that is capable of detecting single molecules within an aqueous solution without the need for any amplification. The ARROW biosensor is an ideal platform for infectious disease detection and so this chapter focuses its discussion on the SMD techniques that are most popular for these types of applications. However, SMD covers a much broader spectrum of important applications in today’s world, including genetic analysis [8], controlled drug delivery [9], molecule profiling with atomic force microscopy [10], and studying DNA-protein interactions [11, 12]. These applications will not be discussed in this chapter. It should be noted, that when the sensitivity of any device is discussed throughout this work, that it is referring to the minimum molecular concentration of a particle or input signal that is required to produce a measurable output signal.
There are a plethora of techniques and methods available for detecting microorganisms, such as bacteria and viruses, at the single molecule level. In this chapter, we discuss only some of the techniques that have been studied for use in infectious disease detection. The chapter is divided into two sections. The first section discusses SMD techniques that rely on some type of amplification process in order to be capable of detection with single molecule sensitivity. The second section focuses on those methods that do not require amplification processes in order to achieve SMD.

2.1 Amplification Methods

Amplification in this instance, means the capture of a small number of molecules, potentially all the way down to a single molecule, and then forcing these to be multiplied into many more copies of the same molecule. In some cases, the particles can be amplified into millions or potentially billions of copies [13]. The goal of amplification is that once so many copies have been created, detecting their presence becomes as simple as noticing macroscopic, easy to detect, potentially even visible changes to an assay. The term assay in this case refers to a specific laboratory procedure used for performing SMD of a molecule. The sensitivity of an amplification method of detection is referenced to the initial concentration of the molecule required for detection and not the concentration of the molecule after amplification.

2.1.1 Cultures

The oldest amplification technique and still potentially the most common clinical method for amplifying an infectious disease for subsequent detection is called culturing. This method was pioneered in the early 20th century and has, obviously, matured and become a major part of the modern clinical world [14]. Culturing is defined as a method of multiplying microorganisms
by allowing them to reproduce in a specific culture media that is favorable to the reproduction of the microorganism in question.

As culturing is a very mature amplification technique, there are many processes and procedures used to amplify different organisms, such as bacteria and viruses. These include broth cultures, which occur in a liquid solutions, where multiplication of a given agent will produce visible changes in the solution, such as turbidity and color; agar plates or petri dishes, where samples are swabbed onto a petri dish and allowed to grow into cultures; and stab cultures, which are similar to a petri dishes, but the sample is inserted directly into the gel using a needle [15]. Figure 2-1 is an example of what culturing often looks like and is a picture of a petri dish containing anthrax cultures.

![Image of a petri dish or agar plate culture of anthrax.](https://commons.wikimedia.org/wiki/File:Anthrax_culture.jpg)

One advantage of culturing is the simplicity and cost effectiveness of the method. All it requires is a swab of the sample to be investigated, a controlled environment, and time to grow. Obviously, there are some other intricacies involved in the process, but those three requirements
describe many culturing processes. The modern clinical world has come up with newer more sophisticated processes and procedures, including some impressive automated processes that are used to improve the accuracy and speed of the technique; however, in general, culturing remains the slowest clinical method for detecting low concentrations of infectious diseases. Culturing is also a very mature technique and well-studied, for example, there are several international culture collections that exist for referencing [16]. Another advantage of culturing is the ability to not only detect infectious diseases and other micro-organisms but to also determine molecular traits, such as anti-bacterial resistance, by simply adding specific antibiotics into a culture and then observing the cultures over time for any signs of antibiotic effectiveness [15].

However, there are some disadvantages to this method. First, all cultures take time to grow. In general, most cultures grow sufficiently for detection in 24-48 hours; however, there are some cultures that take much longer, passing two to three weeks [14, 17, 18]. When it comes to clinical diagnosis of serious diseases, the diagnosis is a time-sensitive step. Slow diagnosis in many cases can mean mistreatment for a period and can be detrimental to the patient’s health. Shorter diagnosis times help simplify and improve medical treatment [19].

Another shortcoming with culturing stems from the onset of contamination problems in the process [20]. It is now apparent that contaminated blood cultures are common in the clinical world [21]. These contaminated cultures can lead to confusing diagnostic results that are difficult for clinicians to interpret and high medical costs for hospitals and other health-care providers [22-24]. Significant effort has been put into eliminating the contamination problems; however, due to the nature of amplification, culturing will always be susceptible to potential erroneous amplification that leads to false positives and difficult to interpret results [25].
2.1.2 Polymerase Chain Reaction (PCR)

A more modern amplification technique that relies on molecular biology techniques, or in other words the use and manipulation of DNA, is called polymerase chain reaction (PCR). The use of this method is growing rapidly in clinical applications because it is such an effective and yet simple method for detecting low concentrations or even single molecules of microbials and microorganisms. PCR amplifies, or multiplies, single copies of a short segment of DNA by several orders of magnitude. Once the DNA has been amplified to a more significant concentration it is easily detected with cheap low sensitivity methods. The introduction of PCR in medicine has allowed for some new infectious disease assays that have added to the clinical world’s ability to diagnose disease. For example, PCR is now used for earlier detection of the presence of the human immunodeficiency virus (HIV) in a patient than was possible with older techniques [26].

In this section, we introduce the basic process of PCR and the general steps involved in that process. The process is quite simple and is basically just repeated cycles, often as many as 30 to 40 total cycles, of a few important steps. The first step is the acquisition of the DNA template or target to be amplified. The next step is called denaturation and it performed by simply heating the DNA to high temperature (~95°C). This higher temperature causes the double stranded DNA to denature or separate. Once the DNA has separated it becomes two complementary single-strand DNA chains. The next step is to add primers, or short DNA segments, that are meant to bind to a specific DNA sequence that is present on the target single-strand DNA chains. Then, an enzyme, called a polymerase, polymerizes or forms the new DNA strands from added deoxynucleotide triphosphates (dNTPs), the building blocks of the new DNA strand. This is often referred to as the extension or synthesizing step. The three steps, denaturing,
priming, and synthesizing, are then repeated several times in order to create many copies of the target DNA. Each cycle doubles the number of target DNA copies and so it follows that the amount of DNA created by a PCR process grows exponentially with each repeated cycle.

![Diagram of the basic steps of polymerase chain reaction (PCR) process.](image)

Figure 2-2: Diagram of the basic steps of polymerase chain reaction (PCR) process.

PCR has been heavily researched and has seen many advances since it was initially developed in 1983 by Kary Mullis [13]. Some of the more important advances include multiplexed PCR, which allows for the amplification of multiple, distinct DNA targets at the same time [27-29]. Another major advance in PCR is the addition of real-time, or quantitative PCR (q-PCR) [30, 31]. In q-PCR, specific fluorescent dye molecules, called fluorophores, are bound to the target DNA in a PCR solution. As more DNA is synthesized, dye molecules bind themselves to the new DNA chains. The fluorescence given off by the solution is detected in real-time by a photodetector. The intensity of the fluorescence correlates with the amount of DNA that has been amplified or copied and allows this DNA to be quantified throughout the
entire process. Because the dye molecules can be designed to attach to specific genes within a DNA strand, assays can be created that determine the presence of genes within a microorganism, i.e. anti-biotic resistance genes that are sometimes present in bacteria. All of this, including the fact that many of the PCR assays can be run to completion in a matter of hours, or sometimes minutes for simpler assays, has made PCR a very popular method for detecting infectious diseases.

However, there are some limitations to the method of PCR. First is that PCR uses amplification. As discussed with culturing, amplification always introduces the possibility of false positives or false negatives. If erroneous DNA enters the process, it can mistakenly be amplified and therefore report a positive, when the target microorganisms were not truly present [32, 33]. False positives, are somewhat acceptable for quick and dirty, first round diagnostics, but that means the PCR method should be followed up with another method for more sure detection.

Some other limitations of PCR include its cost, especially when considering bringing the technique into resource limited settings; the need for clinician education, each PCR assay can be quite complicated and require specific procedures to be performed with exactness; potential for false negatives, which are unacceptable in disease diagnostics; and time to detection [33]. Even though the fastest PCR assays can detect their intended diseases very quickly, assays used to determine bacteria or virus resistances can still take multiple days and results do not come in quick enough for rapid treatment [30].

2.2 Non-Amplification Methods

When it comes to research and development, often, the best answer is simply to “simplify” a process. One of the largest limitations shared by both the amplification techniques
discussed above (cultures and PCR) is the added complexity of the amplification process itself. As discussed above, amplification takes time and introduces the possibility of false positives and negatives in the detection method. In the example of culturing, contamination becomes a very large problem throughout the amplification process and for PCR, sometimes the amplification process can lead to mutations and unexpected oddities in the DNA being copied. Because of these facts, direct detection of a bio-molecule is preferable to amplification.

However, direct detection can be difficult to achieve, because of the small molecular nature and low concentrations of the molecules to be detected. For this reason, most of the direct detection SMD techniques discussed in this section are still in a research and development phase and have not entered mainstream clinical use; however, these methods represent a major potential advance in infectious disease detection because they do not require any amplification process to sense the presence of a microorganism on an individual molecule level. There exist a wide variety of methods being researched for performing direct SMD. In the next sections, we discuss three of the more promising methods: optical cavity resonance (OCR), surface-enhanced Raman spectroscopy (SERS), and fluorescence techniques.

2.2.1 **Optical Cavity Resonance**

OCR is a very popular method being researched because it does not require any optical "labeling" of target particles with a fluorescent dye. Instead, the method relies on detecting changes in the resonant frequency of an optical cavity that are caused by small perturbations to that cavity, such as the binding of a target molecule to its surface. In order to detect with high sensitivity, OCR requires high-Q optical cavities, or optical cavities that possess very sharp resonant peaks. OCR cavities have been made using many different structures including ring
resonators [34-36], Fabry-Perot etalons [37], whispering-gallery mode resonators [38, 39], and microtoroid resonators [40].

Typically, SMD is performed using OCR by focusing light from the optical cavity onto a photodetector to measure the resonant peaks generated by the cavity. The molecules to be detected are then introduced into the system with the goal of forcing them to interact with the optical cavity. The most common interaction is simply causing the molecule to bind to the optical cavity, which is usually specially treated to bind to only that specific molecule. When the molecules interact with the optical cavity, they cause changes in its resonant peaks. These changes are detected by the photodetector. One advantage of this method is that recent SMD have been able to tune their cavities with such specificity as to be able to determine particle size as well as its presence [39].

OCR techniques are very dependent on integrated optics components because they require high-Q optical resonators or cavities that can be interfaced with microfluidic systems for the introduction of a solution for probing. The need for components to be small in OCR pairs it well with optofluidic techniques and platforms.

2.2.2 Surface Enhanced Raman Scattering

Raman spectroscopy is used to directly measure the vibrational characteristics of molecules in a system. It is typically performed by illuminating a sample with a very low bandwidth or monochromatic light, such as a single-mode laser. Much of this laser light will undergo elastic or Rayleigh scattering with the sample, which means that the incoming photon and the outgoing photon will have the same energy. However, some of the photons will undergo inelastic or Raman scattering with the sample, meaning that the incoming photon has interacted with the sample’s polarizable electron cloud in some way that transferred energy. Either the
photon has lifted an electron from a ground state to an excited vibrational state or the photon has caused an electron to fall back down to a ground state. These are called Stokes and Anti-Stokes shifts, respectively. Raman spectroscopy is dependent on filtering out the Rayleigh scattered photons and passing any Raman scattered photons to a detector. The resulting optical spectrum can be analyzed in order to determine a structural fingerprint for a given sample.

In standard Raman spectroscopy, the cross section of a single particle is too small and therefore SMD cannot occur. However, the cross section is greatly enhanced on silver and gold nanoparticles due to their increased surface-to-volume-ratio [41]. Particles to be detected are typically adsorbed onto the silver or gold nanoparticles and then their Raman spectrum can be enhanced sufficiently to be detected at SMD concentrations [42]. Because molecules have a specific fingerprint, they can be detected and identified using the obtained Raman spectrum. This method has also been used to detect DNA [43, 44], for sensitive chemical analysis [45], and yeast cells [46].

Per one paper, the requirement of using silver and gold nanoparticles in solution or on a substrate has greatly limited the capability of the Raman scattering method [46]. A newer method that is seeking to reduce the limitations of SERS is called tip enhanced Raman scattering (TERS). In this technique, the sample can rest on a generic substrate, and then a nanoscale gold or silver tip is brought into proximity of the sample using precision instrumentation. The presence of the nanoscale gold or silver tip enhances the Raman scattering signal. This method has seen some promising results detecting single carbon nanotubes [47] and single dye molecules [48, 49]; however, it does not amplify the Raman signal quite as effectively as SERS and can only be used for SMD of particles that possess large Raman cross sections [46].
2.2.3 Fluorescence

Fluorescence-based SMD methods are said to be the “gold standard” of detection [50-52]. There are a variety of optical SMD schemes that involve fluorescence for detection and many of these have found application in the biomedical and chemical worlds. These methods are reliant on attaching fluorescent dye molecules, often referred to as fluorophores, to a specific target molecule [53-55]. The dye molecules possess no fluorescence before binding; however, once bound to a target molecule, they will emit photons when excited by an optical power source. Single mode lasers are the most popular excitation source because they can produce high signal-to-noise ratios (SNRs) [55].

Fluorescence-based SMD saw its origins in a method called fluorescence correlation spectroscopy (FCS) [56-59]. This method involves the measurement of changes in fluorescence intensity or a sample over a given time. The fluorescence data is correlated to a parameter such as molecule concentration. The standard setup for FCS is to illuminate a small volume of a sample containing target particles. The particles are “labelled” before any illumination using fluorescent dyes. It is important that the concentration of the molecules is dilute enough that only a small number of particles is illuminated by the excitation beam at any given time. This technique can easily provide measurements for the study of changing particle concentrations. This typically includes studies of particle motion, such as diffusion or flow.

