Multiplexed Optofluidics for Single-Molecule Analysis

Matthew Alan Stott
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Multiplexed Optofluidics for Single-Molecule Analysis

Matthew Alan Stott

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

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ABSTRACT

Multiplexed Optofluidics for Single-Molecule Analysis

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The rapid development of optofluidics, the combination of microfluidics and integrated optics, since its formal conception in the early 2000’s has aided in the advance of single-molecule analysis. The optofluidic platform discussed in this dissertation is called the liquid core anti-resonant reflecting optical waveguide (LC-ARROW). This platform uses ARROW waveguides to orthogonally intersect a liquid core waveguide with solid core rib waveguides for the excitation of specifically labeled molecules and collection of fluorescence signal. Since conception, the LC-ARROW platform has demonstrated its effectiveness as a lab-on-a-chip fluorescence biosensor. However, until the addition of optical multiplexing excitation waveguides, the platform lacked a critical functionality for use in rapid disease diagnostics, namely the ability to simultaneously detect different types of molecules and particles.

In disease diagnostics, the ability to multiplex, detect and identify multiple biomarkers simultaneously is paramount for a sensor to be used as a rapid diagnostic system. This work brings optofluidic multiplexing to the sensor through the implementation of three specific designs: (1) the Y-splitter was the first multi-spot excitation design implemented on the platform, although it did not have the ability to multiplex it served as a critical stepping stone and showed that multi-spot excitation could improve the signal-to-noise ratio of the platform by ~50,000 times; (2) a multimode interference (MMI) waveguide which took the multi-spot idea and then demonstrated spectral multiplexing capable of correctly identifying multiple diverse biomarkers simultaneously; and, (3) a Triple-Core design which incorporates excitation and collection along multiple liquid cores, enabling spatial multiplexing which increases the number of individual molecules to be identified concurrently with the MMI waveguide excitation.

In addition to describing the development of optical multiplexing, this dissertation includes an investigation of another LC-ARROW based design that enables 2D bioparticle trapping, the Anti-Brownian Electrokinetic (ABEL) trap. This design demonstrates two-dimensional compensation of a particle’s Brownian motion in solution. The capability to maintain a molecule suspended in solution over time enables the ability to gain a deeper understanding of cellular function and therapies based on molecular functions.

Keywords: Matthew Alan Stott, Aaron Hawkins, optofluidics, single-molecule analysis, fluorescence, biosensor, lab-on-a-chip, integrated optics, microfluidics, multimode interference waveguides, Y-splitter waveguides, Anti-Brownian Electrokinetic trap, ARROWs, microfabrication, optofluidic multiplexing
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1 INTRODUCTION

Since its introduction in the early 2000’s, the field of optofluidics has found an important application in single-molecule analysis for lab-on-a-chip platforms. Optofluidics can be defined as the integration of microfluidics and optics on the same miniaturized system. The ability of optofluidics to guide light and small detection volumes (fL-nL) of fluid in the same system make them advantageous for biological and chemical analysis.

In recent years, with global outbreaks of Ebola hemorrhagic fever, Zika virus and the growing threat of antibiotic resistant bacteria, there has been a call for rapid, highly sensitive and accurate disease diagnostic systems [1, 2, 3]. These systems could be used without sending the biological sample to expensive laboratories or needing highly trained professionals. The optofluidic platform described in this dissertation will provide a diagnostic system that can be readily used at the doctor’s office without having to ship biological samples to a diagnostic lab.

1.1 Project Overview

The end goal of the work in this dissertation is the development of a rapid single-molecule analysis system. Specifically, the work focuses on the detection and analysis components of the system, rather than on the blood filtration, genetic selection, and fluorescent labeling components that such a system would require. The research involved in this dissertation includes work in the
fields of microfabrication, microfluidics, integrated optics, and biophotonics while relying on other biomedical fields such as organic chemistry and biology.

There is a wide range of optofluidic platforms being researched for use in single-molecule analysis. They can typically be broken up into two subcategories: (1) those used for detection and (2) those used for trapping and manipulation. Such platforms rely on a change in optical properties such as refractive index, absorption, polarization, or fluorescence to generate the signal used for sensing the molecule [4]. Those of the trapping variety exploit such signals to manipulate the particle either by direct optical forces — as in the case of optical tweezers [5], surface-plasmon-enhanced optical traps [6], and fluorescent activated cells sorters (FACS) [7] — or by indirect forces used in devices such as optoelectronic tweezers [8], light-actuated electroosmosis traps [9], and Anti-Brownian Electrokinetic (ABEL) traps [10]. Both detection and manipulation are important for single-molecule analysis, and both can be implemented on the platform described in this work.

The focus of this dissertation is the liquid core anti-resonant reflecting optical waveguide (LC-ARROW) optofluidic platform. It has been studied extensively at Brigham Young University for over a decade [11]. The basic LC-ARROW platform has proven capable of both trapping and detection at the single-molecule level [12, 13]. The signal detection works by labeling molecules with fluorophores which can then be excited, leading to signal that can be collected on chip and directed off chip to a detector. The molecules are detected directly because they are specifically labeled according to a DNA sequence. This is advantageous because the detection can be done without prior amplification of the molecules, making it ideal for rapid disease diagnostics. The platform has proven its ability to correctly identify single molecules at a time, in order to be useful
for rapid disease diagnostics, however, it also needs the ability to multiplex, or in other words, detect multiple biomarkers simultaneously.

When sending off a blood sample to the lab, that blood undergoes a “test panel”, which is a series of tests administered for diagnostic purposes. These tests can be run simultaneously and some examples of infectious disease diagnostics tests are: rapid enzyme immunoassays (EIA) [14], real-time polymerase chain reaction (RT-PCR) [15], culturing [16, 17], rapid antigen tests [18], blood agar plates [19, 20] and phenotypic assays [21]. The choice of test depends on the disease in question [22, 23]. For example, when testing for various virus infections, RT-PCR is the gold standard [24] and four to eight pathogen types are screened for simultaneously [25, 26]. Current diagnostic panels have excellent sensitivity and specificity but their complexities make them unsuitable for use in a typical doctor’s office and are slower than desired when dealing with certain infections. The LC-ARROW platform will be simple to use, but in order for it to eventually replace expensive laboratories, it must have the ability to multiplex (test multiple pathogens simultaneously).

Until recently, the LC-ARROW platform suffered from being capable of testing only one biomarker at a time. The research in this dissertation brings the ability to multiplex to the biosensor in two different ways, spectral and spatial multiplexing. This was accomplished by introducing new designs to the platform’s excitation waveguides. Before multiplexing, the concept of a multi-spot excitation pattern was introduced by the Y-splitter design. This design was straightforward to implement and to study. It was used to show that multi-spot excitation could improve signal-to-noise ratios through simple signal processing and served as a stepping stone towards the more complex wavelength dependent multiplexed designs. The first wavelength dependent multiplexed design included a multi-mode interference (MMI) waveguide which enabled wavelength division
multiplexing. The second design (which we call the Triple-Core design) allowed additional spatial multiplexing by incorporating multiple liquid cores into which multiple different assays could be introduced simultaneously. The new designs were implemented and optimized through testing and characterization. They were then implemented in full biological tests proving their capability for disease diagnostics.

Precise control of bioparticles improves the analytical capabilities for single-molecule analysis as they can be held securely at a single location for prolonged periods, allowing for observations and experiments on an isolated particle. Previously the LC-ARROW sensor showed its ability to manipulate molecules as a 1D ABEL trap, capable of holding a single molecule for a brief time [27]. This trap was limited to one-dimensional trapping, reducing its efficacy as a molecule trap. In efforts to overcome this limitation new 2D ABEL trap designs were implemented in the LC-ARROW platform.

1.2 Organization

This dissertation is organized as follows: Chapters 2 and 3 are reviews of the current state of disease diagnostics and an overview of optofluidics. They will give important background information helpful in understanding the strength that the LC-ARROW sensor has as a lab-on-chip platform for single-molecule analysis. Chapter 4 will introduce the reader to the LC-ARROW platform through a brief history and its basic operation without the multiplexing capability. The details of the basic LC-ARROW sensor are discussed in its design and fabrication in Chapter 5. This chapter will end with a discussion on changes made to the microfabrication process in order to implement the more advanced multiplexing designs. Chapter 6 gives an overview of spin-on-glass and the structures I built in order to overcome a water absorption problem. These designs
were not kept for various reasons and the discussion is only included as a reference for future students, they were part of my research and studies. Chapter 7 will focus on the Y-Splitter design, its purpose, implementation and successes in improving the overall signal-to-noise ratio of the LC-ARROW platform. It served as a stepping stone to the future multiplexed designs. Chapter 8 introduces the design to implement wavelength specific multiplexing through implementation of the MMI waveguide. Initial frustrations with the spot pattern fidelity are explained and experimental details are presented in how they were overcome. Design specifications are given. The MMI design has been used in many biological tests and their results are also presented. Chapter 9 expounds on spatial multiplexing and the Triple-Core design. The engineering challenges overcome to implement the design are discussed along with the design specifications. Biological testing results are then included. A shift in focus happens in Chapter 10 from molecule detection to molecule trapping. The chapter starts with an overview of optical trapping basics, including a review of direct and indirect trapping methods. This will also give context to the need for particle traps. In order to understand the 2D ABEL trap, one must first understand its predecessor, the 1D ABEL trap. I began research on particle traps by first redesigning and building previously demonstrated 1D traps before I attempted to implement the 2D trap. These results will be given followed by an introduction to the 2D ABEL trap. The 2D trap went through many design changes while attempting to implement it. The changes will be given with pros and cons for each. To conclude Chapter 10, the 2D trapping results will be discussed, with ideas on how to improve them. The concluding Chapter of this dissertation will be a summary of results with concluding remarks.

This dissertation also includes appendices that comprise useful information not contained in the main body. Appendix A is a timeline and literature review of the LC-ARROW platform,
including papers the research group has published over the years. Appendix B is comprised of two parts. The microfabrication recipe I started with in 2013 will be given, followed by the recipe I concluded with, showing the improvements made within the full process. Appendix C will include MATLAB code used in the various waveguide optimization experiments.

1.3 Contributions

My main contributions to this work are in the successful implementation of multiplexing, both wavelength and spatial, into the LC-ARROW optofluidic platform. Their success has given rise to a flood of publications, both journal and conference. I also brought optimized Y-splitter waveguides to the platform to incorporate multi-spot excitation capable of improved signal-to-noise ratios, increasing the overall sensitivity of the platform. Further, I brought the initial results of 2D molecule manipulation to life through implementation, testing and redesign of multiple 2D ABEL trap designs. I owe most of the success to the many months spent at the optical testing bench and in the cleanroom constructing devices. Through testing I was able to pinpoint design flaws that prevented the creation of high fidelity spot patterns from the MMI waveguides output, and Y-splitter angle optimization. My expertise in the cleanroom enabled me to build the devices and create a new rib waveguide fabrication process in order to implement the new designs. The LC-ARROW platform has been studied extensively at BYU for almost twenty years, and as graduate students come and go, there were many inconsistencies left in fabrication processes. I reconciled these inconsistencies through much trial and error in order to successfully build clean and highly sensitive optofluidic chips.

The contributions listed above are what I consider my main contributions to the work in this dissertation and the project. I have also contributed in other ways that have helped to move research
on the LC-ARROW forward. I have consulted with and mentored many graduate students on microfabrication and optics in order to help them understand and build LC-ARROWS. As part of my studies I was made the tool owner for the Trion Minilock Phantom III used exhaustively by our group for research. Along with my work on the Trion, I assisted in the repair and maintenance of many of the machines in the cleanroom in order to use them to successfully build LC-ARROWS. I also studied the incorporation of spin-on-glass to the LC-ARROW biosensor, along with researching many other potential materials for the sensor such as frit glass and silicon oxynitride.