Since FCS, many other fluorescence-based SMD methods have emerged in academic research with a broad range of applications [51, 60, 61]. These techniques generally share a few things in common: they rely on fluorescent dyes for signal generation, they use laser light, within the visible or near infrared spectrums, for excitation, and they filter out the excitation signal and only pass the fluorescent signal to a detector. There are many fluorescence-based SMD platforms
that exist on the macroscale level, using full table-top optical setups for full functionality. Many of these setups make use of immersion microscope objectives to maximize collection efficiency and avalanche photodiode (APDs) or photomultipliers for sensitive detection. However, fluorescence-based SMD lends itself very well to miniaturization and the field of optofluidics. There are myriad lab-on-a-chip SMD systems that are fluorescence based. The next chapter will detail some of the devices and applications being researched on fluorescence-based SMD. The ARROW biosensor, which is the focus of this dissertation, is a lab-on-a-chip fluorescence-based SMD platform. It is also described in more detail at the end of the next chapter.
3 LAB-ON-A-CHIP TECHNOLOGY

Lab-on-a-chip technology is becoming very popular in today’s research world. The primary goal of research in this field is to shrink macroscale laboratory processes into total miniaturized systems that can be fabricated using the fast and cheap parallel fabrication techniques that have been developed for microelectronics. Lab-on-a-chip platforms are ideal for optofluidic SMD schemes because the miniaturization helps improve sensitivity of optical techniques and new optofluidic components and techniques are now moving these once fantasized lab-on-a-chip ideas closer and closer towards portable instrumentation. A major focus of lab-on-a-chip research is the creation of platforms for SMD applications, but they are not limited to only these applications. Lab-on-a-chip systems are also used to create miniature fuel cells [62, 63], organ-on-a-chip devices [64], and assist in the fabrication of controlled delivery drugs [9]. However, this chapter only focuses on lab-on-a-chip platforms that are being researched for SMD applications, and even more specifically, lab-on-a-chip systems that make use of SMD methods discussed in the previous chapter.

The purpose of this chapter is not to provide all of the details for each lab-on-a-chip system discussed, instead it is intended to give a broad literature review of much of the research that is being done on SMD lab-on-a-chip systems. These techniques all represent other strategies being used to accomplish a similar task to the ARROW biosensor. The end of this chapter
includes a brief section on the ARROW biosensor lab-on-a-chip biosensor and describes some of its advantages and disadvantages compared to the other systems describes.

The first example of a lab-on-a-chip SMD system makes use of PCR. In fact, the platform is not only capable of basic PCR, it can be used for performing real-time PCR or q-PCR. The authors report on the successful detection of unpurified methicillin-resistant *Staphylococcus aureus* (MRSA) using this system [65]. The lab-on-a-chip was made with standard soft-lithography techniques on Polydimethylsiloxane (PDMS), a typical polymer. Soft lithography and PDMS are popular in lab-on-a-chip fabrication because they provide simple fabrication processes that work with cheap polymer materials, such as PDMS. A major advantage of this PCR lab-on-a-chip system is that it eliminates some of the complexity that can be involved in PCR by providing an automated process that does not require much training to learn. The miniature nature of the system allows for the use of a very simple DNA cleaning technique, called the boil-prep technique, for PCR prep. This eliminates the need to purchase a more expensive PCR DNA cleaning kit that is typically used in PCR.

The lab-on-a-chip does not have an internal heating component and requires mounting to a complex heating element to provide temperature control inside the chip. This heating element is used to perform the required PCR cycling. The chip also has no internal optical waveguides; instead an external objective lens was used to focus any fluorescence from the chip onto an off-chip detector. The PCR lab-on-a-chip is made up of four wells and two overflow channels that are patterned into the PDMS. The overflow channels are present to ensure no air bubbles are trapped in the wells during the PCR cycling. There is no sample preparation feature on the chip and so all sample preparation has to be done off-chip. The PCR solution that is prepared for the chip contains the DNA template for amplifying, deionized water, primers, fluorescent probes,
dNTPs, and Taq polymerase. 10 µL of the PCR solution is then introduced into the wells using a micro pipette and then a PDMS lid is placed over the top of the chip to seal the solution into the wells. The heating element is then activated and cycled through the denaturation, annealing, and DNA synthesizing steps. Figure 3-1 shows the PCR lab-on-a-chip mounted onto the heating element used for thermal cycling.

Figure 3-1: A PCR lab-on-a-chip that was used to demonstrate the successful detection of unpurified methicillin-resistant *Staphylococcus aureus* (MRSA). Reprinted from [65] with permission from Elsevier.

There are many other lab-on-a-chip systems that use PCR in order to perform SMD. Here, we introduce just one more example of an on-chip PCR system. Just like the previous system, this PCR lab-on-a-chip system is not only capable of basic PCR but can also perform real-time PCR. The authors report on the successful amplification and subsequent detection of very low concentrations of E. coli O157 within a solution [66].

This lab-on-a-chip possesses a single, long hollow channel that runs back and forth along the length of the entire chip. There are two individual heating elements that are attached to the underside of the chip. These heating elements create a sharp temperature gradient between the
two sides of the chip. The path of the hollow channel passes through this temperature gradient several times, which causes any solution that flows down the hollow channel to experience cycles of two distinct temperatures and provides the mechanism for PCR cycling. There is no pumping required for this lab-on-a-chip, instead it relies on capillary action to pull the PCR solution through the hollow channel. Because the temperature of the heating elements does not have to be adjusted mid-process and because there is no required pumping, the process runs itself all the way to completion on its own, without any outside intervention.

This lab-on-a-chip is fabricated out of a cyclo-olefin polymer (COP) using soft-lithography techniques. The COP material is important because it was determined that PDMS and other polymers could not easily be made hydrophilic. This chip requires hydrophilic surfaces throughout the length of the channel, because these surfaces provide the capillary action that pulls the PCR solution through the chip. This PCR lab-on-a-chip also has no internal optical waveguides and is instead integrated with a macroscale fluorescence detection system in order to be capable of performing real-time PCR. Figure 3-2 shows the design for this lab-on-a-chip.

After performing PCR, the chip must be rinsed well and retreated with specific surfactants to ensure that its surface remains hydrophilic. If it is not retreated after each PCR process the surface loses its hydrophilic nature and the capillary action that is used to pull the PCR solution through the channel fails. As with the previous PCR lab-on-a-chip, this system is not capable of any on-chip mixing or PCR sample preparation. The PCR solution must be mixed off-chip before it can be introduced as a droplet at the entrance to the hollow channel. Capillary action then pulls the PCR solution through the hollow channel and its path cycles it through the basic temperature steps of PCR. In this report, E. coli was the specific target for amplification and detection and the paper reports that the system performed successful real-time PCR in just
18 minutes from when the PCR solution entered the hollow channel until the process was finished. This time did not include the DNA preparation, cleaning, and mixing time. Still, this fast time of only 18 minutes helps demonstrate the value of performing these SMD on microscale systems versus in a macroscale setting, where this process can take as many as 24 hours.

![Autonomous microfluidic lab-on-a-chip used for real-time PCR. Reprinted from [66] with permission from Elsevier.](image)

Figure 3-2: Autonomous microfluidic lab-on-a-chip used for real-time PCR. Reprinted from [66] with permission from Elsevier.

The next lab-on-a-chip platform for SMD that is presented uses optical cavity resonance as its detection method, which requires no amplification for detection [67]. The basic design of this lab-on-a-chip is depicted below in Figure 3-3. The chip is made with a planar design, suitable for standard microfabrication processes and consists of a 100 µm radius microring.
resonator brought in to close proximity with a through-chip waveguide. This proximity causes evanescent coupling to occur between the two waveguides and transfers the resonant frequency peaks of the microring resonator to the through-chip waveguide. Both the through-chip waveguide and the microring resonator are fabricated out of silicon with a silica base beneath and were patterned using standard photolithography. The through-chip waveguide is then protected with the addition of a silica cladding layer, while the microring resonator is left exposed. A hollow channel, formed using PDMS, is then placed directly over these two waveguides, the through-chip waveguide and microring resonator. This channel is used to introduce the analyte into the waveguide system.

Figure 3-3: Lab-on-a-chip with a microring resonator to perform SMD using OCR. Reprinted with permission [67].

In order to perform SMD, an external laser is focused onto the chip and coupled into the through-chip waveguide. The light travels through the waveguide, interacts with the microring resonator and then is focused off-chip, using a macro objective, onto a photodetector. The resulting optical spectrum is recorded with specific resonant peaks present, corresponding to the microring resonator. The analyte solution is flowed through the hollow channel. The through-
chip waveguide is protected from any changes by its silica cladding layer; however, the microring resonator is unprotected. The analytes interact with the resonator and cause changes in the resonant absorption peaks of the resonator. These changes are recorded by the photodetector and indicate the detection of any analyte in the solution that was flowed through the hollow channel.

The paper reports that this OCR lab-on-a-chip was used to demonstrate the measurement of the absorption spectra of N-methylaniline from 1460 to 1610 nm. Figure 3-4 shows the measured absorption spectra. The solid line represents a commercial method used for measuring the absorption spectra and the dots represent the points measured by the lab-on-a-chip.

![Figure 3-4: The absorption spectra of N-methylaniline from 1460 to 1610 nm using the OCR lab-on-a-chip discussed above. Reprinted with permission [67].](image)

The 100 µm radius microring resonator was capable of measuring the absorption spectra with a 1 nm resolution. The measured Q-value of the microring resonator was greater than
100,000. This Q-value can be raised by simply increasing the radius of the microring. In the same sense, if less resolution is needed for a specific application, then the microring can be shrunk down even farther in order to allow for a more compact chip design.

The next SMD lab-on-a-chip presented also uses OCR for its detection mechanism [68]. However, this system makes use of multiple microring resonators, instead of just one, in order to allow for multiplexed detection of molecules, or detection of different molecules in parallel. Figure 3-5 shows the basic design for the lab-on-a-chip system. On this chip, one through-chip waveguide is split into three separate waveguides. These waveguides all pass by four individual microring resonators, making twelve resonators in total. As with the previous OCR platform, a hollow microfluidic channel is placed over the top of the microring resonators. This hollow channel is used to flow analyte solution over the microring resonators to perform SMD.

![Figure 3-5: Another lab-on-a-chip using OCR in order to perform SMD. This chip is capable of multiplexed SMD. Reprinted from [68] with permission from IEEE.](image)
The working principle of this OCR lab-on-a-chip system is the same as discussed above; however, this system is much more complicated because of the use of multiple resonators for multiplexed detection. Each microring resonator is prepared to detect or interact with a different molecule. The paper describes the mathematical processes in more detail that are used in order to separate results out from all of the individual optical spectra that arrive at the detector. Even with the added complexity, multiplexing in SMD platforms still represents a major advantage. This is because in medical diagnosis, a sample must be tested for the presence of many different biomolecules. If a system can only probe for the presence of one particle at a time, then it takes additional time to run several tests to cover all of the molecules that must be tested. This requires more sample be obtained from a patient in order to run each individual test. Multiplexing allows for all the molecules to be tested for in parallel and makes the process faster, cheaper, and requires less sample from a patient.

The next lab-on-a-chip system is depicted in Figure 3-6 and uses surface-enhanced Raman spectroscopy (SERS) to perform SMD [69]. The chip in this system consists of a microfluidic channel where a silver (Ag) colloid is mixed together with the analyte solution. An objective is focused onto the microfluidic channel and table top optical elements are used to focus laser light onto the channel, filter out any Rayleigh scattering and deliver all of the Raman scattering from the analyte to an optical spectrometer.

The paper reports on the direct measurement of two different organic analytes: Thiophenol (TP) and 2-naphthalenethiol (2-NT). Both the TP and 2-NT analytes were prepared in ~50 nM solutions and introduced into the microfluidic channel. The advantage of this lab-on-a-chip system is its simplicity. It is made on top of a glass slide with only one microfluidic channel running through a PDMS layer. The disadvantage is that all of the complicated optics,
sample preparation, and test setup is performed off-chip. The only function of the actual chip is
the microfluidic hollow channel.

Figure 3-6: SERS lab-on-a-chip. Reproduced from [69] with permission of The Royal Society of
Chemistry.

The above lab-on-a-chip could potentially be miniaturized into a portable device; however,
it would be complicated and expensive because of how many processes take place off-chip in
order for the system to function properly.

All the above examples of SMD lab-on-a-chip platforms have one thing in common: they
either use completely external waveguides for any optical routing and detecting or the integrated
waveguides being used are traditional solid-core waveguides that function based on the principle
of total internal reflection (TIR). TIR is an optical phenomenon that only occurs when a higher
refractive index material is bounded by lower index of refraction material. Then, if the angle of
incidence of light at the waveguide’s boundary is below the critical angle, defined by Snell’s
law, all the real optical power will be completely reflected into the core of the waveguide and
none of the power will transmit through the boundary and out of the core. Figure 3-7 shows the side profile of a typical waveguide that uses TIR as its waveguiding principle.

![Diagram of waveguide](image)

**Figure 3-7: Profile depicting waveguiding using typical total internal reflection (TIR).**

The discovery of TIR waveguides changed the world over the past decades. These types of waveguides make up most of the optical fiber networks and systems that have been used to connect the entire world through high-speed, high-bandwidth internet. They are also very prevalent in the field of integrated optics. However, in optofluidic applications, TIR waveguides have a major disadvantage: the guiding core generally must be made of a solid material to satisfy the optical requirements of TIR. Plainly stated, this means that a standard TIR waveguide cannot be used to guide light in a liquid. The ability to guide light through a liquid is made possible with the advent of other more sophisticated optical waveguides.