1.4 List of Publications

1.4.1 Peer-Reviewed Journal Articles


### 1.4.2 Conference Papers and Presentations


2 DISEASE DIAGNOSTICS

Ever since germ theory was first proposed in the mid-1500s by Girolamo, disease diagnostics have undergone many changes over the years [28]. The advent of the microscope in 1683 helped in the visualization of microorganisms, but it wasn’t until the mid- to late-1800s that viruses were identified and the first vaccines implemented [29]. Today, our knowledge of infectious diseases is remarkable as we can identify differences in DNA down to a single base pair [30]. With all the advancements in disease diagnostics, current methods are still costly, requiring large labs and highly trained personnel to properly diagnose clinical samples. These methods often require a sample to be shipped from the doctor to the laboratory, adding time to diagnose, which in some instances can be fatal. Further, underdeveloped locations which lack the expensive laboratories have limited options in detection of infectious diseases, making wide spread outbreaks a common occurrence. In recent years, we have seen the need for rapid clinical diagnostics due to the global outbreaks of Ebola hemorrhagic fever and Zika virus along with the growing need for rapid detection and analysis of antibiotic resistance in bacteria [1, 2, 3]. This chapter will give a brief overview of the state of the art information in virus and bacteria infectious disease diagnostics. It will be followed by an introduction to lab-on-a-chip technology which is at the forefront of providing compact diagnostic solutions.
2.1 Virus Diagnostic Tools

Methods to diagnose infectious diseases continue to develop over time (Figure 2-1). However, few of these methods actually get implemented routinely into diagnostic laboratories. For example, microarray-based technologies initially showed great promise for amplification-free multiplexed detection. Unfortunately, their low sensitivities kept them from clinical diagnostics [31]. Current gold standards for viral disease diagnostics, such as enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR), have excellent sensitivities and are robust in their ease of use in the lab [32, 33]. They are both capable of multiplexing due to fluorescent probe labeling and parallel testing. However, they require amplification steps of the viruses to enable detection.

The discovery of diseases has been accelerated in molecular biology thanks to the push of the human genome project. The effort to map the genome enabled the ability to sequence genetic information at rapid rates, thanks to high-throughput sequencing (HTS) technologies. Currently, genetic information can be determined down to the single base pair. Although HTS is a great method for disease discovery, its high cost and operational complexity has kept it from being implemented in clinical diagnostics [34]. The recent development of the virome capture sequencing platform for vertebrate viruses (VirCapSeq-VERT) shows great promise in becoming an amplification-free diagnostic tool as it can achieve sensitivities comparable to RT-PCR [35]. The system enhances the efficiency of HTS for characterizing host by utilizing nearly two million 50-mer to 100-mer nucleotide probes, making it capable of multiplexed testing for every known vertebrate virus and their variants. VirCapSeq-VERT has also shown high sensitivity in clinical samples (such as nasal swabs) that have low numbers of viral sequences compared to the background of host sequences. Although this system shows promise, it is still in its infancy and is
currently limited to vertebrate viruses. Only time will tell if VirCapSeq-VERT will become an industry standard format in virus diagnostics [36].

![Figure 2-1: History of virus diagnostics. Timeline showing how the development of different technologies (shown above the timeline) enabled standard methods for diagnosing virus infection (shown below the timeline). Serology and PCR subsequently enabled detection of hundreds of viruses, largely individually, while modern HTS technology platforms, such as VirCapSeq-VERT, allow detection of thousands of viruses simultaneously. Reprinted with permission from reference [37]. Copyright 2015, American Society for Microbiology.](image)

2.2 Bacterial Diagnostics

The diagnosis of bacteria tends to be more challenging than that of virus detection as they require, in many cases, a positive blood culture before an assay can be performed [38]. Testing becomes more challenging as some bacteria are unculturable, thus infections of such strains do not result in a positive culture [38]. The need for fast and accurate diagnosis of infection has gained increased attention due to the rising occurrence of antibiotic resistance in bacterial pathogens. Phenotypic assays are currently the standard for profiling antibiotic resistance in bacteria. It can take between twelve and twenty-four hours to acquire the first results of a standard test [21]. However, when more detailed characterizations are needed, multiple analyses are performed and can take days to months depending on the complexity of the question. These types of analyses are
outlined in the timelines of Figure 2-2 for reference and ease of outlining the complexities involved.

Figure 2-2: Processing of clinical samples in the diagnostic laboratory: schematic representation of the timeline for the processing of clinical samples with classical pathogens (a) or slow-growing bacteria such as Mycobacterium tuberculosis (b). Clinical samples are directly submitted for antigen detection (immune chromatography), PCRs, Gram stain and/or serology while the bacterium is being cultured. Once a pure bacterial culture is obtained (red star), a second panel of analyses is performed. Direct sequencing on clinical sample allows shortening of the ‘time to result’ (red dashed arrow), especially for fastidious bacteria or slow-growing bacteria. Techniques are colored according to their application for bacterial detection (yellow), species identification (green), antibiotic susceptibility testing (pink), and strain typing (orange). The main steps of genome sequencing are highlighted in purple. MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; POCT, point-of-care tests; RFLP, restriction fragment length polymorphism. Reprinted with permission from reference [38]. Copyright 2013 European Society of Clinical Microbiology and Infectious Diseases.
Long turnaround times of the test can have fatal consequences for sepsis patients as the survival rate can drop by ~8% per hour [39, 40]. Correct diagnoses in these circumstances are vital as improper treatment can result in increased antibiotic resistance and undesired side effects, such as kidney failure [41]. Evaluation of the resistance profile can be performed by a variety of techniques. Some techniques include phenotypic assays, fluorescence in situ hybridization, mass spectrometry, and PCR [21, 42, 43, 44]. Current PCR assays for analyzing gram-negative bacteria reports lower limits of detection just below $10^6$ CFU/mL (colony-forming unit/milliliter) [45, 46]. These limits are not ideal because sepsis can become a threat at levels as low as 10 CFU/mL [47, 48]. There is a growing need for improved diagnostic tools, as evidenced by the National Institutes of Health’s search during their Antimicrobial Resistance Diagnostic Challenge for rapid diagnostics to combat the spread of drug resistant bacteria [49].

### 2.3 Lab-on-a-Chip

Lab-on-a-chip (LoC) or Micro Total Analysis Systems (μTAS) are devices that integrate one or many laboratory functions on a single, integrated microfluidic chip. LoC technologies will blaze the trail towards sensitive, portable, affordable, and easy-to-use disease diagnostic techniques. The lab-on-a-chip field is truly multidisciplinary, requiring cross-disciplinary contributions including biology, chemistry, physics and multiple engineering disciplines. The basic construction relies heavily on microfabrication techniques developed from microelectromechanical systems (MEMs) and microfluidics studies. The telecommunication industry has also played a role in their development in the advent of integrated optics. Many LoC devices now rely heavily on the integration of microfluidics and integrated optics (optofluidics) to form optical diagnostic systems. One such system is depicted in Figure 2-3. The LoC uses microfluidic channels patterned in a PDMS layer to extract plasma and then performs a multiplexed assay to detect various biomarkers.
This device highlights some of the advantageous that lab-on-a-chip systems can bring to disease diagnostics. The LoC in Figure 2-3 was developed by Dimov et al., who called it a self-powered integrated microfluidic blood analysis system (SIMBAS) [50]. The system integrates sample volume metering, plasma separation from whole human blood, multiple immunoassays, and flow propulsion into a single LoC device. SIMBAS chip effectively draws the blood into the device using potential energy from the vacuumed PDMS. Once removed from the vacuum, 5 μL of blood was dispersed amongst the five reservoirs and the blood was drawn to the suction chambers. The plasma in the detection region was then able to be analyzed with a standard streptavidin biotin assay by removing the top glass slide with captured biotin and inserting the slide into a standard microarray scanner. Within ten minutes, the biotin was readily detected in whole blood.

Figure 2-3: Self-priming, self-contained, tether-free SIMBAS integrates volume metering, plasma separation from whole-blood, multiple biomarker detection and suction chambers for fluid propulsion. Reprinted with permission from reference [50]. Copyright 2010 The Royal Society of Chemistry.

From this SIMBAS, we can see some prime advantages to LoC for point-of-care applications. First, the device is portable and easy to use; simply remove it from the vacuum pouch, insert blood, remove the glass slide, and place it in the scanner. Second, the required sample volume is greatly reduced. Typical blood tests require milliliters of blood; this test only required 5 μL, which can be acquired from a finger prick system. Third, the analysis was relatively fast—approximately ten
minutes to receive results. The LoC systems’ advantages are its portability and ease of use. A prime example of LoC diagnostic systems that are commercially available are the standard pregnancy tests and diabetes blood glucose tests. Another less known diagnostic tool is the Triage® cardiac panel (BiositeInc, USA), which is used to determine the cardiac proteins creatine-kinase-MB (CK-MB), myoglobin, and troponin I (cTnI) in whole blood [51]. The further development of LoC and/or μTAS systems for rapid detection of infectious diseases will help to reduce patient treatment costs, decrease time to diagnose, and open the doors to diagnostic instruments in underdeveloped areas.

From this brief review, we can see the need for improved diagnostic instruments capable of rapid, amplification-free diagnostic tools. Lab-on-a-chip (μTAS) devices capable of clinical diagnosis are at the forefront of development to meet that need. The work in this dissertation discusses the development of a lab-on-a-chip multiplexed detection system capable of detecting various pathogens, such as bacteria or viruses, with high sensitivity and selectivity. It takes advantage of small excitation volumes and fluorescent labels to detect single-molecules—such as DNA strands—thus avoiding slow amplification steps. The chip can also be readily attached to additional microfluidic systems for blood filtration and preconcentration allowing for integration of all the necessary components [52].
3 OPTOFLUIDICS

Optofluidics is the research field that combines integrated optics and microfluidics in the same system. The field has developed over the years from being considered a subdiscipline of optics and/or microfluidics in the early 2000s to its own analytical field with an emerging industry [53, 54, 55, 56, 57]. Optofluidics can date back to as early as 1972 when fluid-filled fibers were considered for long-haul fiber communication systems [58]. However, the term did not arise in publications until 1985 in a keynote paper entitled “Optical fibre sensors and systems for industry” in which optofluidic converters were considered “under development” [59]. Thanks to the symbiotic integration of microfluidics and photonics, optofluidics provides unique abilities for chip-based bio/chemical analysis at the single molecule scale. This chapter highlights some optofluidic bio/chemical sensors, with emphasis on systems with multiplexing capabilities. Single-molecule analysis will then be overviewed, pinpointing the advantages that optofluidic systems bring to the field. This chapter concludes with an overview of the liquid core anti-resonant reflecting optical waveguide (LC-ARROW), which is a fundamental component of the systems described in this dissertation.