To get around this disadvantage, the lab-on-a-chip systems described above either use evanescent coupling, bringing waveguides into close proximity with solutions and molecules in order to be detected or they rely on off-chip macroscopic optical components for the routing of
light. Evanescent coupling does play well with lab-on-a-chip integrability; however, it does not provide direct light-molecule interactions, which can lower the sensitivity of a system.

Simply, routing light off-chip is also very popular; however, it does not fit well with the ideal of true total system, lab-on-a-chip technologies. The chip can perform much of the microfluidic routing on a microscale, but the external optics required for functioning are macro components, making them more difficult to integrate into a portable device.

The anti-resonant reflecting optical waveguide (ARROW) does not function on the principle of TIR and so it does not require a high refractive index guiding. The ARROW makes use of multilayer, dielectric stacks to create highly reflective optical mirrors [70]. The dielectric stacks are designed to provide high reflections at glancing angles for a broadband of optical wavelengths. The dielectric stacks are placed around the core of the waveguide and force light to reflect back into the core with very little optical loss. Figure 3-8 shows the profile of a typical planar, solid-core ARROW design. As shown, there is an ARROW stack placed below the core

Figure 3-8: Profile of a standard ARROW waveguide.
in order to provide an optical barrier at the bottom of the core and the core is bounded by air on the top. The low index of the air barrier on top causes light to remain in the core at the top due to TIR.

However, the major advantage of the ARROW is that the core can be made of a low refractive index material, such as a liquid or even air. Figure 3-9 shows an example of a hollow core ARROW. The design adds a dielectric ARROW over the top of the hollow core as well as beneath the core.

![Figure 3-9: Example of a hollow-core ARROW waveguide.](image)

Both figures above show simple two-dimensional ARROW designs. However, in real life applications, a strictly two-dimensional waveguide is not realistic. Real waveguides require optical confinement on their sides as well as on the top and bottom. Figure 3-10 shows profiles of both hollow-core and solid-core ARROW design that are often used to form on-chip, planar ARROWs.
The next example of lab-on-a-chip SMD platform uses both liquid-core and solid-core ARROWs in order to perform on-chip SERS [71]. The lab-on-a-chip was made on silicon substrates with ARROW layers made of silicon nitride and silicon dioxide. There were six ARROW layers used for the top and bottom claddings. Sacrificial etching was used to form a hollow channel through the ARROW layers. These layers were all deposited at low temperatures using plasma enhanced chemical vapor deposition (PECVD). Figure 3-11 shows an (a) SEM image of the liquid-core ARROW profile and (b) the experimental setup used to perform SERS on-chip.

![Figure 3-10: Schematic profiles of hollow-core and solid-core ARROWs.](image)

SERS is performed on this SMD platform using the following steps. Laser light from a standard 635 nm HeNe laser is coupled on-chip by aligning a single mode optical fiber with the solid-core ARROW on the sensor. The solid-core ARROW is integrated with the liquid-core ARROW in order to provide a mechanism for coupling light into the liquid-core. A solution containing silver nanoparticles (~20 nm in diameter) and the analyte is introduced into the chip
via a reservoir that is attached to the top of the chip. The analyte in the solution adsorbs to the silver nanoparticles and is interacted directly with the laser light. Raman scattering from the analyte is focused onto a Raman spectrometer using an objective. The paper reports on the successful detection of Rhodamine 6G molecules with concentrations as low as 30 nM, which is well into the SMD regime.

The final lab-on-a-chip SMD platform discussed in this chapter is the platform that is the focus of the rest of this dissertation. This lab-on-a-chip system makes use of ARROWs to provide high light-matter interactions within the sample solution. The platform, referred to as the

Figure 3-11: Lab-on-a-chip for SERS. Reprinted from [71] with the permission of AIP Publishing.
ARROW biosensor in this work, uses fluorescence spectroscopy, specifically fluorescence correlation spectroscopy (FCS), to perform SMD. The use of fluorescence means that the platform relies on the “labelling” of specific molecules with fluorescent dyes for detection. This represents a disadvantage when compared to the OCR, SERS, and TERS, which are label-free methods. However, the addition of the fluorescence dyes and the integrated design of the ARROW biosensor have proved to be very effective for SMD on this platform. The platform has already been used to demonstrate SMD of various biomolecules, including the H1N1 virus and other related flu viruses [72-74], λ-DNA [75, 76], the Ebola virus [77-79], cancer biomarkers, and other particles [80-82].

The ARROW biosensor saw its origins in the early 2000s as preliminary experiments were performed to collect proof-of-concept results [1], [83-86]. The next chapter contains more details on the basic ARROW biosensor platform, how it is used to perform SMD, and some more of the results achieved using the platform.
4 ARROW BIOSENSOR PLATFORM

This chapter discusses in further details the ARROW biosensor platform, which is the focal point for this dissertation. The ARROW biosensor has been being researched and improved upon since its inception in 2004 [1]. This chapter discusses the basic design of the ARROW biosensor platform. It also discusses the most recent methods used to “label” analytes with the necessary fluorescent dyes in order to perform SMD. The end of the chapter reports on some of the more recent biosensing results using the biosensor.

4.1 ARROW Biosensor Structure

The layout of the ARROW biosensor has remained quite constant in its basic design. The device is made up of two different types of anti-resonant reflecting optical waveguides (ARROWS). There are solid-core ARROWS that are integrated with hollow-core ARROWS. The hollow-core ARROW provides the hollow on-chip channel where an aqueous solution can flow. Because the hollow-core ARROW must remain enclosed to prevent any solution from leaking out of the channel, there are two sets of solid-core waveguides to interface with it. The first set of solid-core ARROWS intersect the hollow-core ARROW orthogonally at its center. These waveguides provide the optical path to direct off-chip laser light to a specific excitation point at the hollow-core ARROW. This excitation point is where the fluorescently “labeled” particle will pass by and emit a fluorescent signal. These solid-core ARROWS are called the excitation waveguides. The second set of solid-core ARROWS are coupled to the two ends of the hollow-
core ARROW and are used to collect the fluorescent signal from the hollow-core ARROW and direct it off-chip. This design is illustrated below in Figure 4-1(a).

![Figure 4-1: (a) Top view schematic of the basic ARROW biosensor design. (b) The side-view profile of the critical excitation point at the hollow-core ARROW.](image)

The hollow-core ARROW is shown in the figure in blue. The green line indicates the excitation path of the light. This light is used to optically excite any fluorescent particles that are present within the liquid solution as they pass through it. Once a particle is excited and give of an optical signal, the red lines indicates the path of that fluorescent signal as it is collected and guided off-chip. Figure 4-1(b) shows a cross-sectional view of the excitation point, where the excitation solid-core ARROWs intersect the hollow-core ARROW.

The ARROW biosensor can only detect particles that pass through the excitation point if they are fluorescent. The biological molecules to be detected by the biosensor, such as viruses and bacteria, are generally not fluorescent at all. This means that the molecules must be “labeled” with a fluorescent marker before they are introduced to the biosensor. This is typically done using fluorescent dyes that can bind themselves to biological molecules, called
fluorophores. There are various processes used for “labeling” biological molecules, the next section discusses two of these methods in some detail.

4.2 Fluorescent Tagging Methods

4.2.1 Intercalating Dyes

This section discusses two basic methods used to fluorescently tag the molecules before they are introduced into the ARROW biosensor for detection. The first method involves the use of intercalating dyes. Intercalating dye molecules attach to the DNA with a biological molecule by a process called intercalation. Intercalation is the process of inserting particles between the planar bases of a DNA strand.

The intercalating dyes do not possess any fluorescence in an unbound state, but after intercalating into DNA they become fluorescent. In this way, they can be used to probe a sample for the presence of any DNA. The use of intercalating dyes is already very common in biochemistry and there are many commercial dyes that are available for a variety of fluorescence applications. There are dyes designed to intercalate with single-stranded DNA, while others are designed to work better with double-stranded DNA. Intercalating dyes are designed to fluoresce at specific wavelengths of light. Some of the more commonly used dyes are ethidium bromide, SYBR Green, SYBR Gold, and Alexa dyes.

Figure 4-2 shows the fundamental principles of how intercalating dyes function. On the left, the unbound dye molecules, shown in red, will not emit a signal when excited because they have not intercalated into a DNA strand yet. On the right, the dye molecules have successfully intercalated into the DNA strand and have become fluorescent.
One of the major disadvantages of intercalating dyes is that the dye molecules do not selectively bind to only the target DNA, based on its DNA sequence. The dye molecules will intercalate with any strand of DNA present within a solution, regardless of its sequence. In some applications, this is not a very significant issue, because they simply probe for the presence of any DNA molecule and are not looking for a specific molecule. However, many SMD applications aim to detect the presence of a specific molecule, such as the Ebola virus.

In these cases, intercalating dyes can still be used in a selective DNA “labeling” process. It just means that the labeling process will be more complicated than simply mixing the solution containing the target DNA with a solution of intercalating dye. Instead, the target DNA must be isolated from all other DNA sources that may be present in the sample solution. The simplest method for accomplishing this is by using what is called a “capture and release” process. First, the solution is forced to flow by a surface that possesses a specific capture sequence for binding to the target DNA. As the solution passes by this surface, target DNA particles bind to this
surface and are pulled out of the solution. The rest of the solution is then washed away, which is typically called the rinse step. After the old solution has been evacuated from the chamber being used, the intercalating dyes can be introduced into the process. The dye molecules bind to any of the captured DNA. The DNA is then released from capture and can be introduced into the ARROW biosensor for detection. This leaves a solution containing “labeled” target DNA molecules with no other DNA molecules present.

Although intercalating dyes can be complicated to work with for selective “labeling” processed, they possess a major advantage: a dye molecule can intercalate between all of the base pairs of a DNA molecule. This means that multiple dye molecules can bind to a single DNA strand, as shown in the figure above. Depending on the length of the DNA strand, 100s of dye molecules can be used to “label” a single DNA molecule. Each dye molecule provides another fluorescent signal during excitation and improves the intensity of the fluorescence. By simply increasing the concentration of dye molecules being mixed with the target biological molecule, more dye molecules can intercalate per DNA strand and the signal obtained can be increased.

4.2.2 Molecular Beacons

The next method used to fluorescently tag biological molecules makes use of molecular beacons. The basic structure of typical molecular beacons is shown in Figure 4-3. Each molecular beacon possesses a dye molecule and a quencher molecule connected by a single-stranded DNA strand. When the quencher and dye molecules are in close proximity, then the fluorescence will be “quenched” and the beacon will not give off a fluorescent signal. However, when the two molecules are separated, then the dye molecule is no longer “quenched” and will emit a signal when excited by a given wavelength of light.
Whereas intercalating dye are non-specific and will attach to any DNA source, molecular beacons are designed to only attach to a specific DNA sequence. When unbound, molecular beacons generally have a ring shape. This shape occurs because the beacon is designed to have complementary DNA sequences near the dye and quencher. In the absence of the target DNA sequence the beacon closes up on itself and forces the quencher to be near the dye molecule. Once again, the proximity of the quencher to the dye particle "quenches" or eliminates any possible fluorescence from the beacon. In the presence of the target DNA sequence, the beacon will preferentially attach itself to that target, which causes the beacon to open up. This process forces the dye molecule and quencher away from each other, thus allowing the dye molecule to fluoresce under excitation.

Compared to intercalating dye, molecular beacons offer a much simpler mechanism for specific DNA "labeling". This can be an advantage when trying to simplify and eliminate steps in the sample preparation process. Any complexities in the processing can lead to user error or
make it very difficult or cumbersome to automate the process. However, the molecular beacons are limited in the fact that only one fluorescent dye molecule can bind to a given DNA target sequence, which limits the signal emitted that can be detected. Table 4-1 is a brief summary of the main characteristics of using intercalating dye versus molecular beacons.

Table 4-1: Summary of differences between intercalating dye and molecular beacons

<table>
<thead>
<tr>
<th>SPECIFICITY</th>
<th># OF DYE MOLECULES PER TARGET</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTERCALATING DYE</td>
<td>Non-specific</td>
</tr>
<tr>
<td>MOLECULAR BEACON</td>
<td>Specific</td>
</tr>
</tbody>
</table>

4.3 Biosensing Using the ARROW Platform

This section describes the standard procedure used to perform successful SMD on the ARROW biosensor platform. First, the biosensor is placed onto an optical setup. The excitation waveguide is manually aligned to a single mode optical fiber and the collection waveguide is brought into the focus of a collection objective lens. Once the chip is successfully aligned, the aqueous sample is introduced into the hollow-core ARROW and forced to flow through the channel. Depending on the experiment being performed, the sample may be allowed to flow through the channel with simple capillary action, which leads to low flow velocities or a vacuum is applied to the tail end of the channel to increase the flow velocities of the molecules in the solution. Before these molecules are introduced into the biosensor they are “labeled” with fluorescent dyes using either intercalating dyes or molecular beacons. These fluorescent dye molecules and “labeling” techniques are described in the above section.

While the solution is flowing through the liquid-core ARROW of the biosensor, excitation laser light is directed onto the chip through the single mode optical fiber. The
wavelength of light used depends on the type of fluorescent dye that has been used on the sample. Historically, 488 nm, 635 nm, and 735 nm have all been used for different biosensing applications. This light then guides down the excitation solid-core ARROW and passes through the hollow-core ARROW at the intersection point. When target biological molecules pass through this point, they emit a direct fluorescent signal that is then guided down the hollow-core waveguide. The signal passes into the collection solid-core ARROW and subsequently off-chip where it is routed to an avalanche photodiode (APD) for detection. An APD is used because of its ability to detect extremely low optical signals. Figure 4-4(a) illustrates this process and (b) shows an example of the data that is obtained by the APD during a biosensing experiment. Each peak in the (b) represents a fluorescent molecule passing through the excitation point and giving off a fluorescent signal, thus demonstrating the ability of the ARROW biosensor platform to perform SMD.