3.1 Multiplexed Optofluidics

The goal of multiplexed optofluidics is to provide systems that are small, fast, easy to use, versatile, and has the required sensitivity to detect across multiple analyte types (e.g. DNA strands, proteins, single viruses and other biomarkers). The concept of a multiplexed immunoassay was
first described by Feinberg in 1961 in his letter entitled “A ‘Microspot’ Test for Antigens and Antibodies” [60]. Now, multiplexed immunoassays are the standard in clinical disease diagnostics. One common diagnostic system is ELISA (enzyme-linked immunosorbent assay) [32]. ELISAs are versatile, simple to use, highly sensitive, robust, and repeatable, and they have the ability to multiplex. These characteristics make it an ideal testing scheme. ELISA detects targets from enzyme catalysts or fluorescent tags that provide high signals capable of large dynamic ranges [32]. Chip-based optofluidic approaches would like to mimic the characteristics that make ELISA a great diagnostic tool but improve upon it through the reduction of sample volumes and the increase of attainable molecular information. The lab-on-a-chip approach ultimately improves reagent and sample consumption, automation of analysis, cost, precision, and ease of use. Thus, in recent years, research focus has been on bringing optofluidic systems for biological and chemical analysis to life. A few companies have recently implemented portable systems. However, these systems are still limited in one or more of the following ways: high operating costs, low flexibility, limited multiplexing capability, high sample consumption, expensive chip production, or bulky instrumentation [61, 62, 63, 64, 65]. Multiplexed optofluidics attempt to overcome these limitations. The approaches to multiplexed optofluidic detection technologies can be lumped into two categories: array- (chip-based) and bead-based (suspended). These technologies achieve multiplexing in a couple of ways, namely spatial separation (separate channels or wells) or unique labels (such as fluorescent dyes or distinctive beads). As there are several reviews on the topic of optofluidic systems for bio/chemical analysis [4, 66, 67, 68, 69] and other multiplexed diagnostic systems [70, 71, 72], I will only briefly describe some multiplexing approaches and give an example of each.
A DNA microarray, also known as a biochip, is a collection of microscopic DNA spots attached to a surface. These spots contain specific oligo probes that can bind to specific targets. The hybridization of probe to target is then typically detected by fluorescence given off by a label. The fluorescence light is used to quantify the abundance of the target sequences. DNA microarrays are one example of the array-based approach to multiplexing. Many optofluidic systems have been developed following this approach. Optical microarray systems typically rely on fluorescence or chemiluminescence detection, but they can also be developed to rely on a change in refractive index as is the case in label free systems [73, 74, 75]. Array systems typically use a CCD camera or CMOS camera as the detector. An example of such a label free multiplexed system is shown in Figure 3-1 [75]. With this device Ymeti et al. uses a young interferometer (YI) sensor to detect viruses directly (without the need of fluorescent labels) by characterizing the output interference pattern of the four waveguide channels. Three of the waveguides are functionalized with biorecognition probes while the fourth acts as a reference waveguide. As target viruses attach to the specific probes, they cause phase shifts due to the change in the effective refractive index of the waveguide, and thus, a change in the interference pattern. Through analysis of the interference pattern, the sensor was able to attain sensitivities of $10^{-7}$ refractive index units corresponding to a protein mass coverage of approximately 20 fg/mm$^2$ [75]. Further the device demonstrated that three targets could be detected simultaneously. However, when using the YI design, it is difficult to push beyond that due to the long interrogation lengths on the order of a centimeter.
Localized surface plasmon resonance (LSPR) is another example of an optofluidic array-based label-free system. It also detects binding on metallic nanoparticles and has shown multiplexed applications [76]. Fluid handling technology advancements have made handling small droplets on the order of picoliters a reality and is advantageous for array-based applications. Droplet microfluidics (DMF) utilizes electrowetting of droplets to aid in the precision control of reagent and sample droplets. It is being readily applied to chip-based clinical diagnostics and clinical assays [77]. Another fluid handling technology enabling multiplexed array-based optofluidics is that of inkjet micro printing. This allows for high-density arrays of microspots to be easily implemented and subsequently analyzed [78, 79, 80]. Fluid handling technologies have enabled array-based technologies to increase in parallel analysis, providing superior throughputs to that of bead-based systems. Array-based systems are also advantageous in ultra-high-density analysis, such as in virus detection when there are large quantities of analytes to probe. However,
when there are few targets to be detected (as is the case in antibiotic resistance detection), the bead-based approach is preferred as it can attain lower limits of detection with high sensitivities.

Bead-based systems utilize microbeads (available in many types and sizes [72]) to encode electrochemical [81], magnetic [82], graphical [83, 84], physical, [85] or spectrometric [86, 87] information inherent in the bead to the attached target analyte. The multiplexed capacity can be increased by sending beads through separate channels. The large variety of encoding methods provides the advantages of detecting multiple targets in large populations, providing higher sample throughput, ease of modification, and high-quality control [88]. The bead-based system approach can be expanded into particle analysis where the use of a microbead is not required. In such a system, the target is directly labeled rather than attaching it to a microbead. The lack of beads enables the system to achieve single-molecule detection limits. This approach allows for high sensitivity and selectivity, by eliminating the noise of the population and interrogating single particles in parallel. The most prominent example of a bead-based system, dating back to the 1960s, is the flow cytometer [89, 90]. Flow cytometers are readily available in diagnostics labs and provide high-throughput multiplexed detection [70]. Research continues in creating on-chip optofluidic flow cytometers capable of small sample (μL-fL) volumes, particle focusing abilities, and the integration of optical light sources [91]. An example of an optofluidic flow cytometer system was developed by Kim et al. (Figure 3-2) [92]. The microfluidic channels were structured in PDMS using standard soft lithography techniques. CARVO and syringe pumps were used to inject sample and sheath flow, respectively. Laser lights at 532 nm and 635 nm were coupled to the interrogation region, which then excited the sample as it passed excitation beams. Other optical fibers, also enclosed in the PDMS chip, guided the fluorescence signal to four photomultiplier tubes (PMTs). This system demonstrated multiplexed immunoassays of six different bacteria and
toxins simultaneously at limits of detection (LOD) comparable to commercially available systems. Ultimately, multiplexed systems that are capable of single-molecule analysis will achieve the best LOD and provide for exciting advances in many fields.

Figure 3-2: A schematic system layout for the microfabricated flow cytometer for multiplexed detection of bacteria and toxins. Sizes are not to scale. Reprinted with permission from reference [92]. Copyright 2009 American Chemical Society.

3.2 Single-Molecule Analysis

Optofluidic devices provide on-chip solutions with small excitation volumes for single-molecule analysis (SMA). Typically, SMA is accomplished through devices such as confocal fluorescence microscopes and through techniques based upon optical cavity resonance (OCR), surface-enhanced Raman scattering (SERS), and fluorescence. Of these techniques, fluorescence is the most commonly used and developed because it offers high sensitivity. The platform described in this dissertation is fluorescence based, thus fluorescence systems will be the focus of this section. Fluorescence single-molecule detection methods rely heavily on the chemical attachment of individual fluorescent dyes and other biological probes that absorb and emit light at specific wavelengths. The spectrally distinct labels typically emit light in the near ultraviolet to near infrared range and selectively identify the single molecules to study. A typical bench top
The experimental setup (Figure 3-3) consists of an inverted microscope, which allows for the excitation light to dissipate into the above free space and reduces background noise. The excitation light sources used can range from a broadband illumination source or a monochromatic source such as a laser. The laser source is preferred when SNR limitations require maximum fluorescence collection, as it can controllably excite the attached fluorophore at maximum absorption [93]. The microscope objective typically has numerical apertures greater than one to maximize photon collection at the detector, which is usually a single-photon counting avalanche photodiode (SPC-APD) or photomultiplier tube (PMT). The filter design is another important consideration in the experimental setup of SMA. An efficient filter design helps to reduce background noise while effectively passing the fluorescence signal. Maximizing signal-to-noise ratio is critical for single-molecule detection as the fluorescence intensity varies while it is being recorded over time. Fluorescence signal is usually recorded in a fluorescence trace that is integrated over a certain time period called a “bin”. Along with the bin time, a threshold is defined to eliminate the false detection of molecules. This threshold is generally defined by the background noise (influenced heavily by the buffer solution). With high noise, fluorescent bursts can be lost in the background and be eliminated in the threshold. Optofluidic approaches help to minimize background noise by shrinking the excitation volume of the buffer solution.

### 3.2.1 Optofluidic Single-Molecule Detection

To understand some of the advantages to shrinking SMA systems in optofluidic devices, a brief mathematical review will be given here. Further mathematical theoretical details can be found in “Theory of Single-Molecule Spectroscopy: Beyond the Ensemble Average” by Barkai et al. [95]. Poisson statistics are used to model single-molecule detection since detection events occur infrequently compared to the total number of possible observations. The Poisson probability-
density function is determined by the concentration of the specific target molecules and the excitation volume and can be given as

\[ P_c(x) = \frac{c^x e^{-c}}{x!}, \quad (3-1) \]

where \( c \) is the average number of molecules per unit volume and \( x \) is the number of molecules that instantaneously occupy that volume [96]. In most circumstances, the probability of observing more than one molecule at a time is nonzero \((P_c(x > 1))\). This leaves a finite probability of detecting more than one molecule at a time, which can produce errors in the experiment [97]. Detecting a single molecule implies that the molecule is separate from other interfering molecules, thus solutions of molecules need to be highly diluted and/or the excitation volume needs to be very small. The minute volume excited will minimize the probability of detecting more than one molecule at a given time as \( P_c(x > 1) \) approaches zero. Microfluidic channels and integrated optical waveguides can provide volumes capable of single-molecule detection with near-zero probability of detecting more than one molecule. Optofluidics provide a variety of advantages for single-molecule analysis such as accelerated, diffusion-controlled reactions, reduced sample consumption, and reduced background noise. In addition, the microfluidic channels provide dimensions in which single-molecule counting can occur in rapid succession with minimal multi-burst events. For example, Stavis et al. demonstrated single-molecule detection at rates of over 1000 molecules/min using an electokinetically driven flow procedure not typically feasible in bulk systems [98, 99]. The LC-ARROW platform developed by Hawkins, Schmidt, et al. discussed in this dissertation is an excellent example of a planar SMA system capable of single-molecule detection [12].
There have been a variety of optofluidic systems which have shown suitable for SMA. In “Microphotonic Control of Single Molecule Fluorescence Correlation Spectroscopy Using Planar Optofluidics”, Yin et al. demonstrates on-chip fluorescence correlation spectroscopy (FCS) for single-molecule detection, on a full planar integrated chip [100]. FCS is a correlation analysis of temporal fluctuations in the fluorescence intensity given off by the molecules. FCS can provide indirect measurements of the size, the shape, the molecules’ diffusion constants, the concentration, and the viscosity of the environment. The systems demonstrated by both Stavis and Yin are fluorescence approaches to SMD. The fluorescent labels provide higher signal-to-noise ratios (SNR) and selective identification of the molecules but at the cost of extra preprocessing procedures. Other label-free approaches have also been implemented on-chip with whispering-gallery microcavities [101] and plasmonic metamaterials [102]. These approaches eliminate the
preprocessing procedures making them faster for high throughput applications but trade SNR, resulting in lower sensitivities.

There are a variety of applications in the detection of individual molecules such as kinetic studies of irreversible reactions, DNA analysis on a single molecule scale, molecular screening and sorting for drug development and disease diagnostics, manipulation for prolonged observation and precisely controlled experiments, high throughput immunoassays, array-free hybridization measurements, and even flow characterization of the microfluidic channel [94]. The applications utilized by the LC-ARROW platform described in this dissertation are for molecule manipulation and disease diagnostics. However, the platform could be adapted to a variety of applications. These applications are facilitated by the fluorescence detection approach due to the needed sensitivity, which is provided by the increased SNR. The LC-ARROW platform is highly dependent upon the anti-resonant reflecting optical waveguide, which gives it the ability to guide light in both solid and liquid cores. Because of this dependency, the following section of this chapter gives an overview on anti-resonant reflecting optical waveguides as a key optofluidic component.

3.3 Reason for ARROWs

In 1854, John Tyndall demonstrated light confinement in liquid by streaming water from a bucket in the sun light. In this simple experiment, as shown in Figure 3-4, observers saw light reflecting at the water-air interface as it guided in the water stream. This light confinement at the boundary is now commonly known as total internal reflection (TIR).
Willebrord Snellius, Augustin-Jean Fresnel, and others helped contribute to the theory of total internal reflection. Snellius derived Snell’s law which is used to describe the relationship of refraction at two media’s interfaces,

\[ n_1 \sin \theta_i = n_2 \sin \theta_t, \]

where \( n_1 \) and \( n_2 \) are the refractive indices of the two media, \( \theta_i \) is the angle of incidence, and \( \theta_t \) is the transmitted angle. These angles are measured from the normal of the boundary. Fresnel, also a contributor to our understanding of TIR, later promoted the wave theory of light, contradicting the widely accepted corpuscular theory (particle theory). This gave an understanding of how phase changes at the boundary when reflected in TIR.
Light will achieve TIR when it is incident upon a medium at an angle greater than a critical angle. At this angle, light will not transmit into the medium but will be reflected. This critical angle is defined where $\theta_t = 90^\circ$ in Snell’s Law and is given below.

$$\theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right)$$

(3-3)

Notice that for $n_1 < n_2$ the fraction is greater than one and the sine inverse is therefore undefined. This means that for total internal reflection to occur, the refractive index of the first medium must be greater than that of the second for a critical angle to be found. In Tyndall’s demonstration, TIR criteria is satisfied because the refractive index of water ($n_1 = 1.33$) is greater than the refractive index of air ($n_2 = 1$). This experiment may have been the first demonstration of a fluidic waveguide. Tyndall was facilitating sunlight guiding in a liquid medium. For most optofluidic waveguides, it is difficult to confine water within a medium where TIR is satisfied, $n_1 > n_2$. The refractive indices of liquid substances ($n \approx 1.33$) are typically lower than the media that could easily confine liquid to a planar silicon substrate ($n = 3.8$) such as silicon dioxide ($n = 1.47$) or silicon nitride ($n = 2.016$). Some types of Teflon AF can obtain indices as low as 1.28 in the visible regime [103], but they would not be suitable for sacrificial core fabrication procedures. Since low-index, single-optical cladding layers are difficult, if not impossible, to attain for liquid core waveguides, one can apply Fresnel’s understanding of the phase shift at the boundary to achieve a type of pseudo TIR. This is done by choosing the layer’s thickness such that the round-trip phase shift fulfills an anti-resonance condition, $\Phi_{RT} = m\pi$, where $m$ must be an odd integer and $\Phi_{RT}$ is the round-trip phase shift. These layers we call “ARROW layers” as they are used to make ARROWs.
3.3.1 Understanding ARROWs

Anti-resonant reflecting optical waveguides are interference-based waveguides that can achieve low-loss propagation in a low-index core. Interference based cladding layers are produced by single- or multiple-dielectric layers that have a specific thickness \( t \) which achieves an antiresonance condition. This thickness is given by

\[
t_1 = \frac{m\lambda}{4n_1}\left[1-\frac{n_C^2}{n_1^2}+\frac{\lambda^2}{4n_1^2d^2}\right]^{-0.5},
\]

where \( m \) is an odd integer, \( \lambda \) is the wavelength in vacuum, \( d \) is the thickness of the core, \( n_C \) is the core index, and \( n_1 \) is the ARROW layer index [104]. ARROWs are considered interference-based because anti-resonance is caused by destructive interference. Since the core is low index and the cladding layers are high index, light is not reflected at the core cladding interface by TIR. Instead, it can be said that the field is confined by anti-resonant Fabry-Pérot reflections [105]. This confinement is because the transmission characteristics are similar to the Fabry-Pérot resonator and high reflection occurs at the anti-resonant wavelengths. This Fabry-Pérot analogy, however, falls apart when discussing spectrum because, unlike Fabry-Pérot resonators, anti-resonant reflections are spectrally broad. Figure 3-5a attempts to illustrate the ARROW principle. Light is refracted into the cladding layer, and because of the thickness chosen for anti-resonance, light destructively interferes in the cladding and is reflected back into the core with high efficiency. The ARROW principle works well when the fundamental mode is propagating at a glancing incident angle, but with one ARROW layer, the mode is leaky. By adding multiple layers that meet the anti-resonance condition, as shown in Figure 3-5b, the guiding efficiency of the waveguide can be increased as long as the reflected light from each layer remains in phase. One final note about this guiding structure: it gives a good loss discrimination against higher order modes. The higher order
modes have a higher loss, and as such, are filtered out over the distance of the waveguide, giving it a single-mode like functionality [106].

Figure 3-5: (a) ARROW waveguide principle with single ARROW layer. (b) ARROW waveguide principle with multiple ARROW layers near TIR.

3.3.2 History of ARROWs

The first ARROW device demonstration was published in 1986 by Duguay et al., a group from AT&T Bell Laboratories [106]. During that time, substantial research and interest went into building low-loss optical waveguides on planar substrates. The desire of the time was to produce optical waveguides on silicon wafers and to promote their integration with electronic circuits, not to develop optofluidics. ARROW’s first application in optofluidics was not until 1995 when Delonge and Fouckhardt demonstrated the use of ARROW layers to confine light in planar-integrated hollow-core capillaries [107]. Since 2002, Dr. Hawkins’ research group at BYU, in collaboration with UCSC, has been investigating the use of ARROWs for their use in liquid and gas sensors. An Italian research group led by Bernini, is also currently researching ARROW applications in optofluidic devices and, in a recent review, has outlined them as a “promising building block for optofluidic sensor development” [108]. This indeed is true. In the past few years we have demonstrated their usefulness in detecting viruses, cancer biomarkers, single nucleic acids [109, 110, 111].
4 ARROW PLATFORM

The basic Liquid Core Anti-resonant Reflecting Optical Waveguide (LC-ARROW) design consists of a liquid core waveguide orthogonally intersected by a single rib waveguide for the excitation beam and two wider rib waveguides coupled to the ends of the liquid core for signal collection (Figure 4-1). This layout is by definition an optofluidic device and facilitates fluorescence correlation spectroscopy (FCS), the counting of fluorescent peak signals correlated to the passing of a fluorescently-labeled bioparticle. This simple design has some advantages in that it is easy to construct and is simple to understand. However, this design lacks the ability to detect multiple biomarkers at a time with relatively low achievable sensitivity, limiting its potential as a marketable disease detection system.

Figure 4-1: Pictorial representation of the basic LC-ARROW biosensor under test.
This chapter lays out the basic operation fundamentals of the LC-ARROW optofluidic platform. A brief history will be provided initially as a stepping stone to understand the foundation of the system and the progress made up to 2013. Since the platform has been extensively studied in Dr. Hawkins’ research group for many years, a comprehensive timeline of publications about the platform advances will be provided in Appendix A. This will help future researchers quickly gain a broad understanding of what changes have been made to the basic structure and of its fabrication processes since research began in 2002 [112]. This section will end by explaining the design limitations of the basic sensor that prevent it from becoming a reasonable analysis system for disease diagnostics.

4.1 Brief History of the LC-ARROW Integrated Fluorescence Sensor

In 2002, Dr. Aaron Hawkins started researching the basic LC-ARROW. Due to Dr. Hawkins’ prior experience with the technique, wafer bonding was avoided, and the search for the most effective sacrificial core material began. Dr. Hawkins et al. gained sufficient results to publish on three materials: aluminum, positive photoresist, and SU8 (Figure 4-2) [113, 114, 115]. Although the reflow resist arch-shaped structure produced the best throughput, they did not bode well with the addition of intersecting solid core waveguides, and the SU8 rectangular structures were kept.

![SEM images of hollow cores made with (Left) SU8 © 2004 [113] (Middle) positive photoresist © 2005 OSA [115] and (Right) aluminum © 2005 IEEE [114]. Figures reprinted with permission from OSA and IEEE.](image-url)
In 2005, the first optical sensors based on hollow-core ARROW waveguides were proposed, introducing the intersecting hollow and solid cores (SC) [116]. The hollow-core ARROW was optimized in the following years. A self-aligned pedestal (SAP) step was implemented to provide further structural integrity to the liquid core (Figure 4-3) [117]. This eliminated a crevice formed by the oxide deposition along the liquid core except at the location where the LC to SC intersected on top the pedestal. The rectangular hollow core structures created difficulties in the deposition of the top ARROW layers over the core as they were deposited non-conformally on the side walls. By 2007, the top ARROW layers (alternating films of silicon nitride and silicon dioxide) were eliminated and a single silicon dioxide (SiO₂) overcoat (SOC) layer was implemented [118]. This SOC design, however, was not entirely embraced until much later; students still published on devices with top layers after it was initially introduced.

Figure 4-3: SEM image of the hollow core with self-aligned pedestal © 2010 IEEE [117].

The solid core waveguides were initially one micron rib structures etched into three microns of oxide [12]. These early structures showed their ability to detect a single molecule despite the simple design [119]. Later yield experiments showed that thicker oxides reduced cracking of the hollow cores [120]. The thicker oxides also improved a challenge with a crevice at the liquid to solid core juncture, resulting in the ribs becoming three microns etched into six microns of oxide—
the thickest oxide achievable at the time. This brief history highlights some of the basic changes made over time to the basic LC-ARROW device; the interested reader is encouraged to review the publication timeline in Appendix A.

4.2 Basic Sensor Operation

The basic sensor’s ability to guide light directly into the path of a passing molecule, in an excitation volume on the order of femtoliters, gives the LC-ARROW platform the ability to detect single molecules without amplification from the emitted fluorescence signal [119]. The sample filtration and labeling happens prior to loading the analyte into the sensor. For blood diagnostics, filtration of the red and white blood cells, platelets, and other undesired specimens happens first and then followed by the labeling of target biomarkers. Target molecules are labeled off-chip with specific fluorophores in a variety of ways. Intercalating dyes or stains, oligo probes, and molecular beacons are just a few of the techniques used to attach the fluorophores to a target molecule (Figure 4-4). These techniques will briefly be described, as they are vital to the operation of the optofluidic sensor.

There are many commercial dyes available such as ethidium bromide, SYBR Green, SYBR Gold, and Alexa dyes [121]. Some dyes can be attached to molecular beacons for specific labeling of DNA or RNA, while others are used as intercalating dyes. Intercalating dyes however are a nonspecific way to attach fluorophores to any DNA target. The type of probe used will depend upon the application and pre-concentration procedures. When they can be used, intercalating dyes are the preferred method as they are cost-effective, can save time, and can easily attach multiple fluorophores to a single target providing high intensity signals. Intercalating dyes have been used in virus detection where clinically relevant concentrations range over many orders of magnitude
One disadvantage of intercalating dyes is that it can add to the background noise of the sensor if the unbound dyes are not properly eluted out prior to loading the optofluidic chip. Another disadvantage to intercalating dyes is that they are non-specific. When using intercalating dyes to detect the presence of specific particles, further steps are needed. Oligo probes can be used to capture specific molecules prior to introducing the stain. Oligonucleotide probes are short strands of single-stranded (ss) DNA or RNA used to detect or capture complementary nucleic acid sequences by hybridization. Oligo probes can be attached with fluorophores to enable detection or solely be attached to a substrate for capturing the desired sequences. We use magnetic beads with bound oligo probes to capture the target sequence in many of our tests. In applications where very few targets are available, magnetic beads may not be ideal as the bead could block the excitation light from reaching the trace number of targets on the bead. In these situations, molecular beacons or unbound oligo probes are more applicable. Molecular beacons are short, synthetic oligonucleotides that form the hairpin or stem-loop structure. The loop sequence can provide a single-base mismatch detection, making molecular beacons highly selective. Once the loop sequence hybridizes with the target DNA, the loop unfolds, separating the fluorophore from the quencher and allowing fluorescence. The close proximity of the quencher to fluorophore when unbound to target DNA keeps unbound molecular beacons from adding to the noise floor of the sensor. This quality is attractive when doing blind tests (when the scientist does not know the concentration of the target molecule in solution), and is the case for any clinical diagnostics. Different labeling techniques are represented in Figure 4-4.
Pre-concentration and labeling currently happens off-chip. However, on-chip, full-blood preparation can be accomplished using a PDMS microfluidic layer called an automaton, which can be integrated onto the biosensor [110, 111]. Once the analyte solution is preconcentrated and labeled, it is injected into the liquid core reservoir (Figure 4-5a). Negative pressure is applied to the opposite reservoir causing the particles to flow towards the excitation beam. Once the excitation light hits the passing labeled molecule, it fluoresces and gives off light differing in wavelength than that of the excitation beam. This light can then be efficiently captured and guided in the liquid core to the integrated collection waveguides and then directed off-chip via free space.
optics to the coupled avalanche photo diode. An optical filter is placed between the objective lens and single-photon counting avalanche photodiode (APD) to filter out wavelengths not corresponding to the fluorescence signal. An example of the fluorescence signal recorded by the APD is given in Figure 4-5b. Each peak indicates a labeled target molecule as it passes the excitation beam. Variation in peak intensity is attributed to the various locations in the channel that the particle could be located as it passes the excitation beam. Those particles which pass through the center of the channel are believed to cross the highest intensity portion of the Gaussian beam, causing the target to fluoresce more and provide higher signal. The noise floor, as seen in Figure 4-5b, is affected by photoluminescence of the materials used to make the sensor, fluorescence from the analyte solution, and dark noise intrinsic from the APD. There are many points of loss and attenuation of the signal in the optofluidic platform. The end of this chapter is dedicated to reviewing those points and to what I and past researchers have done in attempt to compensate for or improve them.