Figure 4-4: (a) Basic experimental setup used for biosensing. (b) Typical data recorded during biosensing experiments.
The ARROW biosensor platform has recently been used to demonstrate SMD of several different biological molecules. Figure 4-5(a) shows the “labeling” process that was used to detect Ebola virus oligonucleotides [77]. In this instance, the ARROW biosensor was connected to an automatic microfluidic device used for on-chip sample preparation and delivery. This device was developed by our collaborators working at UCSC and was called the “automaton”. The “automaton” was set up to perform all the necessary pre-detection processing to “label” the Ebola oligonucleotides.

Figure 4-5: (a) Sample preparation process used to bind multiple target Ebola DNA strands, with molecular beacons attached, to magnetic beads for detection on the ARROW biosensor platform. (b) Signal at the APD when no target Ebola DNA strands were present and (c) when they were present. Reprinted from [77] with the permission of AIP Publishing.
First, magnetic beads were introduced into the chip that were attached with a capture or pull-down DNA sequence. Analyte was added to the solution and the target DNA strands specifically bound to the pull-down sequence on the magnetic bead. Next, molecular beacons were added into the process that were designed to attach to the target DNA sequence. Once this entire process was done the solution was rinsed and the magnetic beads were released and introduced into the ARROW biosensor. Each magnetic bead had several molecular beacons attached to it. Figure 4-5(b) shows the fluorescence detected by the APD when no target DNA strands were included in the sample preparation. This demonstrates that there are no false positive signals detected. Figure 4-5(c) shows the detected signal at the APD when target Ebola oligonucleotides were included in the solution, demonstrating successful SMD on the ARROW biosensor platform.

Figure 4-6: SMD of the Ebola virus. Each line on the figure represents the detection of an Ebola virus being detected. Reprinted from [79]. Figure is under Creative Commons CC-BY license.

The next example of SMD on the ARROW biosensor is shown below in Figure 4-6 [79]. The figure is data of the ARROW biosensor detecting the Ebola virus again. However, this data has been converted into binary using a threshold value. This Ebola detection was accomplished
at much lower concentrations than the previous report. Detecting lower concentrations was made possible with a biosensor that had slightly better SNR than the previous ARROW biosensor and using a new pre-concentration step on the automaton during the sample preparation. As shown in the figure, Ebola viruses were detected at concentrations as low as 210 pfu/mL.

The next example of SMD using the ARROW biosensor is the sensitive detection of the H1N1 virus. The viruses were “labeled” using a similar process to the ones above; however, they were detected on a special ARROW biosensor that had multiple excitation spots along the liquid-core ARROW. The paper showed that by having multiple excitation point for each biological molecule, a shift multiply algorithm could be used to greatly enhance the SNR of the data set.

![Figure 4-7: SMD of the H1N1 virus using an ARROW biosensor with multispot excitation. Reprinted from [74] with permission from IEEE.](image)
5 BASIC BIOSENSOR PLATFORM FABRICATION

This chapter describes the major fabrication steps and processes used to make the ARROW biosensor. Microfabrication process recipes often require tweaking and changes over time to ensure that they continue to perform correctly and lead to a high fabrication yield. Due to this fact, only the main fabrication principles, techniques, and major steps of the fabrication process are included in the section and many of the minute details are omitted. The most up-to-date process recipe available when this dissertation was written is included in Appendix B for reference.

The full fabrication process of the ARROW biosensor can be broken down into seven major steps: bottom ARROW layer deposition, sacrificial core patterning, pedestal etch, top oxide deposition, solid-core rib etch, sacrificial core expose, and sacrificial core etch. Each of these fabrication steps is described in its own section below.

5.1 Bottom ARROW Layers Deposition

The first step in the fabrication process is to deposit the bottom ARROW layers. These layers make up a dielectric stack that is designed to be highly reflective in the entire visible wavelength spectrum at glancing angles [70]. The thicknesses of these layers are also designed to allow for constructive interference within the core of the ARROW [87]. The ARROW layers are deposited directly on top of a silicon substrate. When designed correctly the ARROW layers provide an optical buffer to keep light away from the absorptive silicon substrate and force it to
guide within the core of the ARROW. Figure 5-1 illustrates what the biosensor looks like after the bottom ARROW layers are deposited.

![Figure 5-1: Illustration of bottom ARROW layer deposition.](image)

Table 5-1: List of the material, index of refraction, and exact thicknesses of the bottom ARROW layers.

<table>
<thead>
<tr>
<th>LAYER #</th>
<th>MATERIAL</th>
<th>INDEX</th>
<th>THICKNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Ta$_2$O$_5$</td>
<td>2.107</td>
<td>102</td>
</tr>
<tr>
<td>5</td>
<td>SiO$_2$</td>
<td>1.47</td>
<td>265</td>
</tr>
<tr>
<td>4</td>
<td>Ta$_2$O$_5$</td>
<td>2.107</td>
<td>102</td>
</tr>
<tr>
<td>3</td>
<td>SiO$_2$</td>
<td>1.47</td>
<td>265</td>
</tr>
<tr>
<td>2</td>
<td>Ta$_2$O$_5$</td>
<td>2.107</td>
<td>102</td>
</tr>
<tr>
<td>1</td>
<td>SiO$_2$</td>
<td>1.47</td>
<td>265</td>
</tr>
</tbody>
</table>

The most recent design of the ARROW biosensor calls for six ARROW layers. Specifically, these are alternating layers of SiO$_2$ and Ta$_2$O$_5$. Table 5-1 lists the specific indices of refraction and thicknesses used for each of these initial six ARROW layers. In the past, SiN was
used instead of Ta$_2$O$_5$, but it was discovered that Ta$_2$O$_5$ has a lower photoluminescence than SiN. Changing to Ta$_2$O$_5$ helps decrease the background noise in the biosensor [80, 88].

The bottom ARROW layers are deposited onto the silicon substrate using the deposition technique called sputtering. This method involves driving ions towards a target, made of the deposition material, using an electric potential [89]. The ions strike the target and cause target atoms to break off or “sputter” off the target. The precipitated atoms are then allowed to redeposit directly onto the desired substrate. This process is depicted below in Figure 5-2. The targets used for sputtering the bottom ARROW layers are made of SiO$_2$ and Ta$_2$O$_5$.

![Schematic for a typical sputtering process.](image)

**Figure 5-2: Schematic for a typical sputtering process.**

### 5.2 Sacrificial Core

Next, the hollow-core ARROW structure needs to be formed; however, hollow channels are not made easily using microfabrication. There are only a few methods for making a hollow channel in microfabrication and they all involve multiple step processes. The method used for ARROW biosensor fabrication is the sacrificial material etch method, which is simply done by depositing a solid, sacrificial material, in the shape of the desired hollow channel, onto the
substrate to provide a structural layer. That material is then covered with the structural layers until all the fabrication processes are complete. Then, as a last step, a small portion of the sacrificial material is exposed and etched out using a wet etch.

The sacrificial core is deposited and then patterned using standard photolithographic processes. The material used for the sacrificial cores is SU8, a standard microfabrication, negative photoresist. Figure 5-3 shows a diagram of the basic steps of photolithography. The first step is to apply a photosensitive material, called photoresist to the substrate. This is typically done by applying some drops of the photoresist onto the center of the substrate and then spinning the substrate at high speeds. The centrifugal forces of the spin cause the photoresist to spread over the substrate and create a uniform thin layer of the photoresist over the entire wafer. By controlling the acceleration of the spin, the final speed, and the spin time, the photoresist film can be spun on at very accurate and precise thicknesses.

As stated above, a photoresist, is sensitive to the light that strikes it. Typically, photoresist is designed to be most sensitive in the UV spectrum. There are two main types of photoresist, positive and negative. A positive resist is structurally weakened when exposed to UV radiation, while a negative resist is gains structural integrity, through cross-linking, with UV exposure. SU8 is an example of a negative photoresist. The next step in the photolithography process is to mask, or cover the substrate, where UV exposure is undesired. The substrate is then exposed to the UV radiation.

Once the photoresist has been exposed to UV radiation, the next step is to develop away the undesired photoresist. This process leaves patterned features made from photoresist on the substrate. The photoresist can then be used as an actual layer in the structure of the device or act as mask for etching away or depositing other material.
Figure 5-3: Typical process flow for basic photolithography.
For optical purposes, it is important for the hollow-core ARROW to have very straight side walls. This allows for the most efficient coupling of light between the hollow-core and solid-core ARROWS. SU8 photoresist is used for the sacrificial core because it tends to maintain a very straight side wall profile throughout the entire fabrication process [85, 90, 91]. Figure 5-4 shows an ARROW biosensor with a completed sacrificial core.

![Figure 5-4: ARROW biosensor with a completed sacrificial core.](image)

**5.3 Pedestal Etch**

The next step in the ARROW biosensor fabrication process is called the pedestal etch. This step is important in order to make the hollow core have good structural integrity and prevent cracking [92]. Without the pedestal step, the hollow channels in the ARROW biosensors will often crack and break due to a crevice that forms in the top layer later in fabrication. Figure 5-5 shows the ARROW biosensor with a completed pedestal etch.

The purpose of this pedestal depicted in Figure 5-5 is to raise the hollow channel high above the substrate surface. This is necessary because of a crevice that forms in the oxide overcoat used to cover the hollow channel and helps to improve the fabrication yield of the biosensor. Figure 5-6 shows an SEM image of hollow-core ARROW made without (a) and with
(b and c) a pedestal etch. In (a) it is clearly visible that a crevice forms in the overcoat material right next to the hollow-core, which is due to a non-conformal process. This crevice makes a weak point for the hollow-core ARROW and will cause the channel to crack easily.

Figure 5-5: Illustration of pedestal etch step in the ARROW fabrication process.

In Figure 5-6 (b) and (c) the hollow-core ARROW is resting on a pedestal. In (b) the pedestal was formed using a standard photolithographic patterning process. The figure shows that although the hollow channel rests on a pedestal, the mismatch in size between the pedestal and core cause shoulders to appear in the top layer. These shoulders also represent a weak point in the structure of the hollow channel and will break very easily. In (c) a self-aligned process was used to exactly the match the size of the pedestal to the size of the hollow channel.

The self-aligned pedestal step is illustrated below in Figure 5-7. The first step in the process is to spin-on a thick layer of positive photoresist to cover all the features on the wafer. Instead of using a mask to pattern features, the entire wafer is flood exposed with a small amount of UV radiation. A low concentration developer is then used to slowly develop away the photoresist until only the top of the sacrificial core is exposed. The next step is to deposit a thin film of nickel over the entire wafer. Once the nickel is deposited, the wafer is submerged in
acetone to quickly dissolve away the underlying photoresist material. All the nickel attached to photoresist will lift off the wafer, while the nickel adhering to sacrificial cores will stay attached. At the end of the process, a perfectly aligned nickel mask if left resting on the sacrificial core.

Figure 5-6: Illustration of how a self-aligned pedestal, shown in (c), helps raise the structurally weak crevice away from the fragile hollow core.

Figure 5-7: Full process flow for the self-aligned pedestal (SAP) step.
Once the nickel mask is formed on top of the sacrificial core, the pedestal is etched using a dry etcher. A dry etcher is used for this step because it is important for the etch profile to have straight walls. A standard wet etch does not give straight side walls, because the etch is isotropic in nature, meaning the acid etches the material in all directions at the same speed. In order to produce straight walls, an etch must be anisotropic in nature, which means that it etches down much faster than it etches to the sides. Figure 5-8 (a) shows an example of an anisotropic etch profile, and (b) shows the etch profile obtained by using an isotropic wet etch.

![Figure 5-8: Examples of an (a) anisotropic dry etch profile versus the (b) isotropic wet etch profile.](image)

The pedestal etch process makes use of an e-beam evaporator to deposit the nickel etch mask and a dry etch machine for etching the pedestal; the general functions of both tools are described below.

An e-beam evaporator is a physical vapor deposition (PVD) process, where a solid target source is used to produce a vapor that eventually is deposited onto the desired substrate. Sputtering, which was described above is an example of another PVD process. However, in e-beam evaporation processes the solid target is vaporized with the use of high temperatures generated via a high-energy e-beam instead of physical ion bombardment. In the case of e-beam evaporation an electron beam is focused onto the target to heat it quickly and cause it to
vaporize. Another form of evaporation is thermal evaporation, where an electric current through a heating filament is used to heat up the source. Evaporation must occur at very low vacuum levels (~$10^{-5}$ torr) to ensure that the vapor of the source has a direct path to deposit onto the substrate. At higher pressures, the increased number of gas particles in the chamber will collide with the vapor from the source, causing impure and low quality films. Figure 5-9 shows an illustration of a standard e-beam evaporation chamber.

![Figure 5-9: Schematic of typical e-beam deposition machine.](image)

The idea behind dry etching is to use a plasma gas to both chemically and physically etch away a thin film. Figure 5-10 depicts a general dry etch chamber. Source gases are fed into a chamber and a high frequency voltage is applied to the chamber to excite a plasma. In some dry etch processes, just the chemistry of the gases is taken advantage of to etch away the thin film; however, these types of processes generally lead to an isotropic etch profile just like the wet etch. In other cases, however, the ions making up the plasma are used to physically bombard the thin
film and sputter it away. This is done by giving the ions a downward velocity towards the substrate, either by mismatching the top and bottom electrodes or by using an inductively coupled plasma (ICP). An ICP is generated using induction versus a capacitive setup and can create very directional ions within the plasma, which leads to high anisotropic nature in the etch.