Figure 4-5: (a) Top view image of experimental setup of basic sensor during operation, chip enlarged to see detail. (b) Typical fluorescent signal recorded during a biosensing test.
4.3 Basic Sensor Weaknesses and Attempts to Improve

The basic LC-ARROW platform has been improved over time through the efforts of many researchers as discussed previously in the brief history. This section will show some of the main design and fabrication issues that my team and I have faced during my time as a graduate student and the improvements we made to solve those issues.

![Figure 4-6: Basic LC-ARROW platform with points of loss indicated.](image)

4.3.1 Sources of Optical Loss

Sources of optical loss come from the fiber to facet, SC to LC, LC to SC and facet to objective coupling losses, propagation losses of the solid and liquid cores, and fluorescence collection efficiency of the LC (Figure 4-6). The fluorescence efficiency is affected by the location of the particles in the channel as they pass the fluorescent beam. The fluorescence light radiates in all directions. Since we normally only detect from one side of the chip, we lose half of the fluorescence signal, lowering the efficiency. To improve efficiency, we could detect from both sides and cross correlate the signals collected from each APD. Coupling losses at the chip edge can be minimized by a clean cleave, leaving a pristine facet. Since cleaving currently happens by
hand, the value of the loss varies from chip to chip. The other sources of optical loss are intrinsic in the fabrication and materials used in the design.

### 4.3.2 Crevice

The crevice that is formed by the plasma-enhanced deposition process used to deposit the guiding-layer oxide has been a source of major loss at the LC to SC interface. The process can be crude in research where old, poorly maintained deposition machines are the means of film deposition. There have been many attempts to improve this source of loss. Two of which were published prior to the start of my graduate studies. The studies first eliminated the top ARROW layers completely, leaving only a single overcoat layer and the second layer that optimized the thickness of the oxide to produce the best mode coupling between SC and LC [120]. Since then, we have installed a new PECVD machine in BYU’s cleanroom. Recipe adjustments with the new machine have produced very low-stress, conformally grown oxides that have eliminated or significantly reduced the problem [124]. SEM images of both the old, crevice-forming oxide and the new conformal, low-stress oxide are seen in Figure 4-7.

![SEM images of liquid cores coated with (left) non-conformally grown oxide © 2010 IEEE [117] and (right) conformally grown oxide.](image.png)
4.3.3 Water Absorption

We had major issues with the sensor’s guiding layer: PECVD silicon dioxide. The material is porous and has a high affinity to absorb water. The water absorption tends to deteriorate the mode confinement for the excitation and collection waveguides (Figure 4-8). This amplifies the losses of the chip at each location, making the device useless. Initial attempts to anneal the chips up to 300°C showed promising results, but they did not last long as air humidity would quickly age the oxide again [125]. Spin-on-glass (SOG) overcoats were also used as an attempt to protect the underlying oxide from aging, but they did not withstand the strong acid etch nor prevent the oxide from absorbing water [126, 127]. The SOG layers were only a few hundred nanometers thick and were also porous. After much deliberation, buried channel waveguides finally proved an effective solution [128]. The sensor would receive a second PECVD oxide layer to overcoat the rib waveguides preventing the solid core from aging and maintaining well confined modes. The buried devices drastically improved signal-noise ratios of the platform, outperforming the detection capabilities of the unburied versions.

4.3.4 Ultimate Limitation

With all the improvements made to the basic LC-ARROW sensor, the design still lacks the ability to multiplex, i.e. detect and identify multiple different pathogens concurrently, and it has
been unable to achieve high enough sensitivities in assays where few fluorophores are prerequisite. Without the ability to identify multiple biomarkers simultaneously, it can never reach its full potential as a sensor for disease diagnostics. The new designs—Multimode Interference Waveguide and Triple-Core designs—that I have implemented overcome these limitations by introducing wavelength dependent multi-spot patterns, which, through signal processing, can improve SNR and introduce spectral and spatial multiplexing. These designs will bring the LC-ARROW platform closer to the doctor’s office as an easy to use diagnostic system, and will be discussed in future chapters of this dissertation.
5 LC-ARROW DESIGN AND FABRICATION

This chapter reviews the basic design of the LC-ARROW platform, and reviews some of the improvements made over time. The specific materials and design dimensions are discussed for the basic platform, as they were the foundation upon which the multiplexed designs were built. This is followed by a basic overview of the microfabrication process, which is used to build the basic sensor. Fundamental understanding of the process is important for the development of improved designs, as the process limits material choices and achievable structures. For example, a polymer cladding layer could not be used if the devices being fabricated will see a strong acid in a later processes. The details of the recipe will not be given here, since fabrication specifics in a research facility often require adjustment in order to obtain reasonable device yield. Adjustments are typically made because the equipment is not held to industry standards (e.g. a recipe developed on a machine that has rarely been serviced will need to be adjusted after it has been repaired). Recipe specifics including spin speeds, temperatures and etc. are provided in Appendix B for the interested reader. To conclude the chapter, changes made to the fabrication process in order to successfully build the multiplexed designs are presented. To observe a comparison of adjustments made over time, a comparison of the initial recipe (2013) with that of the current recipe (2018) can also be found in Appendix B.
5.1 Platform Materials and Design

Anti-resonant reflecting optical waveguides (ARROWs) are the key component to allowing the functionality of the bio-optofluiddic device. They provide the ability to guide light in a liquid core and facilitate waveguide fabrication on silicon substrates. One attractive feature of ARROWs is that they can be made with a variety of wave guiding materials. The chosen materials depend upon the application and variables such as wavelength of operation and the refractive index of the core material. For the purposes of the LC-ARROW sensor, materials were chosen that would work in the visible regime and do not contribute to the noise of the biosensor through photoluminescence. Photoluminescence is light emission from a material, after the absorption of photons. The excess radiation adds to the background noise, which causes false negatives while attempting to detect a molecule. In order to reduce noise we use materials with low photoluminescence such as silicon dioxide (SiO$_2$) and tantalum oxide (Ta$_2$O$_5$). The ARROW layers have been passed down from over a decade of research in Dr. Hawkins research group [129], and have undergone ample optimization [130]. Thus, they are only listed here for reference, in order from the first layer deposited on the silicon substrate to the last (Table 5-1). They consist of alternating SiO$_2$ and Ta$_2$O$_5$ layers, commercially sputtered by Evaporated Coatings Inc. All devices discussed in this dissertation are founded upon these ARROW stacks. The dielectric layers are deposited on a $<100>$ Si substrate which facilitates diamond scribe cleaving orthogonal to the waveguides when aligned properly to the crystal’s $<100>$ orientation. This removes the need to polish the chip edge (aka waveguide’s facet) after dicing. Another advantage to establishing the device on a silicon substrate, is in the amount of infrastructure readily available. Integrated circuit (IC) technologies have taken advantage of silicon wafers for many years, as silicon is the work horse of the industry [131]. The fully developed infrastructure will help the sensor to be built at a low cost.
There are a few ways to make liquid cores on silicon wafers. One method is by etching channels initially into the substrate followed by ARROW layer deposition. The etched substrate is then bonded to another wafer with ARROW layers [132]. This method was avoided because of the lack of flexibility that comes from wafer bonding, and the inability to easily integrate orthogonal SC waveguides [133]. The alternative is to use a sacrificial material, which shapes the structure during fabrication and then can be easily removed once the devices are complete. Sacrificial cores make for a better option as they are easier to implement with intersecting waveguides. For the LC-ARROW, SU8 is the material used as the sacrificial layer which forms the hollow channels. SU8 is a negative epoxy based photoresist which comes in a variety of densities and viscosities and can be processed using standard UV photolithography techniques [134]. Further, SU8 can withstand high processing temperatures after being hard baked, thus maintaining its shape throughout the fabrication process.

Sacrificial cores are designed to be ~5-6 μm tall x 12 μm wide. Simulations showed that these dimensions produce a pseudo single-mode which propagates in the ARROWs [113]. These dimensions also provide high fabrication yield. The reason for the range of the LC height is that the spin speed used to apply the SU8 to the wafer determines the thickness of the SU8 (feature height). In a research environment the SU8 can sit for a long period of time with varying use. As the SU8 ages its viscosity changes, thus spin speeds require slight adjustments in order to obtain...
the desired height. Getting an exact height can be time consuming since the core must be removed and re-built each time the height is incorrect. To save time we usually accept heights anywhere within the range of 5-6 μm. Because of this range we must note the following: cores closer to five microns give higher yield and cores closer to six microns have improved throughput. Recently this guideline has changed with the new PECVD machine, because we can obtain near zero stress recipes for our oxide [124]. Now six micron cores also produce great yield and is the typical target height. The length of the liquid cores has also changed over time. The first LC-ARROW design to detect a single molecule (Z-Series) was 4 mm long from collection waveguide to collection waveguide, plus an additional 2 mm for each arm to the reservoir, for a total of 8 mm of liquid core to etch (Figure 5-1) [135]. These cores took a few weeks to etch out entirely, as the time to etch scales drastically for longer cores [136]. Later the design changed the length to 300 μm plus the arms, resulting in an etch time of a few days. The shorter core also resulted in a higher collection efficiencies, due to the reduced propagation loss along the liquid core waveguide.

Figure 5-1: SEM image of the original Z-series basic LC-ARROW device with long liquid cores.
A thick dielectric must be deposited over the sacrificial core for structural support and to form the solid core (SC) waveguides. Like the ARROW layers the dielectric which forms the guiding layer needs to be a low photoluminescent material for wavelengths in the visible spectrum. Since oxide has a near zero absorption coefficient in the visible range, very few photons will be absorbed and so it is a low photoluminescent material, making silicon dioxide (SiO$_2$) arguably the best candidate to form the solid core waveguides. The top guiding layer also needs a strong chemical resistivity to Piranha, which is a mixture of hydrogen peroxide (H$_2$O$_2$) and sulfuric acid (H$_2$SO$_4$), the strong acid used to etch out the core. We need this layer to completely enclose the liquid core, ~6 μm tall, without deforming the SU8 structure. In order to meet these specifications a plasma enhanced chemical vapor deposition (PECVD) machine is used to deposit amorphous SiO$_2$ dioxide. PECVD is well suited for our needs as it can grow thick layers, in a short time, at low temperatures. Other common deposition methods such as, chemical vapor deposition (CVD), low pressure chemical vapor deposition (LPCVD), and thermal oxidation all require temperatures well above 300° C, and are unable to attain thick layers in a reasonable time and without multiple runs. The low deposition temperature, 250° C, however does come at a cost, it forms SiO$_2$ films that tend to be less dense with dangling bond defects. Structurally this means that the Si-Si and Si-O bonds are more spread apart resulting in large interstitial sites for water molecules to enter in. As moisture permeates the surface, water molecules become bound as a silanol (O$_3$ $\equiv$Si-OH) and this results in a raised index of refraction at the surface [137]. The raised index deteriorates the optical modes in the rib waveguides, hindering the performance of the detection system. Thermal annealing can control the water susceptibility of the film [137], however temperatures above 300° C tend to crack the ARROW layers limiting use of this method. The raised temperature breaks the silanol bonds and creates a bonding bridges between the host silicon atoms, resulting in film
densification. Annealing at 300° C for a long period of time has shown to improve mode confinement, however this is a temporary fix [125]. To make environmentally stable waveguides, Tom Wall et al. recently developed a buried waveguide design [138]. Initially the basic LC-ARROW platform utilized rib structures without top cladding layers. The excitation and collection waveguides are etched into the top oxide layer and are placed orthogonal to each other in order to reduce the noise from the excitation wavelength. Differing widths are used for the collection and excitation waveguides because of their unique roles, however they are both etched down to the same rib height. The excitation waveguides are kept narrow (~4 µm in the basic design) to eliminate higher order modes and to produce a sharp beam profile that enables single-molecule detection and femtoliter excitation volumes. The narrow mode also limits the amount of photoluminescence from the surrounding material and the analyte solution, lowering the background noise level. Collection waveguides are designed to be wider, 12 µm, to match the mode of the LCs and achieve higher collection efficiencies. Even though a perfect mode match is ideal for optimal coupling [139], a recently conducted experiment showed that it is easier to couple light from a smaller mode into a larger one (Figure 5-2). An optical fiber with ~4 µm core was butt coupled to a chip with 3 µm deep rib waveguides ranging in width from 2 µm to 12 µm. The fiber was aligned to find the maximum throughput, and the resulting throughput was documented. Higher throughputs were recorded for wider waveguides (Figure 5-2) indicating that a larger collection core, ~14 µm, could be implemented in future designs to improve the LC to SC alignment tolerances and reduce signal loss at the LC to SC interface. SEM images of the collection and excitation rib waveguide structures can be seen in Figure 5-3.
Figure 5-2: Normalized throughput versus waveguide width. Each waveguide was excited by an optical fiber with ~4 μm core coupled to a 635 nm laser.

In summary listed below are the basic LC-ARROW platform dimensions:

- LC waveguides 6 μm tall by 12 μm wide by 300 μm long
- Top oxide, $n = 1.51$, and 6 μm thick
- SC are etched 3 μm into the top oxide to form the rib waveguides
- Collection waveguides are 12 μm wide and the excitation waveguides are 4 μm wide.

The basic rib waveguide SC structures were recently replaced by buried rib waveguides (BRW) to mitigate water absorption, with an additional 6 μm oxide cladding, $n = 1.45$. Experiments, with the data in Figure 5-2 as a foundation, determined the dimensions of the SC were not optimal and changes were made for the multiplexed designs discussed later in this dissertation.
5.2 Fabrication Steps Overview

Microfabrication techniques, already developed by the microelectronics industry for manufacturing integrated circuits (IC), have provided infrastructure for the fabrication of optofluidics. Optofluidic fabrication success lies at the center of understanding microfabrication techniques from multiple fields: integrated photonics, microelectromechanical systems (MEMS), and microfluidics. Microfabrication techniques all rely on the ability to planar process, or modify, the whole surface of wafer with each step. The fabrication process for the LC-ARROW biosensor can be broken down into major steps categorized by the structure created in that step. These steps include: pre-core, sacrificial core, self-aligned pedestal, rib waveguide formation, core expose and SU8 core removal. Each process will be quickly summarized as they have already previously been explained in detail [140] [141]. Some steps have been adjusted in order to build the new multiplexed structures and will be explained in detail following the process overviews.
5.2.1 Pre-Core

The pre-core step is used as the initial masking step to create alignment marks for future pattern alignment, as well as to protect the ARROW layers under the reservoir. In some of the designs, electrodes are placed into the reservoir, in order to induce flow into the sample through the liquid core. The dielectric layers prevent electrical shorts through the Si substrate and need to be protected for the latter removal of oxide in the core expose process. The process starts by depositing a thin layer of chrome, ~70 nm, on top of the ARROW layers. Currently we receive commercially grown ARROW layers from Evaporated Coatings Inc on <100> oriented Si wafers. If we were to deposit the alternating SiO₂ and Ta₂O₅ films here, that would be considered the first step. After the Cr deposition, AZ3330 photoresist (PR) is patterned on the wafer by aligning the photoresist mask to the flat edge of the wafer. This alignment is crucial as it allows for orthogonal cleaving across the SC waveguides, providing a clean facet for optical coupling. AZ3330 is a positive photoresist which means that as it is exposed to ultraviolet light, the chemical bonds break and the resist will be removed upon developing. After developing, the resist is hard baked at 110° C. This strengthens the adhesion at the Cr-PR interface to protect the Cr over the features as the wafer is placed in Cr etchant. The Cr is removed entirely in the field, where there are no Pre-Core features (typically large squares or circles). After which the PR is removed with acetone and IPA leaving the pattern on the wafer for many chips (Figure 5-4). Oxygen plasma is then used to clean the surface of the chip from micro debris, for the following steps.
5.2.2 Sacrificial Core

This step outlines how the hollow channel for the liquid core is to be formed. For all designs discussed in this dissertation this process is the same. The sacrificial core is deposited and patterned using SU8-10, a negative photoresist capable of achieving near vertical walls with good aspect ratios [142]. It is spun on at a speed to obtain the desired ~6 μm thickness, the height of the core, across the bulk of the wafer. After the resist is applied, it is baked at a max temperature of 95°C, in preparation for the UV light exposure. This soft bake prevents the mask from sticking to the wafer and prepares the resist for exposure. The photolithography mask is aligned to the alignment marks created during the Pre-Core step, and the wafer is exposed. The SU8 that is exposed to UV radiation is strengthened by its chemical bonds to remain after the develop step. After exposure, a post exposure bake (PEB) is needed to enable cross-linking of the chemical bonds where the radiation penetrated, which further strengthens the bonds. The wafer is then placed in SU8 developer.
(MicroChem) to remove the unexposed resist, leaving the SU8 sacrificial core in place. The developer is rinsed off with isopropyl alcohol (IPA). At this point it is essential to check the dimensions of the core, to save time and effort, as these will be the dimensions of the hollow core after it is etched out. If the dimensions are out of tolerance, the SU8 core is removed in a strong acid bath (Nanostrip at 90° C for 30 minutes). This is followed by placing the wafer in the dehydration bake oven, followed by repeating the step. Once the core measures the desired dimensions, the wafer is hard baked at 200° C and then again at 250° C. The hard bake condenses the SU8 giving it high thermal and chemical stability, which is needed to withstand future steps [143]. After this process, the wafer is again placed in an oxygen plasma to clean off any residue that may have occurred during processing. The process flow diagram and top view of the chip after this process is visible in Figure 5-5.

![Chip Top View](image)

Figure 5-5: Sacrificial core process diagram with top view representation of a single chip after step completion. (a) Si substrate topped with ARROW layers (b) Spin on SU8-10 photoresist (c) Pattern and develop SU8-10 leaving the desired features.
5.2.3 Self-Aligned Pedestal

The pedestal consists of raising the core by etching through the ARROW layers and ~6 μm into the Si substrate. The introduction of the pedestal happened early on in the development of this platform [116]. However, the self-alignment techniques were not introduced until 2010 [117]. Self-aligned pedestal (SAP) gets its name because of the photolithography technique used removes photoresist from the core without the need of a lithography mask, perfectly self-aligning the pedestal to the core. A Ni lift-off step is used to protect the core and pedestal features during the plasma etch process (Figure 5-6). The pedestal provides stronger structural integrity for the LC by lowering the crevice, which is formed by the oxide deposition, to the base of the pedestal. The pedestal also proved to improve LC transmission.

In order to completely submerse the SU8 core for patterning, we spin on AZ4620 photoresist (PR) at a relatively slow spin speed to achieve a high thickness. The PR is then exposed with a pedestal mask which patterns features which protect the ARROW layers under the excitation and collection waveguides. After which the PR is flood exposed (no mask is used) for a short time, which causes the resist to absorb the UV radiation on the surface. The wafer is placed in a low concentration developer to remove the resist quickly where the features were patterned, and slowly over the core. This self-aligns the pedestal features from the previous mask to the core by a partial removal of photoresist over the whole wafer, leaving the SU8 core partially exposed (Figure 5-6c). At this point it is important to measure over the core to know how much of the resist has been removed. Developing the AZ4620 resist too much causes Ni residue to remain on the wafer and not developing it enough causes the Ni to peel off of the SU8 core. A target of ~ 2 μm is ideal for optimal liftoff. After verifying the correct height over the core, Ni is evaporated to coat the devices using an evaporation system (Denton vacuum E-beam evaporator). The Ni adheres to the PR as
well as the exposed areas of the wafer, which includes the SU8 core and pedestal features. An acetone bath dissolves the AZ4620 PR which lifts off the Ni from the areas over the resist, while leaving Ni over the features. The Ni mask protects the features as the wafer is anisotropically etched in an inductively coupled plasma (ICP) reactive ion etcher (RIE) (Trion Minilock Phantom III) to form the pedestal. Finally the Ni is removed using Ni etchant, and the wafer is ready for the next step.

![Figure 5-6: SAP process diagram with after step top view. (a) Process begins after patterning of SU8 core (b) Spin on AZ4620 photoresist (c) Pattern PR with normal and flood exposure (d) Deposit Ni (e) Liftoff Ni in acetone bath (f) Plasma etch through ARROW layers and ~ 6 μm into Si substrate (g) Remove Ni in Ni etchant.](image)

5.2.4 Rib Waveguides

To form the rib waveguides for the collection and excitation solid core waveguides, first six microns of PECVD SiO₂ is deposited over the wafer at a max temperature of 250° C. The thickness of the top oxide was optimized to adequately provide LC structural integrity and optimal coupling.
between the liquid and solid core waveguides [120]. After the top-oxide deposition, SU8 2025 is spun on at a spin speed capable of providing at least 6 μm of SU8 over the liquid and solid cores. After curing the SU8 for a set amount of time, a photolithography mask is precisely aligned to the SU8 core and the SU8 is exposed to UV light. After exposing the resist, the SU8 is baked and then developed in SU8 developer. Since the SU8 is extremely thick in the field compared to over the features, developing is a challenge and takes a good eye for detail. It is easy to under develop which can leave SU8 residue and ruin the subsequent etch. Slight agitation of the developer is helpful to get fresh developer to the SU8 surface, providing for a clean develop. After developing, the SU8 is hard baked at ~200°C to condense and strengthen the resist, so that it can be used as a reasonable mask for subsequent plasma etching. An oxygen plasma “descum” is necessary prior to etching the SiO2 in order to remove micro residue in the field. Failure to do this step results in a “sneeze effect” on the wafer after etching, which basically looks like the wafer was sneezed on before being etched. After the plasma clean, the oxide is anisotropically etched 3 μm deep in an RIE/ICP etcher (Trion Minilock Phantom III). This forms the rib structure for the SC waveguides (Figure 5-7g). Once etching is complete, the remnants of the SU8 mask are removed in Nanostrip at 90°C.

The alignment of this step is the most crucial since misalignment results in high loss at the LC to SC interface, reducing the collection efficiency of the biosensor. Thus checking the alignment before the etching step is necessary. If the pattern is misaligned, the SU8 mask is removed with Nanostrip at 90°C and then the step begins again after the oxide deposition (Figure 5-8c). This step is usually repeated multiple times before a successful rib alignment is achieved. Removing the SU8 each time takes a substantial amount of time, making this step one of the most time consuming parts of the process. Furthermore, SU8 only allows for a 3 μm etch into the oxide.
Etching further eliminates the SU8 mask and starts removing the waveguides. Even with these setbacks this process worked for the basic LC-ARROW design, as etching only 3 μm was sufficient. For the multiplexed designs a deeper etch was necessary, thus a new process using a Ni etch mask was developed, which will be discussed later.

Figure 5-7: SU8 masking process for the patterning of solid core rib waveguides. (a) Process begins after the pedestal step (b) Deposit 6 μm PECVD oxide, sacrificial core cross section (c) Deposit 6 μm PECVD oxide, SC waveguide cross section (d) Spin on SU8 2025 (e) Pattern and develop SU8 (f) Dry etch the oxide forming rib waveguides (g) Remove SU8, SC waveguide cross section (h) Remove SU8, sacrificial core cross section
5.2.5 Core-Expose

The core exposure step removes oxide at the tips of the sacrificial cores while protecting the rest of the features. This step begins by taking the wafer out of the dehydration bake oven and applying a thick layer of positive photoresist, AZ4620. The wafer is then soft baked for 10 to 15 minutes, as the thick resist tends to stick to the patterning mask upon exposure when it is not baked. The PR is then exposed and developed, removing PR at the tips of the LCs. The PR is then hard baked to strengthen the adhesion between the oxide and PR, which protects the waveguides as it is etched in hydrofluoric acid (HF). The wafer is placed in HF to expose the tips of the core for the final step.