![Figure 5-10: Schematic of a typical dry etch chamber.](image)

### 5.4 Top Oxide Deposition

After the pedestal etch is complete, the next step is to deposit the top oxide layer over the top of the entire substrate. Figure 5-11 shows the ARROW biosensor after the top oxide is deposited. SiO$_2$ is the material that is used for the top oxide layer for a few reasons. The first is its chemical stability. SiO$_2$ can withstand long periods of time submerged in a wet etch environment. This is an important feature, because the final step of the ARROW fabrication process is to submerge the entire wafer into an extended acid bath in order to etch out the hollow channels. The second desirable attribute of SiO$_2$ is its transparency and low optical absorption in the visible light spectrum. The top oxide is used in this fabrication process, not only to cover the
hollow channels, but to form the solid-core ARROWs that interface with the hollow-core ARROW. In this way, the top oxide doubles as an important structural layer and the core materials for the waveguides of the ARROW biosensor.

![Figure 5-11: Illustration of top oxide deposition step in the ARROW biosensor fabrication process.](image)

The thickness of the top oxide is very important. First, because it provides the structural walls of the hollow-core ARROW. A thicker top oxide generally makes the structure stronger. The second reason is that the thickness of the top oxide must be designed so that it vertically lines up the optical modes of the solid-core and hollow-core ARROWs on the biosensor. Six microns has been determined via finite difference optical simulations and verified through experimentation [93].

The most common way to deposit high quality SiO₂ in microfabrication is via chemical vapor deposition (CVD). Unlike PVD, CVD processes react gaseous sources to yield a solid products in the form of a thin film on a substrate. A very simple CVD process that can deposit a high quality SiO₂ film is called low pressure chemical vapor deposition (LPCVD). The source gases SiH₄ and N₂O are flown into a low-pressure chamber with the substrates that are to be
coated. The entire chamber is then heated up, using resistive heaters, to encourage the chemical reaction of the two source gases to form a solid thin film of SiO₂. Figure 5-12 illustrates what a typical LPCVD system looks like. SiO₂ films produced using this method tend to have quick deposition rates and are very high quality, with low amounts of impurities in the film; however, they require high temperatures of around 700°C for the reaction to occur. The hollow channels on the ARROW biosensor cannot withstand such high temperatures without developing large cracks and damage.

![Figure 5-12: Schematic of a typical LPCVD setup.](image_url)

Fortunately, plasma enhanced chemical vapor deposition (PECVD) can be used to deposit SiO₂ at much lower temperatures (~250°C). As can be inferred by its name, PECVD makes uses of electric fields to produce a plasma during the deposition process. This plasma helps energize the gas source particles and encourages the same chemical reaction to occur, just at a lower temperature. Figure 5-13 shows a representation of a PECVD chamber.

PECVD is a very common method in microfabrication to deposit a quick and simple dielectric layer when needed. It can have decently fast deposition rates and its low deposition temperature is also a very appealing characteristic. There are some disadvantages to using PECVD for depositing dielectric layers. The main disadvantage is that the low deposition
temperature leads to a low quality thin film being deposited [89]. The film is low quality because it will contain impurities [94], will be a non-stoichiometric film [95], it will have a low breakdown voltage, and it will be hygroscopic [96], meaning that it will absorb water from its environment. The film will also be more optically absorptive than a high quality SiO2 thin film [97-99], which leads to high optical loss in the solid-core ARROWs. Unfortunately, even with all of these disadvantages, other materials have been investigated for use as a top layer and PECVD SiO2 remains the best option for a top layer in the ARROW biosensor.

![Figure 5-13: Schematic of typical PECVD tool setup.](image)

Although PECVD tends to have decently conformal depositions, which means that the film deposits evenly along edges, trenches, or other topology on the substrate, it is not perfectly conformal. Oxide growth over a channel, such as the sacrificial core, causes a bread-loafing effect to occur [89], which leads to the crevice feature that was discussed in the previous section. Figure 5-14 is an SEM image that shows the crevice that forms during the PECVD oxide
deposition. This crevice is the main reason that the pedestal step is imperative to improve the structural integrity of the biosensors.

![Crevice image](image)

Figure 5-14: SEM image showing the crevice formed due to the non-conformal nature of PECVD.

### 5.5 Solid-Core Rib Etch

Once the top oxide has been deposited the next step is to pattern and etch the solid-core ARROWs. The solid-core ARROWs are designed to be rib waveguides and are used to interface with the hollow-core ARROW on the biosensor. This is quite a basic photolithography, mask, and dry etch process. The only complication to this step is that the biosensor has a high amount of topology at this point in fabrication. This requires a thick layer of photoresist in order to effectively cover all of the features already present on the substrate. A thick photoresist layer makes high feature resolution difficult to achieve and limits the size of the features that can be defined correctly. The excitation solid-core ARROWs are typically designed to be 4 µm wide, which is pushing limits of our photolithography processing for this step. Figure 5-15 shows the ARROW biosensor after the rib etch is complete and the solid-core ARROWs are defined on the chip.
Figure 5-15: Illustration of the rib etch step in the ARROW biosensor fabrication process.

5.6 Sacrificial Core Expose

At this point, all the structural layers of the ARROW biosensor are complete. The only problem is that the sacrificial core material remains and is completely covered by the top oxide layer. The next step is to etch away the top oxide layer over the ends of the sacrificial core and expose the sacrificial core for etching. Figure 5-16 shows the ARROW biosensor platform once the top oxide layer has been etched away and the sacrificial core has been exposed.

Figure 5-16: Illustration of the core expose step in the ARROW biosensor fabrication process.
5.7 Sacrificial Core Etch

The last step in the fabrication of the ARROW biosensor platform is to etch away the sacrificial core material to clear out and for the hollow-core ARROW. This is a very slow process because as the hollow channel etches further in, it takes time for new reactive acid to diffuse into the channel and time for the waste products of the etch to diffuse out. Depending on the length of the hollow-core ARROW this process generally takes anywhere from 3 days to 14 days. The SU8 sacrificial cores are etched away using a piranha acid mix, which is a 2:3 mixture of H₂SO₄:H₂O₂. This mix ratio is important in order to control the intensity of the etch and protect the hollow channels from popping due to pressure from lots of waste product being formed too quickly within the channel [100].

Figure 5-17: Illustration of the core etch step in the ARROW biosensor fabrication process.
6  PECVD OXIDES AND WATER ABSORPTION

In this chapter, PECVD oxides and their properties are discussed in more detail. First, we
discuss the methods for tuning a PECVD process to produce films with a desired index of
refraction. The index of refraction of PECVD SiO$_2$ can be manipulated in several different ways
in order to produce the correct film. Next, the intrinsic stress of PECVD SiO$_2$ thin films is
discussed and the methods for tuning the PECVD process in order to minimize the stress in the
thin films. Minimizing the intrinsic stress in the PECVD thin films of the ARROW biosensor is
important to help prevent cracking and defects from forming in more fragile the hollow-core
ARROWS. The last section of the chapter deals with the water absorption properties of PECVD
SiO$_2$ films. Water absorption in the PECVD SiO$_2$ thin films of the ARROW biosensor has
proved to be very problematic because it causes changes in the material properties in the film and
deteriorates the waveguiding nature of the ARROW.

6.1  Refractive Index and Stress Tuning

The refractive index and the intrinsic stress value of a PECVD SiO$_2$ thin film are two of
the more important material properties to consider when using the film for optical waveguiding.
Obviously, the refractive index of the film will determine the behavior of the light as it travels in
the waveguide. The intrinsic stress is also important to consider for a couple reasons. The first
reason is that the waveguides need to be structurally sound; they cannot break. If they do break,
then their waveguiding will be ruined and they will not perform correctly. However, there is
another reason to consider the stress in the film as well. It turns out that lower stress SiO$_2$ thin films tend to absorb moisture into the thin film slower than high stress SiO$_2$ thin films. This fact is discussed in a later section in this chapter.

One advantage of PECVD thin films is that material properties in the film, such as refractive index and intrinsic stress can generally be tuned with simple process changes. The first and simplest method for tuning the refractive index of an SiO$_2$ thin film grown using PECVD is to adjust the source gas flow rate ratio [101]. In this case the source gases being used are silane (SiH$_4$) and nitrous oxide (N$_2$O). In general, by including more silicon reactants in the reaction with an increase in SiH$_4$ flow, the thin film becomes more silicon rich, which causes the refractive index to rise. However, an important characteristic of PECVD is that it can be a bit hard to predict and every deposition chamber can behave quite differently.

It is important to characterize each chamber separately in order to understand the thin films that it will deposit under different conditions for a given PECVD chamber. Figure 6-1 is a graph that shows the index of refraction versus the SiH$_4$:N$_2$O ratio for the two most prominent PECVD chambers in the BYU cleanroom: PECVD2 and PECVD3. For all of these tests, the refractive index of the thin film was determined using a single wavelength (635 nm) manual ellipsometer (Gaertner 1169-AK). It is clear that both chambers follow the rule of thumb that increased silicon reactants available during deposition causes the PECVD thin film to increase in refractive index. It is should be noted, however, that refractive index values for the two different tools are quite different. This variation in process recipe result is typical when dealing with different PECVD tools.
The intrinsic stress in the SiO$_2$ thin films was also measured and used to characterize both PECVD2 and PECVD3 in the BYU cleanroom. The stress in the thin films was calculated by measuring the wafer bow, caused by adding PECVD oxide thin films to a substrate, using an optical 3D profilometer (Zeta 20). The profilometer is capable of determining the height of the top surface across a wafer. The curvature in a wafer was determined using the 3D profilometer, both before and after the deposition of a SiO$_2$ thin film. The change in curvature was then calculated, and knowing the thickness of the substrate, its elasticity, and the thickness of the thin film SiO$_2$, the intrinsic stress of the film can be determined using the Stoney equation \[102\].

Figure 6-2 is a graph that shows the relationship of intrinsic stress to the SiH$_4$:N$_2$O gas flow rate ratio. It is very interesting to note the glaring differences in the graph between the two tools. PECVD2 SiO$_2$ films first decreased in stress with increasing silicon composition and then reached a point where that trend reversed and the stress began to increase with an increase in
silicon. The films grown using PECVD3 typically simply became more negatively stressed with an increase in silicon richness. This graph is an amazing example of how each PECVD chamber can behave quite differently. It cannot be overstated here that with PECVD, each tool must be characterized and studied individually. It should also be pointed out, that each chamber can also change over time and should be re-characterized to keep thin films depositing with the desired material properties.

The refractive index in a PECVD SiO₂ thin film can be tuned and adjusted in many other ways besides changing the source gas flow rate ratio. One common method is to dope the thin film with a new material by adding a new source gas. Common dopants that have been used to change the refractive index in SiO₂ thin films are germanium, fluorine, and nitrogen [103-105]. An additional dopant gas source is convenient in PECVD deposition, simply because its addition can provide more precise tuning and more control over different material properties. The

![Graph showing stress versus SiH₄:N₂O ratio.](image)

**Figure 6-2: Stress versus the SiH₄:N₂O ratio.**
Figure 6-3: Index of refraction versus CF$_4$ flow.

Figure 6-4: Intrinsic stress versus the CF$_4$ flow rate during PECVD deposition.
PECVD2 and PECVD3 tools in the cleanroom have also been characterized with fluorine doping in SiO₂ thin films by using CF₄ as a dopant source gas. Figure 6-3 shows the effects of CF₄ in the chamber during deposition on the refractive index of the SiO₂ thin film. For both tools, the refractive index typically decreased with an increase in CF₄ flow during deposition; however, the PECVD2 saw that trend change for some reason. In literature, typically fluorine doping is reported to decrease the refractive index of PECVD SiO₂ thin films.

The change in intrinsic stress versus the CF₄ flow rate was also studied in SiO₂ thin films deposited in both the PECVD2 and PECVD3 tools in the BYU cleanroom. The intrinsic stress seemed to drift more positive in the thin films with increasing CF₄ flow during deposition.

### 6.2 Water Absorption

PECVD SiO₂ thin films are hygroscopic, meaning they tend to absorb water from their environment [106-108]. The incorporation of water from their environment causes the refractive index of the films to change quite dramatically. In fact, we found that the refractive index in PECVD SiO₂ thin films will increase by as much as 1.6% upon absorbing moisture [2, 109, 110]. This is a fact that was unaccounted for in previous years of the ARROW project. Obviously, if the refractive index of a waveguide’s material is changing, it is very important to understand how it will change and take that into consideration.

In my early years on the project, we began to experience inconsistent and unexplained problems with our solid-core ARROWs. Typically, the waveguides would fail to confine light properly, leading to extremely high optical loss in the waveguides and causing extra noise in the biosensors. Figure 6-5(a) shows the profile of a standard rib waveguide and the expected fundamental mode and (c) shows an actual image of a working waveguide. However, (b) and (d) show examples of the actual mode profiles we were seeing in our waveguides.
Figure 6-5: Mode in rib waveguide with water absorption.

The poor mode profile in the solid-core ARROWs led to high optical losses that were unacceptable for high sensitivity SMD. Obviously, this is because the high optical loss would be detrimental to the SNR possible using the waveguides for biosensing. However, that is not the only reason the poor waveguiding was ruining the SNR during biosensing, the poor confinement also leads to another major problem: errant light that escapes from the solid-core ARROW strikes the liquid-core ARROW where there should be no light. This extra excitement causes the noise floor to increase, greatly decreasing the final SNR of the biosensor. Figure 6-6 shows the top view of an ARROW biosensor and (a) depicts an excitation solid-core ARROW that is confining the excitation light correctly; whereas, in (b) it shows what we were experiencing in our waveguides. The light was not confined well to the core and scattered outwards, leading to very poor SNR values for biosensing.

At first, the origins of this waveguiding problem were unknown. We began a search to try and determine what could be the major cause of our solid-core ARROWS failing. Eventually, we
determined that the waveguides seemed to change over time. We also found that by baking the waveguides at 300°C, we could “fix” the waveguides [111]. It was postulated that maybe the SiO$_2$ thin films were absorbing water, potentially even from the air environment. A quick check of the literature on PECVD SiO$_2$ thin films [112], confirmed that these films are hygroscopic and could be expected to absorb water from their environment.

Figure 6-6: Top view of an ARROW biosensor with a (a) functioning solid-core ARROW and (b) a failing solid-core ARROW.

A quartz crystal monitor was used to experimentally ensure that this was what was occurring in our SiO$_2$ thin films. These monitors are typically used to detect extremely small mass changes in thin film evaporators in order to determine deposition rate. They work on the principle that changes in their mass will change their resonance frequency. A thin film of the PECVD SiO$_2$ was deposited onto the monitor and its mass was monitored. The monitor was left out in standard atmospheric conditions to allow for water absorption from the air to occur in the film. Figure 6-7 shows the data that was collected during this experiment. The figure shows that the mass of the film continued to drift upwards over time, confirming that the film was absorbing something from the atmosphere and changing its mass.
The literature that discusses water absorption in SiO₂ is not very definitive on how water absorption changes the material properties of the oxide. For example, some sources claimed that the incorporation of water in the oxide would eliminate extra dangling bonds and change the chemical structure in a way that would decrease the refractive index [113]. Other sources claimed that the water would fill in low index air pockets or voids present in the thin film and cause the overall index of refraction in the material to increase. Another question about the water absorption is whether it is reversible process or not. Can the water incorporated in the film be expelled or has it changed the material in an irreversible process? We found that the existing literature could not agree on the answer to this question either. Some sources claimed that the process could be reversed with a simple bake out while other claimed the incorporation of water in SiO₂ was an irreversible process [106, 114-117]. An experiment was designed in order to determine how the refractive index of our SiO₂ thin films was effected by water absorption and whether or not incorporated water could successfully be expelled from the film.
A 1 µm thick film of SiO₂ was deposited on a standard silicon substrate using PECVD. The index of refraction was measured directly after deposition using a spectrum reflectometer (Filmetrics 205-0135). The film was then submerged in an 85°C water bath to encourage quick water uptake into the thin film. After 1 day in water the film was measured again on the reflectometer. We found that the index of refraction tended to increase with water absorption by around 1.8%.

Figure 6-8: Transmission of a solid-core ARROW throughout an annealing and acid soak cycle. The transmission increases with each anneal and decreases after the acid soak.

To determine if the uptake of water into our PECVD SiO₂ thin film waveguides could be reversed, we fabricated a normal solid-core ARROW as a test waveguide. The transmission of the waveguide was measured and then the waveguide was submerged in heated acid. After the
acid exposure, the transmission of the waveguide was tested again. The waveguide was then baked at 300°C for 12 hours and tested again. The process was repeated to determine if there were any major changes to the optical transmission of the waveguide with a bake out. Figure 6-8 shows that the waveguide would decrease in optical transmission with water exposure and then revert to a better transmission with a bake out. It is important to note that the transmission decreases a little with each bake out due to structural damage caused by high temperature cycling.

These two experiments demonstrate that, at least with our oxide films, water absorption will cause the refractive index of the oxide to increase and that the process is reversible using an extended 300°C bake out. With the process of water absorption in our PECVD SiO₂ thin films better understood, the next step was to understand its effects on waveguiding and to look for solutions to mitigate the negative waveguiding effects of water absorption in the SiO₂ films of our solid-core ARROWs. The next chapter discusses how the incorporation of water in the SiO₂ films ruins the waveguiding profile and several attempts for eliminating the negative effects.
MITIGATING WATER ABSORPTION WAVEGUIDING EFFECTS

In this chapter, we introduce a more detailed explanation of how water absorption in the PECVD SiO₂ thin film that makes up the solid-core ARROW leads to waveguide deterioration. Several methods are then introduced as possible solutions to the water absorption problems. These include low temperature bakes, stress tuning, and buried waveguides. Specifically, two generations of buried waveguides are discussed. The first generation of buried ARROWs (bARROWs) saw the first promising results for preventing the negative effects of water absorption in the PECVD SiO₂ waveguides. However, these waveguides still showed signs of water absorption affecting their waveguiding characteristics. The second generation of bARROWs was designed and proved to eliminate all of the negative effects of water absorption on waveguiding in PECVD SiO₂ waveguides.

7.1 Waveguide Deterioration due to Water Absorption

It has already been determined that water absorption in the PECVD SiO₂ film of the solid-core ARROW causes the waveguide lose its light confining properties and perform poorly as a waveguide. This section discusses the reasons behind this major change in waveguiding properties.

First, it has been mentioned above, but deserves another reminder, when the PECVD SiO₂ films that we grow absorb water it causes their index of refraction to increase. The water incorporated into the film fills any micro voids that were previously filled with just air. Standard
air has an index of refraction value of essentially 1. The water that replaces the air is assumed to have an index of refraction value around 1.33. This means that the overall effective index of the film will increase. Experiments have shown that the index of refraction of the SiO$_2$ film will increase by around 1.8% as it takes in moisture [109]. This increase in refractive index is not inherently bad for a waveguide; however, the true problem occurs when water absorption causes only localized changes in refractive index throughout the waveguide.

For example, for a 6 µm thick oxide layer, water can only absorb into the film through the exposed surface. As the water diffuses slowly into the oxide layer it creates a higher index top layer than the rest of the waveguide beneath. Interestingly, this higher refractive index top layer is sometimes visible in an SEM when using certain imaging parameters. Figure 7-1 is an SEM image of a solid-core ARROW that clearly shows two distinct layers in the oxide. Only one thick layer was deposited, the presence of the second layer is caused by the water uptake in the upper portion of the PECVD SiO$_2$ film.

![Figure 7-1: SEM image of a solid-core ARROW cross-section that depict a distinct water saturated upper layer in the PECVD oxide film. Reprinted with permission [111].](image)

Due to the principle of total internal reflection, light tends to guide within higher refractive index materials, and so the higher refractive index top layer tends to pull the light up towards the surface of the solid-core ARROW. Simulations were performed on the rib waveguide geometry used for the solid-core ARROWs in the ARROW biosensor to determine exactly how the higher refractive index top layer will affect the guiding properties of the
waveguide. The simulation software used is called FIMMWAVE and uses the finite difference method in order to numerically solve waveguiding modes in different waveguiding geometries. Figure 7-2 shows simulation results with this higher refractive index top layer included in the geometry. It can be seen that the higher refractive index layer tends to cause the optical mode of the waveguide to pull up towards the surface of the waveguide and begins to allow light to guide within the field portion of the waveguide. It is this portion of light that guides within the field of the waveguide that causes that waveguide to scatter light out of the core so readily and deteriorate its waveguiding properties.

Figure 7-2: (a) SEM image of a solid-core ARROW depicting the important regions used in the finite difference simulations. (b) Top images show simulated modes with water absorption layer (left) and with no water absorption layer (right). Bottom images show actual modes observed in a solid-core ARROW. Reprinted from [111] with permission from IEEE.
The first attempt to relegate this problem was to simply develop a lower temperature bake out (~300°C) in an inert nitrogen environment to drive any moisture out of the waveguide. The preliminary results described in the previous chapter were promising that perhaps a simple bake out could be the answer to the water absorption problem. We had already determined that a bake out would remove the moisture from the film and restore the waveguide’s desired waveguiding properties; however, each bake out would slightly damage the devices, causing cracking in the thin films or breaking the hollow-core ARROWs on the biosensor, and lowering the waveguide’s optical transmission. Another problem with the bake out method is that after a bake out the SiO2 films would once again begin absorbing moisture from its environment and after a time would eventually require another bake to drive the moisture out again. Waveguiding behavior changing over time and requiring multiple bakes is not viable for commercialization of a medical device, which is obviously required to be extremely predictable and stable.

At this point in the ARROW biosensor project, our group at BYU had no ability to test any waveguides onsite in the BYU facilities. The waveguides were all fabricated in the BYU cleanroom and then had to be shipped to our collaborators in UCSC for testing and characterization. While this was an ideal situation for difficult biosensing experiments that required much expertise and handling of bio-hazardous materials, skills that were only available at UCSC, water absorption testing was much simpler and could be expedited greatly if simple testing capabilities were brought onsite to BYU. At this point, I designed and built a new optical table setup for waveguide characterization and testing at BYU. Figure 7-3 shows the layout of the optical table setup that was built.

The waveguide to be tested is placed onto a mount on the optical table. The waveguide is then manually aligned to the focusing objective and to a single mode optical fiber using two
different XZY stages. The optical fiber comes from a pig-tail 635 nm single mode laser (Thorlabs S1FC635). This laser was used for all experiments and testing done at BYU. The backlight illumination in the waveguide setup allows for imaging of the waveguide facet from the side. This is important because it aids in the manual alignment of the optical fiber to the waveguide and also allows us to view the mode profile of waveguides on the setup. There is also a photodetector on the table which allows for the power coming through the chip to be measured. In a typical throughput measurement, first, the laser is focused onto the photodetector with no waveguide in place. This power is measured and recorded as the full power level. The chip is then put in place and aligned to the objective and fiber. The light is focused onto the photodetector again and the power is recorded. The optical power throughput is then determined by calculating (power through the waveguide/full power level).

![Waveguide setup built at BYU to be used for water absorption testing.](image)

We also needed a consistent way to induce quick water absorption in the PECVD SiO2 waveguides. Some initial testing was done by simply submerging the waveguides in a water bath.
sitting on a hot plate set to 85°C. The raised temperature and wet environment worked well to expedite the water absorption process in the waveguides; however, it was determined that extended time in a water bath caused precipitates in the water to coat the waveguides, making them dirty and leading to questionable results. It was not clear whether the negative waveguiding results were due to just water absorption or if it was mostly caused by the precipitates coating the surface of the waveguide. Later on, a better solution was discovered. The waveguides were kept on a raised platform inside a standard crockpot. Water was pooled in the bottom of the crockpot and the crockpot was set to the warm setting. This environment kept the waveguides at a constant temperature of ~80°C and a relative humidity of 99%. Water absorption in the crockpot was expedited, but the waveguides showed no signs of foreign precipitates coating their surfaces.

This setup was used to experiment with different bake times, temperature, pressures, and environments; however, all baking tests still showed the same two major problems. The waveguides were still unstable and susceptible to water absorption after a bake and the waveguides showed signs of damage after each bake. This demonstrated that these two problems could not be solved using a simple bake out and other changes were required to stabilize and protect the ARROW biosensors from the negative effects of water absorption in the PECVD SiO₂ thin film.

### 7.2 Intrinsic Stress and Water Absorption

Through all our testing, it was noted that water uptake in PECVD SiO₂ thin films might be affected by the intrinsic stress of the film. The next attempt to eliminate negative water absorption effects in the waveguides was to determine if there was an intrinsic stress level that could eliminate water absorption in the film. The newer PECVD3 tool was used to develop process recipes that had very different intrinsic stress values: -50 MPa, ±0 MPa, +50 MPa, and
+100 MPa. Solid-core ARROWs were fabricated using these different stresses. All of the waveguides were made using a 6 µm thick oxide layer that was patterned and etched to make 3 µm deep rib waveguide geometries that were 4 µm wide. Figure 7-4 shows the geometry used for each rib waveguide used for water absorption testing.

Figure 7-4: Rib waveguide geometry used for waveguide testing.

Once the different waveguides were fabricated for testing they were subjected to an extended 12 hour bake at 300°C in order to ensure all of the water was driven out of the waveguides before testing began. Immediately after baking an initial throughput measurement was taken for each of the waveguides and this was recorded as the day 0 throughput value. Then the waveguides were introduced to the wet environment described in the section above. They were periodically removed and had their throughput retested. Figure 7-5 shows the results of this testing.

At first glance, the +50 MPa stress SiO2 film seemed to perform best in these tests because of its initial high throughput results; however, looking at relative throughput drop, the ±0 MPa stress actually had the lowest throughput drop over time. This means that the ±0 MPa stress proved to be the most resistant to water absorption in this experiment. The differences in
initial optical throughputs on day 0 can be explained by the imprecision of microfabrication. If some of the waveguides were produced with slightly too small features, then the light from the optical fiber would not couple into the waveguide as well as if the waveguides were made to be the right dimensions.

Figure 7-5: Stress vs. water absorption in solid-core ARROWs made using PECVD SiO$_2$ thin films. Reprinted from [5] with permission from IEEE.

The most important result of this testing, however, was not that the ±0 MPa stress SiO$_2$ thin film absorbed the least water, but that all of the films absorbed water. This experiment demonstrates that water absorption will occur in all PECVD SiO$_2$ thin films, regardless of its intrinsic stress value. The negative effects on waveguiding of water absorption in SiO$_2$ films cannot be eliminated by changing the process recipe.

7.3 First-Generation bARROWs

The water absorption versus intrinsic stress tests showed that water absorption will occur in any PECVD oxide film and it cannot be eliminated completely by simply changing the
deposition recipe. A new waveguide design or material was needed to mitigate the negative water absorption effects that were occurring in the ARROWs. The first viable solution tested was the addition of a top cladding layer, made from PECVD SiO₂ as well.

The new top cladding layer is still hygroscopic; however, it helps prevent water absorption from reaching deep enough into the waveguide and effecting the core of the bARROW. Figure 7-6(a) shows the original ridge or rib waveguide design. The core oxide was deposited to be 6 µm thick and have an index of refraction of 1.46. The waveguide was then masked and etched 3 µm into the oxide, leaving a 3 µm oxide field on the sides of the waveguide. Figure 7-6(b) shows the first new bARROW design used in an attempt to mitigate water absorption effects in PECVD SiO₂ waveguides. The core oxide was still grown to 6 µm thick; however, the index of refraction was slightly raised to 1.48 and the waveguide was then etched a full 6 µm to completely remove any oxide around the edges and create a channel. Next, a top cladding layer was deposited over the top of the waveguide to bury it can create a bARROW. The cladding layer had an index of refraction of 1.46.