5.2.6 SU8 Core Removal

The SU8 sacrificial core is removed in a strong acid (Piranha = 60 mL H₂O₂:40 mL H₂SO₄) bath over a period of days to weeks, depending on the channel dimensions, at a temperature of 130° C. The etch removes the residual AZ4620 deposited during the core-exposure step only after the first day in acid. Acid is changed daily to provide fresh chemicals each day. This step was optimized in 2010 [136], to provide fast etch times with good yield. It was found that changing the acid more frequently would decrease the etch time. The cores are checked periodically before cleaving the chips and placing reservoirs, to observe the etching progress and to make sure all of the SU8 is removed. It is important to fill the hollow core with deionized water before placing the wafer in acid to avoid cracking the cores, each time the wafer is removed and checked at the microscope. If the wafer is put back into acid dry, the heat from the Piranha’s initial reaction and the pressure from the initial filling of the cores cause cores to crack. After many days in acid the SU8 is completely removed, leaving a hollow channel (Figure 5-9).
Figure 5-8: Core Expose process diagram for the removal of oxide over the tips of the SU8 sacrificial cores.  
(a) Sacrificial core cross section from on top of the Cr pre-core squares  
(b) Process begins after forming the rib waveguides, SC waveguide cross section  
(c) Spin on AZ4620 PR, Sacrificial core cross section  
(d) Spin on AZ4620 PR, SC waveguide cross section  
(e) Expose and develop away PR overtop of the sacrificial core tips  
(f) Hard bake the PR everywhere else  
(g) Place wafer in hydrofluoric acid to remove oxide over SU8 cores  
(h) PR protects SC and LC waveguides, SC waveguide cross section  

Figure 5-9: SU8 Core removal process.  
(a) Cross section of SU8 sacrificial core  
(b) Hollow core after SU8 is removed.
5.3 Adjusted Rib Step for New Designs

There were some fundamental challenges with using an SU8 mask for the rib mask step, with the main problem being the inability to etch more than 3 µm into the oxide. In order to overcome this problem a new material was needed for the etch mask. This proved to be a challenge because the mask deposition and photolithography occur over tall features of varying heights, and forming optical waveguides requires smooth side walls to reduce scattering loss. This is why a thick photoresist was initially used, since it had the ability to uniformly cover the features. In choosing a material, many of its properties were considered including: etch selectivity, film stress, deposition method, achievable thickness, and deposition temperature. Metal was first thought of as an alternative to the SU8 because a thin layer, ~100 nm, could withstand the entire 6 µm of etching. Metals readily available and easily deposited in BYU’s cleanroom facility (Ni, Cr and Al) were first attempted in patterning the waveguides. Stress in the Cr films caused them to flake off during plasma etching and Al was less appealing to deposit given the available deposition methods in our facilities, thus Ni was kept. Standard lithography techniques were used in attempts to protect the metal with photoresist, and then wet etch the unprotected metal away. This resulted in poor adhesion and rough sidewalls (Figure 5-10). After much experimentation eventually a metal lift-off step was attempted and showed promise. It was further optimized into the new process called the “Ni Lift-off Rib” step. The step is described in full detail below, with explanations of some of the challenges to be overcome in order to successfully implement the process.

5.3.1 Rib Waveguide Ni Lift-off Process

The rib waveguide Ni lift-off process begins with 6 µm of PECVD oxide being deposited over the whole wafer. The oxide is then coated with AZ3330 photoresist (PR) at a spin speed of 1000 rpm. AZ3330 is a thin resist typically incapable of evenly coating features with our platform’s
tall dimensions. In order to completely and evenly coat the tall features, a large initial application is dropped on prior to spinning. Initially AZ4620 PR was used because it could easily coat the tall features, however when the excitation waveguides were designed to be 2 µm the recipe no longer worked, as small feature sizes were no longer attainable. A balance of soft bake, exposure and developing times were crucial in this process development. Longer bake times allowed for longer exposure times without reducing feature resolution. I attempted to keep the time in the developer to approximately one minute, similar to the standard AZ3330 recipes. After the PR is spun on, it is baked at 90º C for 7 minutes. A long exposure time is required to remove the thick resist piled up next to the tall liquid cores atop pedestals. The small waveguides are on top of the pedestal and so the resist is thinner. The long bake dries the resist so as to limit the effects of over exposing, which cause wider features. The exposed resist is then removed in AZ 300 MIF developer leaving the waveguides defined. Ni, ~100 nm, is then evaporated over the whole wafer. The wafer is then placed in a sonicated acetone bath which quickly removes the PR and undesired Ni. The Ni is left to protect the LC and define the SC waveguides. With a Ni mask we are able to etch deeper into the thick oxide. For the multiplexing designs we aim for 5 µm. After the etch, Ni is removed in a wet etch, using Ni etchant and the rib waveguides are defined. The full process is represented in Figure 5-11. With this overview of the step we can now discuss some of the specific challenges.
that were overcome in developing this recipe, and compare this masking process to the old SU8 process. The full details to the Ni lift-off step are also provided in Appendix B.

Figure 5-11: Ni masking process for the patterning of solid core rib waveguides. (a) Process begins after the pedestal step (b) Deposit 6 μm PECVD oxide, SU8 core cross section (c) Deposit 6 μm PECVD oxide, SC waveguide cross section (d) Spin on AZ3330 photoresist (e) Pattern and develop resist (f) Deposit Ni (g) Liftoff Ni in acetone bath with sonication, SC waveguide cross section (h) Ni protects the SU8 core structure (i) Dry etch into the oxide forming rib waveguides (j) Remove Ni, SC waveguide cross section (k) Remove Ni, SU8 core cross section.
5.3.2 Lift-off Step Details

A lift-off step for optical waveguide formation is an atypical process to use, as such, there are some fine details to pay attention to while performing this step in order to get it right. First and foremost, this step is challenging due to the uneven tall features sizes, which cause the resist to be thinner on top of the pedestal and thinnest over the core. The short height over the core is desirable, as we wish to remove the resist, to protect it with Ni. The thin resist makes this step easier. In order to use AZ3330 and attain an even coat without gaps in the photoresist, the majority of the wafer needs to be covered with resist before spinning it at 1000 rpm for 60 seconds. The high core causes resist buildup at its edge, which forms a ramp for the resist to pass over the core. This creates a challenge in the lithography. Since the resist is uneven next to the cores, using a standard AZ3330 lithography process [144] creates tapers in the waveguides next to the cores. This happens because the UV radiation quickly passes through the thin resist away from the core, but does not fully expose the resist at the edge of the core (Figure 5-12). It also causes partial removal of the resist next to the cores as they branch toward the reservoir (Figure 5-12). The exposure has to be just right in order to remove the taper next to the core. For small features this is extremely challenging, as the feature size approaches the wavelength of the light. Initially this step was developed with a 4 μm wide waveguide as the smallest feature. This required a soft bake for 5 minutes at 90° C in order to maintain features without taper. Since its conception the excitation waveguide has reduced to 2 μm wide requiring further baking and a flood expose step. After spinning on the resist, a soft bake of 90° C for 7 minutes is needed to attain the desired 2 μm wide waveguides. A rule of thumb is, the longer you bake, the longer you can expose without compromising the resolution of the features. This is because the resist is more dried out and condensed. This allows for a 30 second exposure which eliminates the taper and lack of core protection in the field. Since the resist is
baked on, I found that a 1 second flood exposure, after the initial 60 seconds in developer, could be used to thin the resist and ensure the 2 μm features were clear of residual resist. The flood exposure is followed by developing for 15 seconds. This step ensures that the resist is removed near the tall core, and eliminates Ni liftoff in undesired locations (Figures 5-12c, d and 5-13a, b).

Figure 5-12: Top view images showing the challenges caused by the tall and uneven feature heights after the Ni deposition. (a) Taper created next to the core with the original recipe without a long soft bake and long exposure. (b) Waveguide without taper using the adjusted step. (c) Core branch to the reservoir without the full Ni protection due to the thick resist. (d) Core with full protection after adjusting exposure and bake times.

After the resist is exposed and developed, Ni is deposited over the wafer. The wafer must be attached to the spinners, that are connected to the E-beam planetary, in order to ensure an even coat next to the tall cores. The wafer is then placed in an acetone bath to etch away the resist and
liftoff the Ni. Initial results showed that Ni residue would remain like flaps on the waveguides causing undesired roughness (Figure 5-13c). In order to overcome this problem the acetone bath was sonicated during the lift-off step (Figure 5-13d). After optimization of the sonication time, it was determined that 1 minute of sonication would liftoff the Ni and remove all residue. Over sonication results in removal of the small Ni features that form the waveguides and under sonication would leave Ni flaps. Once the features are etched into the oxide, the Ni is easily removed in Ni etchant. Care must be taken to ensure that all the Ni is removed. Residual Ni on the waveguide causes optical power attenuation and negatively affects the mode confinement.

Figure 5-13: Top view images showing the challenges caused by under developing the photoresist and lifting off the Ni. (a) Under developed residual resist next to core creates a gap at the LC to SC intersection. (b) Top view of a successful liftoff step (c) Without sonication Ni flaps connected at the edge of the waveguide leaves undesired roughness and residue. (d) Top view demonstrating the good results of sonication.
5.3.3 Ni Mask Versus SU8 Mask

There are many advantages to using the Ni mask over the SU8 mask including: improved process time, smoother edges, realignment ease, reduced time in Nanostrip and most important, the ability to etch deeper into the film. The tradeoff is the need to pay close attention during the step, to avoid forming tapers in the excitation waveguides. Overall I would argue that the Ni mask outperforms the SU8 mask in all aspects when completed correctly without the taper. The ability to check the alignment after performing basic lithography steps makes the Ni mask far superior to the SU8 (Figure 5-14a). Misalignment for the lift-off process results in a 30 second removal of the resist using acetone and isopropyl alcohol (IPA), compared to the 30 minute Nanostrip removal of the SU8. This allows for better performance overall as the Nanostrip has proven to degrade the optical performance of the oxide [124, 125]. The thin Ni film also eliminates the shadowing that occurs when using the thick SU8 mask, this is visible in the SEM image (Figure 5-14c) taken after having etched only 3 μm into the oxide. The shadow of the tall feature causes a partial protection of the oxide near the sidewall, creating a rough edge in the lowest part of the waveguide. Overall the reason for switching to a Ni mask was its ability to etch deeper into the oxide. With only 100 nm of Ni deposited as a mask, it can withstand etching all the way through the oxide and ARROW layers on the pedestal. However exhaustively etching into the silicon eventually removes the Ni mask, and forms undesired micro pillars. The Ni lift-off mask to form SC waveguides has proven to be very useful for various designs and applications for the LC-ARROW biosensor.
Figure 5-14: Top view and SEM images of etch with SU8 and Ni masks. (a) Top view image of a properly aligned device etched through to the silicon substrate. (b) Top view image of a successful alignment between collection waveguide and liquid core, checked just before the deposition of Ni. (c) SEM image of an etch that used an SU8 mask. (d) SEM image of an etch that used a Ni mask.
6  SPIN-ON-GLASS

At the start of my graduate degree program the basic LC-ARROW platform had a problem with water absorption and a crevice at the LC to SC intersection. I attempted to compensate for these issues by applying a cladding layer of spin-on-glass (SOG). The planarized film would fill the crevice with a material whose refractive index was closer to that of the rib waveguide and provide a water barrier. The initial results showed promise but after further investigation we determined that the cladding layer would not yield the desired results. The learning process jump started my graduate research as I gained valuable experience in the cleanroom and learned details in microfabrication. I include this section to record the work that was done with SOG as a cladding layer for the optofluidic platform even though the cladding was not kept as the optimal structure.

6.1  A Brief Overview of Spin-on-Glass

Spin-on-glass is a sol-gel thin film technology that is simply applied by spinning, dipping or spraying a solution which contains the desired oxide precursor. The film coating is subsequently heated to condense the material into its solid form. There are two main types of SOG materials, silicates and siloxanes. The silicate variety solidifies into pure SiO₂ matrices after annealing, making it resistive to the strong acid (Piranha) etch which we use to etch out the cores. Siloxane however contains organosilicon compounds making it susceptible to the acid etch. These organosilicon dopants help siloxanes to planarize better over existing features, reduce film stress and help achieve thicker films at similar spin speeds compared to those of silicates. Both
commonly have low initial refractive indices ranging from 1.39 to 1.44 making them great options for optical cladding layers. SOG has been considered for optical systems for many years, however its use is limited by the maximum thicknesses achievable with one coat. This tends to be around 500 nm for silicates and ~1 μm for siloxanes [145, 146].