Figure 7-6: (a) Standard solid-core rib ARROW design used for old single-oxide ARROWs. (b) First bARROW design using a channel geometry under a protective top cladding layer.
The same testing procedure that was used to test water absorption versus stress was used to test these new bARROW designs. The waveguides were fabricated; baked out at 300°C for 12 hours or overnight; aligned on the waveguide testing setup at BYU; and tested for optical throughput, mode profile shape, and confinement properties. The bARROW design was the first successful attempt to mitigate any negative water absorption effects from the SiO₂ waveguides. Figure 7-7 shows mode images of (a) the standard rib ARROWs and (b) the channel bARROWs before and after the waveguides were introduced to wet environments. The ARROWs had significant changes in mode profiles over time. Specifically, the modes would move up towards the surface of the waveguide, where the high index layer formed due to water absorption. The channel bARROWS experienced less change due to water absorption; however, it is clear from the figure that there were some negative effects. The initial mode is very sharp and clear with no aberrations. The mode image after water absorption shows some distorting effects making the mode less perfect.

Figure 7-7: (a) Mode images of a single-oxide rib ARROW before water absorption (top) and after water absorption (bottom). (b) Mode images of bARROW before water absorption (top) and after (bottom). Reprinted from [4] with permission from SPIE.
The optical confining properties of the waveguides was also tested over time with water absorption. This was done by taking images of the waveguides during testing from above. Typically, as the waveguides absorbed more water, they began to scatter more light and be less confining. The FWHM of the top profiles of the waveguides was measured near the output end of the waveguides. Figure 7-8 shows a graph of the FWHM, measured at the yellow line of the waveguides versus days in water. The ridges or standard rib ARROWs increased in width throughout the entire experiment, while the buried channel waveguide (BCWs) or bARROWs remained constant.

![Graph showing FWHM versus days in water](image)

Figure 7-8: Graph depicting the width of the waveguide light as seen from above versus the number of days in water. Reprinted from [4] with permission from SPIE.

The last test performed on the first-generation bARROWs was to test the optical throughput of the waveguides versus days in water. Figure 7-9 shows the relationship of throughput versus days in water. The bARROWs or BCWs, as they are labelled in the figure, started around 40% throughput and after 20 days in water still had a throughput of around 20%. The ridges or standard rib ARROWs decreased from around 40% at the start of the experiment to
less than 5% after only 6 days of testing. While the first-generation bARROWs did not perform perfectly, they definitely showed a major improvement in resiliency over standard rib ARROWs.

![Graph of the optical throughput of the buried channel waveguides (BCW) and the single-oxide ridge waveguides versus time in water. Reprinted from [4] with permission from SPIE.](image)

**Figure 7-9:** Graph of the optical throughput of the buried channel waveguides (BCW) and the single-oxide ridge waveguides versus time in water. Reprinted from [4] with permission from SPIE.

### 7.4 Second-Generation bARROWs

It was determined that the first-generation bARROWs were not 100% effective at preventing waveguiding loss due to water absorption because of one design flaw. The index of refraction of the core and cladding layers were close enough that water absorption in the cladding layer could raise the cladding index above that of the core index. Once this occurs, light can couple out of the core layer and begin to guide in the cladding layer, which is much more lossy and less confining, allowing more light to scatter out of the waveguide.

Two distinct bARROW designs were tested to determine this. The first design was called low index difference (LID) bARROWs and the second was called high index difference (HID)
bARROWS. The LID bARROWS had a core index of 1.46 and a cladding index of 1.456. The HID bARROWS had a core index of 1.51 and a cladding index of 1.448. Figure 7-10 shows the results of the water absorption testing for the second-generation bARROWS. The LID bARROWS decrease from around 45% throughput down to almost no throughput at all in less than 10 days. In fact, the LID bARROWS almost performed worse than standard unburied ARROWS. The HID bARROWS had no decrease in throughput at all over 40 days of testing. The initial optical throughput value was around 60% and after 40 days the throughput value was still right around 60%. This was the first waveguide design that was capable of completely removing any negative effects of the water absorption for an extremely extended period while in an accelerated wet environment. The next step after successfully making water resistant bARROWS was to incorporate the bARROW design into an actual biosensor.

Figure 7-10: Graph of the optical throughput of the bARROWS versus time in water. Reprinted from [5] with permission from IEEE.
This chapter discusses the fabrication changes, challenges, and biosensing results of integrating the second generation, HID bARROWs in an actual biosensor. The chapter begins by discussing the major fabrication changes required to integrate the new design. It also discusses some difficulties that arose in that fabrication process with the new steps. Specifically, cracking caused by the introduction of another oxide layer over the entire biosensor is discussed. The chapter explains the relationship of intrinsic stress and thickness in PECVD SiO$_2$ thin films. Finally, in this chapter we present exciting results demonstrating that the new bARROW biosensors are impervious to water absorption effects and also show an improvement in signal to noise ratio.

8.1 Fabrication

The basic idea of incorporating the second-generation bARROW design into an actual biosensor is not very complicated. Essentially, all the fabrication steps that were discussed in chapter 5 can remain that same with an additional oxide deposition step in order to grow the cladding oxide layer. Figure 8-1 shows the full process flow for making a bARROW biosensor. The only new step is shown in (f) and is the additional cladding layer that goes over the core oxide after the waveguide has been etched.
Figure 8-1: Basic fabrication steps for making the new bARROW biosensor. The new step (f) is the addition of a protective cladding layer.

However, the addition of a thick cladding layer, while the idea is simple, brought about some unforeseen complications. The largest of these was that the additional thickness of the cladding layer caused high stress in the oxide layers and caused them to crack. In fact, several initial attempts were made to fabricate the bARROW biosensors and they all ended with the oxide films cracking throughout the entire wafer, leading to very low yields and low optical throughput because of the additional scattering points in the waveguides.

We investigated the problem and discovered that thicker oxide layers grown in the PECVD3 machine increase in intrinsic stress. Several different recipes were tested and the results are shown in Figure 8-2. All the recipes showed a general trend upward in stress with increased thickness. The orange recipe, which is the recipe we had been using for the cladding layers, cracked after only 12 µm of oxide growth on a completely flat and smooth silicon wafer. A new PECVD process recipe was needed to deposit a low index and low stress oxide.

The task of depositing a low index, low stress PECVD oxide was actually not a very simple one. All our experience and testing of PECVD oxides to this point had indicated that low stress oxide films typically had high index values. However, using some of the trends that had
previously been determined with PECVD parameters and films characteristics we were able to create a new recipe that maintained relatively low intrinsic stress values (~12 MPa) while having a very low index value (1.448). This new PECVD process recipe is what made the fabrication of bARROW biosensors possible and without a low stress, low index oxide layer, the bARROWs could not have been integrated on the biosensors without much more significant changes to design or potentially to the materials used.

![Figure 8-2: (a) Graph of measure intrinsic stress versus the thickness of the PECVD oxide film grown. (b) Microscope image of an oxide that cracked due to excess intrinsic stress in the film.](image)

There was another problem that occurred when trying to integrate bARROWs onto the biosensor platform. The initial bARROW design that was tested used a channel design for the waveguide core. This waveguide shape is shown below in Figure 8-3(a). However, the biosensor made with these channel bARROWs performed very poorly when being used for any biosensing. There are a couple potential reasons for the poor performance of the channel bARROWS. The first is depicted in Figure 8-3(c) which shows an actual SEM of the channel bARROW on a biosensor. The side walls of the cladding layer grew in an odd geometry. This is most likely due to the rough surface that the cladding layer is growing over. To make a channel bARROW, the
core oxide is etched a full 6 µm into the oxide, to completely remove the field oxide on the sides. This etch creates a very rough surface for the cladding layer to deposit over and causes some serious problems. Compare this to the new rib bARROW design that is shown in Figure 8-3(b) and (d). To create the rib core profile, the core oxide only has to be etched half-way through, leaving a less rough surface for the cladding layer to grow over. As can be seen in the SEM, the cladding oxide covers the core rib geometry much more thoroughly than the cladding layer over the channel core.

Figure 8-3: (a) and (c) Schematic and SEM image of the buried channel bARROW design. (b) and (d) Schematic and SEM image of the buried rib bARROW design.
Another potential reason for the poor biosensing results for the channel bARROWs is that the crevice that forms at the liquid to solid core interface of the waveguides seems to be more dramatic in the channel bARROWs. This is shown in Figure 8-4, which shows SEM images of the crevice in both a (a) channel bARROW and a (b) rib bARROW. The crevice in the channel bARROW is much more dramatic and is a significant reason for the low SNR measured by the channel bARROW biosensors compared to rib bARROW biosensors. In fact, Figure 8-4(c) and (d) show actual data, sensing fluorescent microbeads. The channel bARROW biosensor only recorded a SNR of 9.8, while the rib bARROW had a much better SNR of 47.

![Figure 8-4](image)

**Figure 8-4:** (a) SEM image of the hollow-core intersection of a buried channel bARROW. (b) SEM image of the hollow core intersection of a buried rib bARROW. (c) Signal during SMD from the buried channel bARROW biosensor. (d) Signal during SMD from the buried rib bARROW biosensor.
8.2 Results

The new bARROW design represented a major change in waveguide profile as well as in fabrication processing. In order to assure that the biosensor still functioned correctly and had decent coupling efficiency between the liquid and solid core waveguide, the new waveguide geometry was simulated using the FIMMPROP (© Photon Design) software. The most important parameter measured was the core oxide thickness. If the core oxide is too thin, then the solid-core bARROW waveguides will lie too low to couple well with the liquid-core ARROW. The opposite is true if the core oxide layer is too thick. This will cause the solid-core bARROW to be too high above the liquid-core ARROW and limit the coupling efficiency. It has already been shown previously that the solid to liquid core ARROW interface is one of the most important parameters to keep the SNR high for the ARROW biosensors. Figure 8-5 shows the geometry that was used to simulate the bARROW biosensors for core oxide height. The top illustration is the side view of the excitation waveguides, all the way from the optical fiber aligned to the chip to the liquid-core interface. The middle illustration is the top view of the same waveguides. Below that shows the waveguide profile that was simulated for the bARROWS. The core oxide was given an index of refraction of 1.51 and the cladding oxide was assigned an index of 1.448. Air was surrounding the core with an index of refraction of 1.

The simulations were run with geometries shown above. The results are shown in Figure 8-6 and Figure 8-7. The former shows pictures taken from the simulations. These pictures help visually depict whether or not the optical fiber or liquid to solid core interface is optimized. For example, in Figure 8-6 it is clear that the core oxide thickness is almost perfect for the simulated crevice. The crevice in the simulation directs the waveguide mode directly into the liquid-core with almost perfect dimensions.
Figure 8-5: Specific geometries used for the FIMMWAIVE simulations.

Figure 8-6: FIMMWAIVE simulation results.
Figure 8-7 shows the coupling efficiency results for all of the core oxide thicknesses simulated. The blue line in the figure shows that the ideal core oxide thickness is somewhere between 6 and 7 microns. These simulations represent simulations run using the exact desired dimensions for each feature in the biosensors. SEM images were taken of two different bARROW biosensors, one with a 6 µm core oxide and another with a 7.2 µm core oxide. Measurements of all of the actual dimensions were taken of these biosensors and simulations were run with these actual dimension values. The results are shown in Figure 8-7 with the “x” and “+” symbols. A major take away from these simulations is that the coupling efficiency of the solid to liquid core interface on the bARROW biosensors is quite forgiving. As long as the core oxide height is somewhere between 6 and 7 microns thick, the coupling efficiency remains relatively high and does not drop off quickly.

![Figure 8-7: Graph of the FIMMWAVE simulation results for coupling efficiency versus oxide thickness.](image)

Full bARROW biosensors were fabricated with both 6.0 and 7.2 µm thick core oxides. These bARROW biosensors were made using the new low index, low stress PECVD process recipe. The bARROW biosensors also included the new rib bARROW design instead of the channel bARROW design. To test the SNR of the biosensors they were aligned in a setup like
the one shown in Figure 8-8(a). Fluorescent microbeads were introduced into the liquid-core and flown through using a vacuum pressure on one end of the liquid-core. The signal emitted by the microbeads as they passed through the excitation point was guided off-chip and collected using an avalanche photodiode (APD). Figure 8-8(b) shows data for one of the 6.0 µm core oxide bARROW biosensors. Each peak represents a microbead as it passed by the excitation point. The figure also clearly shows the noise floor for the biosensor.

![Diagram of microbead detection experiment](image)

Figure 8-8: (a) Depiction of the microbead detection experiment for testing the biosensors. (b) Example data from a bARROW biosensor.

The height of the signal collected varies with each biosensor as well as the height of the noise floor. The signal-to-noise ratio (SNR) is calculated by determining the average peak height and dividing that number by the height of the noise floor. Several bARROW biosensors were tested in order to obtain an average SNR for both the 6.0 and 7.2 µm bARROW biosensors. An older single-oxide ARROW biosensor was also tested as a control for this experiment. Table 8-1 shows the results for each of the biosensors tested.

The single-oxide ARROW biosensor was first tested before it was annealed at 300°C after fabrication. As expected, the SNR of this biosensor was extremely low, with a value of only...
3.75. This is not a high enough SNR to always accurately determine whether a given peak is simply a result of an abnormally high noise value or if it represents a microbead passing through the excitation point. The SNR of this biosensor was low due to water uptake in the unprotected PECVD oxide ARROWs of the biosensor. The single-oxide ARROW biosensor was then annealed at 300°C in order to drive all of this moisture out of the ARROWS and retested. Once again, as expected the SNR increased dramatically because of the removal of the moisture with the 300°C anneal. The SNR of the single-oxide ARROW increase from 3.75 to 39.7 with the 300°C anneal.

The average SNR for the 6.0 µm bARROW biosensors was 57.0 and the average SNR for the 7.2 µm bARROW biosensors was 70.0. These SNRs were achieved without any 300°C annealing post-fabrication. This demonstrates that the bARROW design successfully protected the biosensor waveguides from any negative water absorption effects that lower its SNR, like that of the single-oxide ARROW biosensor. Also, the higher SNRs obtained for the two different bARROW biosensor design most likely stems from the fact that the bARROW biosensors were never annealed at 300°C. As discussed earlier, the high temperature required for this anneal is typically damaging to the fragile ARROW layers and liquid-core of the biosensor. The damage is typically, not visible; however, the microcracks caused by the high temperature anneal are optically significant to the wavelengths used for biosensing and introduce lossy scattering points into the waveguides.

Table 8-1: Results of microbead testing for single-oxide ARROW biosensors and bARROW biosensors.

<table>
<thead>
<tr>
<th>Sensor type</th>
<th>Annealed?</th>
<th>Core oxide height</th>
<th>Average SNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-oxide</td>
<td>No</td>
<td>7.2 µm</td>
<td>3.75</td>
</tr>
<tr>
<td>Single-oxide</td>
<td>Yes</td>
<td>7.2 µm</td>
<td>39.7</td>
</tr>
<tr>
<td>bARROW</td>
<td>No</td>
<td>6.0 µm</td>
<td>57.0 ± 12.0</td>
</tr>
<tr>
<td>bARROW</td>
<td>No</td>
<td>7.2 µm</td>
<td>72.0 ± 22.0</td>
</tr>
</tbody>
</table>
This chapter demonstrated that the bARROW designs were successfully incorporated into actual biosensors. In order to accomplish this a new PECVD process recipe was developed that was capable of producing low index, low stress oxide films. This lower stress cladding film was capable of being deposited directly over the core oxide without introducing any cracking to the biosensor. Also, a new rib bARROW design was used to keep the SNR high for the bARROW biosensors. Channel bARROWs led to rough oxide layer that increased the loss of the waveguides and degraded their ability to guide light. The new bARROW biosensors achieved the highest SNR values recorded to date for any ARROW biosensor without the need for any damaging anneals at 300°C.
9 CONCLUSION

9.1 Project Summary

When I began research work on this project in 2013, the ARROW biosensor had already been used to demonstrate some SMD capability. However, since that time, the ARROW biosensor has seen some significant improvements and been demonstrated as an even more capable SMD biosensor. Some of these improved results came from my early work in fabrication and process recipe improvements. However, the most important contribution of my work is the solution to water absorption in the ARROWS.

It was unknown that water absorption even occurred in PECVD oxide films when I started work on the ARROW biosensor. All we knew was that there was an odd problem occurring with the confinement of the solid-core ARROWS. As part of my work, we discovered that this waveguiding problem was caused by water absorption. Several solutions to this problem were subsequently tested and eventually the bARROW design idea was presented by me after a thorough literature review of integrated optical waveguides. This design was fabricated, tested, and proved to be the most effective method for mitigating negative water absorption effect.

The second-generation of bARROWS was developed in order to improve upon the initial bARROW design. Experimentation showed that this new generation of bARROWS were completely impervious to any water absorption effects whatsoever.
Complete biosensors implementing the second-generation bARROWs were then designed and fabricated in order to create the first water-resistant biosensors to be fabricated. It took some time to successfully fabricate the new bARROW biosensors due to some complications that arose with intrinsic stress in thick PECVD oxide films. These complications were resolved and eventually complete bARROW biosensors were successfully fabricated with high yields. The new biosensors were tested by detecting fluorescent microbeads. The bARROW biosensors required no post-fabrication annealing or bake and yet outperformed the SNR values of older single-oxide ARROW biosensors.
REFERENCES


APPENDIX A. LIST OF PUBLICATIONS

A.1 Peer-Reviewed Journal Articles


A.2 Conference Papers and Presentations


APPENDIX B. BARROW BIOSENSOR PROCESS RECIPE

Bottom ARROW layers

- Six layers are commercially deposited via sputtering, typically by Evaporated Coatings, Inc. (ECI)

<table>
<thead>
<tr>
<th>Index</th>
<th>SiO₂</th>
<th>Ta₂O₅</th>
<th>SiO₂</th>
<th>Ta₂O₅</th>
<th>SiO₂</th>
<th>Ta₂O₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (nm)</td>
<td>2.107</td>
<td>1.47</td>
<td>2.107</td>
<td>1.47</td>
<td>2.107</td>
<td>1.47</td>
</tr>
<tr>
<td>102</td>
<td>265</td>
<td>102</td>
<td>265</td>
<td>102</td>
<td>265</td>
<td></td>
</tr>
</tbody>
</table>

- Grow 20nm of SiO₂ as an adhesion promoter

Pre-Core

- E-beam Cr 80nm
- AZ3330, 5000rpm, 60s
- Soft bake: 90°C, 60s
- Exp 10s
- Develop ~90s in 300MIF
- Hard bake: 110°C, 2min
- Descum: 150W, 60s
- Cr Etchant
- Remove PR with Acetone and IPA
- Descum: 150W, 60s
- Dehybake
Sacrificial Core

- SU-8 10, 500rpm @ 100rpm/s, 6s; 4400rpm @ 1200rpm/s 60s; 6000rpm @ 6000rpm/s 2s
- Soft bake: 65°C 5min -> 95°C 5min-> 65°C
- Exp 20s on filtered aligner
- PEB: 65°C 4min -> 95°C 4min -> 65°C
- Develop ~60s in SU8 Developer
- 1st Hard bake: 65°C -> 200°C 10min -> 65°C
- Descum: 50W, 60s
- 2nd Hard bake: 65°C -> 250°C 5min -> 65°C
- Descum: 50W, 60s

Pedestal

- AZ4620, 2800rpm @ 1100rpm/s, 30s; 6000rpm @ 6000rpm/s, 2s
- Softbake: 70°C 60s; 90°C 60s; 120°C 20s
- Exp 55s; Flood Exp 7s on unfiltered aligner
- Develop ~3min in 400K:H2O 1:4
- Descum 50W, 30s
- HCL Dip: (HCL:H2O, 1:2) for a few seconds
- E-beam Ni 75nm
- Liftoff with acetone
- Descum 50W, 30s
- Trion "SAPoxide", target: 1.3µm
- Soak in TC1 (RS6:H2O2, 10:1) 10min @ 55°C
- Trion "Dummy Cycle"
- Trion "Bosch", target: 6µm
- Trion "Si_isotropic" 60s
- Soak in TC1 (RS6:H2O2, 10:1) 10min @ 55°C
- Ni Etch
- Soak in TC1 (RS6:H2O2, 10:1) 10min @ 55°C
- Descum 50W, 30s
- Dehybake

**Solid-Core Oxide**
- Deposit 7.2µm in PECVD3 using “Zero Stress” recipe, index 1.51
  - 250°C, 1900mtorr, 16W HF, 164sccm SiH4, 88sccm N2O
- Dehybake

**Rib Etch**
- SU-8 2025, 500rpm @ 100rpm/s, 6s; 3700rpm @ 1200 rpm/s, 60s; 6000rpm @ 6000rpm/s, 2s
- Soft bake: 65°C 4min -> 95°C 5min -> 65°C
- Exp 12s on unfiltered aligner
- PEB: 65°C 5min -> 95°C 8min -> 65°C
- Develop 90s
- Hard bake 65 -> 180 10min -> 65°C
- Descum 100W, 90s
- Trion "SAP Oxide", target 5400nm
- TC1 (H2O2:RS-6=1:10, 55C) 10min
- Nanostrip, 30min @ 90°C
- TC1 (H2O2:RS-6=1:10, 55C) 10min
- Descum: 100W, 60sec
- Dehybake
Cladding Oxide

- Deposit 6.0µm in PECVD3 using “Steve’s Low Index, Low Stress” recipe, index 1.46
  - 250°C, 1900mtorr, 40W HF, 20W LF, 200sccm SiH₄, 500sccm N₂O
- Dehybake

Core Expose

- AZ4620, 1500rpm, 60s
- Soft bake 80°C 20min
- Let rest for a long time to ensure water dissipates out of rough SiO₂ pillars
- Exp 30s
- Dev 400K:H₂O 1:4 (~3min)
- Hard bake 100°C at least 2hr
- Descum 60s @ 150W
- BHF Etch ~700

Core Etch

- Place in Piranha @ 130°C, change acid daily
  - Piranha: 60ml:40ml H₂O₂:Sulfuric Acid
- H₂O @ room temperature for at least 6 hours
- Nanostrip @ room temperature for at least 6 hours
- H₂O @ room temperature for at least 6 hours
APPENDIX C. MATLAB CODE

```matlab
function [x_axis, profileX, y_axis, profileY, FWHM] = fwhm(picName)
    close all;
    clc;

    % Calculates the horizontal and vertical FWHM of
    % a waveguide based on a side view image
    % *************************************

    conversion = .03333; %um/px
    pic = strcat(picName, '.jpg');
    fileName = strcat(picName, 'X', '.csv');
    fileName2 = strcat(picName, 'Y', '.csv');

    % Import image
    I = imread(pic);

    %Crop the image around the entire mode
    K = imcrop(I, [0, 200, 1000, 1000]);
    [m,n] = size(K);
    H = 1;
    figure(H);
    imshow(K);
    [centerX, centerY] = ginput(1)
    centerX = round(centerX)
    centerY = round(centerY)
    K = im2double(K);
    K = 255*K;
    [m,n]=size(K);
    n = n/3;
    n = fix(n)-1;

    % Set up vectors that will contain all of the profile data
    profileX = zeros(m, 1);
    profileY = zeros(n, 1);

    x_axis = zeros(m,1);
    y_axis = zeros(m,1);
    FWHM = zeros(m,1);

    % Fill in x profile vector
    for i = 1:m
        profileX(i,1) = K(i,centerX,1);
        x_axis(i) = i;
```
% Fill in y profile vector
for j = 1:n
    profileY(j,1) = K(centerY,j,1);  
    y_axis(j) = j;
end

x_axis = x_axis .* conversion;  
y_axis = y_axis .* conversion;

% Calculate FWHM
[maxValX, maxPosX] = max(profileX);  
[maxValY, maxPosY] = max(profileY);  

widthY = length(find(profileX >= maxValX/2)) * conversion  
widthX = length(find(profileY >= maxValY/2)) * conversion  
FWHM(1) = widthX;  
FWHM(2) = widthY;

figure
subplot(1,2,1)
plot(profileX, x_axis, '.');  
xlabel('Width (um)');  
ylabel('Pixel Value');  
title(['Vertical FWHM: ' num2str(widthY)]);

subplot(1,2,2)
plot(y_axis, profileY, '.');  
xlabel('Width (um)');  
ylabel('Pixel Value');  
title(['Horizontal FWHM: ' num2str(widthX)]);

dataX = [x_axis, profileX, FWHM];  
dataY = [y_axis, profileY];  
csvwrite(fileName, dataX);  
csvwrite(fileName2, dataY);
function [pixels,averageInt] = autoscatterAveraged(picName,theta, divSize, thresh)
close all;
clear;
%******************************************************************************
% This function takes in a waveguide top view image and calculates the loss
profile and loss coefficient of the waveguide. It is capable of averaging
(divSize) adjacent pixels together in order to clean up the data.
%******************************************************************************
pic = strcat(picName, '.jpg');
fileName = strcat(picName, '.csv');

%import image and convert to grayscale intensity.
I = imread(pic);
I = rgb2gray(I);
figure, imshow(I);

%rotate image and display rotated and unrotated versions.
K = imrotate(I,-theta);
figure, imshow(K);
[m,n] = size(K);
figure, imshow(J);
intensity = sum(J);

%crop image to appropriate length and 30 pixel width.
K = imcrop(K);
[m,n] = size(J);
K = imcrop(J,[1 top n width]);
[m,n] = size(K);

%find pixel vertical mean and store it in intensity vector.
intensity = [0:(length(intensity)-1)];
figure, imshow(K);
K = im2double(K);
K = 255*K;
[m,n] = size(K);
intensity = zeros(1, n);
for i = 1:n
  count = 0;
  for j = 1:m
    if K(j,i) > thresh
      count = count + 1;
      intensity(i) = intensity(i) + K(j,i);
    end
  end
  if count > 0
    intensity(i) = intensity(i)/count;
  else
    intensity(i) = 1;
  end
end
subDiv = length(intensity)/divSize;
subDiv = fix(subDiv)-1;
averageInt = zeros(1,subDiv);
for i = 0:subDiv
    temp = 0;
    for j = 1:divSize
        temp = temp + intensity(divSize*i + j);
    end
    temp = temp / divSize;
    averageInt(i+1) = temp;
end

% intensity = sum(K);
intensity = log(intensity);
pixels = [0:(length(intensity)-1)];
averageInt = log(averageInt);
pixels = [0:(length(averageInt)-1)];
averageInt = averageInt';
pixels = pixels';
multFactor = ones(length(averageInt), 1);
multFactor = multFactor .* (2300 / divSize);
averageInt = multFactor .* averageInt;
threshMat = zeros(length(averageInt), 1);
threshMat(1,1) = thresh;
data = [pixels, averageInt, multFactor, threshMat];
csvwrite(fileName, data);

%take the log and find linear fit, display exponentiated data and fitted %line.
fit = polyfit(pixels, averageInt, 1);
fit1 = polyval(fit, pixels);
figure, plot(pixels, averageInt, '.')  */, pixels, fit1, 'red', 'Linewidth', 2);
% aout = exp(fit(2))
% bout = fit(1) * 800
%[p2,i] = autoscatter('IMG_20150227_134708813.jpg',-2.35,2790,100);