![Figure 6-1: Chemical structure of (a) silicate SOG and (b) siloxane SOG, the molecule R indicates –CH₃ and -C₂H₅ types of organic dopants.](image)

6.2 Silicate Initial Experiment

The idea behind the initial test was to protect the PECVD oxide guiding layer from water absorption and to fill the undesired crevice that forms at the liquid-to-solid core intersection (Figure 6-2). To demonstrate how atmospheric moisture and water from further microfabrication processes can accumulate in thin glass layers, two separate experiments were performed. One used a quartz crystal monitor, typically used to measure deposition growth in a vapor deposition system. The other tracked how the refractive index of oxide films changed over time while in water kept at 85ºC.
The first water absorption test demonstrates that porous glasses absorb moisture from the air, which is to be expected as oxide films are commonly used in humidity sensors [147, 148]. Two oxide films, 2 µm thick, were grown by PECVD over two crystals (Maxtek) at a temperature of 250° C. One of the crystals was then placed into a thin film deposition monitor (INFICON XTM/2) in normal room conditions, ~25° C and 40% humidity. The other crystal had a thin ~500 nm SOG overcoat applied (Desert Silicon P-640) cured, annealed (250° - 300° C) and then placed into the monitor. A third crystal was used as a control, which was placed into the monitor without any layers being deposited on it. Both the PECVD oxide and the SOG coated oxide showed an increase in mass per area in the crystal over time. The trend in added mass matched a diffusion-limited profile (Figure 6-3). This figure suggests that atmospheric water molecules are indeed diffusing into the SOG and PECVD oxide at the same rate. Humidity absorption is expected in both films since both are amorphous SiO₂ and are quite porous without high temperature annealing. When annealed at high temperatures (~1000° C) the micro porosity can be reduced [146, 149]. Unfortunately for our application annealing at such high temperatures cracks the liquid cores and underlying ARROW layers leaving the fluorescence sensor inoperable.
Figure 6-3: Data points are the quartz crystal measurements in normal room conditions, mass per area increase over time, PECVD oxide (dots) PECVD oxide with SOG overcoat (diamonds), and control crystal without any coating (triangles). Line is a proportional fit to the $\sqrt{\text{time}}$ matching a diffusion limited profile. Inset - Representation of quartz crystals with (top) and without (bottom) oxide coating.

The second water absorption experiment tracked how the refractive index of oxide (SOG and PECVD) films changed over time while kept in a water bath kept at 85º C. Both films were deposited on plain Si substrates. The PECVD oxide film was deposited at 250º C at a pressure of 1100 mTorr. The film was 5 μm thick with an initial refractive index of 1.46. The silicate SOG was spun on and cured to a max temperature of 250º C providing a thickness of 180 nm and initial index of 1.42. After soaking in water for about two weeks their refractive indices were measured again using a reflectometer for the thick PECVD oxide and spectoscopic ellipsometer for the thin SOG film. The PECVD oxide’s index had increased by 1.8% and the silicate’s index increased by .3 %. Since the silicate film did not surpass an index of 1.46, the initial target index for our guiding layer, the silicate proved to be an attractive cladding layer.

The effects of water absorption raised the index of refraction in both films. One can describe structurally why this is the case. An unannealed PECVD oxide film tends to be less dense, with
dangling bond defects. The Si-Si and Si-O bonds are thus spread wider apart resulting in an
interstitial site for water molecules to enter. Water in the porous regions can be bound as a silanol
(O$_3$≡Si-OH) in the oxide structure [150]. This adjusts the electronic polarization which is a function
of the polarizability of the ionic species and the number density per unit volume. The electronic
polarization contributes to the dielectric constant and results in a raised index of refraction, since
\[ n = \sqrt{\varepsilon_r} \]
where \( \varepsilon_r \) is the dielectric constant and \( n \) is the refractive index of the film.

Both experiments demonstrate that water absorption occurs in the rib waveguides which are
used to excite and collect fluorescence on the optofluidic platform. To summarize the discoveries
discussed in [124, 125, 128, 138] the water absorbs into the surface of the guiding layer which
raises the refractive index and pulls the propagating mode up. The first experiment to correct this
problem deposited a thin film of silicate (~500 nm, \( n=1.42 \)) over the top of the 6 \( \mu \)m PECVD oxide
optical guiding layer on a basic sensor, as a cladding to prevent water absorption in the core. The
fabrication of the basic sensor followed the steps outlined in the design and fabrication section of
this dissertation. The rib waveguides were patterned using the SU8 ridge mask recipe and etched
3 \( \mu \)m into the oxide layer, with the silicate overcoat leaving a structure similar to that of Figure 6-
4. The silicate did not completely protect the waveguides as water could still diffuse into the sides
of the rib structure and over the slab where the silicate cladding had been etched through. This LC-
ARROW sensor was tested against a similar platform without the cladding layer and showed an
average increase in optical throughput of 47%. Rib waveguides without intersecting cores 4 mm
in length were also tested and the SOG coated waveguides showed an average increase in optical
throughput of 28%.
To understand why the silicate improved the sensors and rib waveguides throughput, simulations were conducted using Photon Design’s FIMMWAVE software. The rib waveguide was constructed to show the effects of the water absorption in the surface for both cases, with and without silicate overcoat, with the same dimensions (Figure 6-4). This was done by adding a thin layer of higher refractive index material, $n = 1.48$, at the surface of the rib waveguide for the simulation where the PECVD oxide’s refractive index is 1.46 and the silicate has a refractive index of 1.42. The rib without the SOG overcoat pulls the mode up towards the material with higher refractive index; however, the rib with the silicate maintains the majority of the mode low (Figure 6-5). Maintaining the mode lower was desired, however the hybrid structures allows additional multimodal behavior, which was undesired. Further simulations were run by modeling how the throughput of the overall sensor would be affected by the different mode locations. A model of the rib waveguide passing through the crevice and liquid core was made with the higher and lower propagating modes as sources. The raised mode showed a throughput of 1.3% whereas the lower confined mode showed a throughput of 8.2%. This indicates that the mode which was confined lower experienced less loss as it passed the crevice into the liquid core. The simulations and experiments gave argument that a silicate cladding layer improved the overall throughput of the sensor. However the experimental and simulated data did not entirely match in the magnitude of
the improvement. Further investigation revealed discrepancies in the device dimensions between the SOG coated LC-ARROW chips and the uncoated chips used in the experiment. The SOG coated LC-ARROW devices had a taller liquid core as well as wider excitation waveguides. We have found that taller liquid cores provide better optical throughput as they allow for better mode coupling. Further the wider rib waveguides mode match better to the optical fiber allowing more light to be coupled in, and thus resulting in increased throughput. These structural variations could possibly account for the mismatch between experiment and simulated data.

Figure 6-5: FIMMWAVE Simulations of the mode propagating through the crevice. (Insets) Simulation of fundamental mode of the rib structure for water absorbed rib without silicate (a) and with silicate (b).

This initial experiment showed that silicate SOG could potentially be a useful cladding layer. Unfortunately this design did not fix the initial problem of the crevice and the detrimental water absorption. The high stress that silicate SOG placed on the devices while it cured also weakened the cores which caused them to readily crack reducing device yield (Figure 6-6). For this purpose we pursued a hybrid cladding layer of siloxane SOG and PECVD oxide. The SOG would fill the crevice and provide a low index while the PECVD layer would prevent the siloxane from etching away during the Piranha acid etching of the cores.
In order to overcome the faults of the silicate SOG experiment a hybrid siloxane device was created. In hopes that siloxane would survive etching in Piranha (60 mL H₂O₂: 40 mL sulfuric acid) we coated SU8 cores with siloxane SOG and placed the wafer in Piranha for a few hours. Top view images of the core before and after being placed in Piranha are shown in Figure 6-7. The cores and siloxane SOG started to etch in the acid as evidenced by Figure 6-7b. The organic material in siloxane makes it susceptible to Piranha as we confirmed with this experiment. In order to prevent the siloxane from etching a second layer of PECVD oxide was deposited overtop of the siloxane to protect it.

It was thought that the second layer of PECVD oxide would hurt the mode confinement of the rib waveguide as the index of refraction would be higher, due to water absorption, than that of the core. We used FIMMWAVE simulations to verify the mode would be well confined (Figure 6-8). The rib core with refractive index, \( n = 1.46 \), was simulated with cladding layers, the first
being 500 nm thick and \( n = 1.42 \) that of siloxane, and the second being 1 \( \mu \text{m} \) thick with \( n = 1.48 \). The simulations showed that the mode would be well confined (Figure 6-8 inset).

![Figure 6-7: Top view images of SU8 core with siloxane SOG overcoat layer (a) before and (b) after etching in Piranha.](image)

An experimental device was fabricated using the same procedures found in the fabrication section of this dissertation and in the original recipe in Appendix B. After the ribs were etched into the oxide, a siloxane SOG coating was spun on at 1000 rpm for 1 minute. The SOG was cured on a hotplate starting at 125\(^\circ\) C which was ramped up by 10\(^\circ\) C every 2 minutes until it reached a max temperature of 200\(^\circ\) C, then the temperature was reduced back to 125\(^\circ\) C by the same process. The SOG overcoat was protected from the Piranha at the core exposure points by an initial core exposure step, which was used to controllably eliminate the SOG where the core exposure would normally happen. This step used the same core exposure recipe and mask, found in Appendix B, with the only change being that the wafer was placed in buffered hydrofluoric acid (BHF) for an extended time to widen the area of exposure. After the wafer was removed from BHF, the photoresist mask was removed with acetone and IPA followed by the deposition of a 1 \( \mu \text{m} \) layer of PECVD oxide. The wafer underwent a second core exposure step, which had a smaller area, to remove the oxide from the tips of the cores without exposing the siloxane cladding layer. This
oxide layer did protect the SOG during the etching of the cores as the structure remained intact (Figures 6-8 and 6-9).

Unlike the silicate layer the siloxane overcoat did not stress the device upon curing, and devices were able to be successfully built with high yield. Tests were performed to see if the throughput improved for these devices with their crevices filled and minimal water absorption. These tests surprisingly showed that the SOG overcoat did not improve throughput by any significant amount. SEM images were taken of the siloxane devices which revealed that the crevice was no longer an issue as the new PECVD machine was capable of growing low stress films with good conformity (Figure 6-9) [124]. Using the new machine my colleague Tom Wall was able to incorporate a buried rib waveguide design [128]. This ended the pursuit of a SOG cladding layer as it no longer had any use in the LC-ARROW platform. However the lessons learned from the
experiments, fabrication and material science studies helped me to accomplish the rest of the work in this dissertation.

Figure 6-9: SEM image of the siloxane overcoat device at the solid to liquid core junction where the crevice should be.
7 **Y-SPLITTER**

The main goal in implementing the Y-splitter design was to improve the sensitivity of the basic sensor. For the basic sensor, we attached hundreds to thousands of fluorophores to the target which enabled high signals to achieve single-molecule detection [110, 119]. The luxury of attaching many fluorophores on a single target is not always feasible, which is the case when attempting to detect very low concentrations of antibody-labeled proteins or labeled nucleic acids [151]. When attempting to detect low-fluorescent signals, a new approach was needed to improve the signal-to-noise ratio (SNR) of the overall sensor [152]. The simple method boosts SNR using a simple signal processing algorithm that takes advantage of multiple excitation spots and the time difference between spots. As the biomarker passes through the multi-spot excitation region, multiple peaks will be produced in the fluorescent signal. We can then apply a simple signal-processing algorithm to the fluorescent signal, which enhances SNR by amplifying the signal and canceling the noise. We call it the “Shift-Multiply Algorithm”.

This section will outline the need for multi-spot excitation, give an overview of the chosen Y-splitter devices, show their design and fabrication, expound on the experiment used to optimize the design, and finally, display the results of using the optimized Y-splitter excitation platform.
7.1 Multi-Spot Excitation

Using only one excitation spot often makes it difficult to distinguish low signals from the background noise, especially when low amounts of fluorophores are used. Figure 7-1 shows the single excitation fluorescence signal collected from a basic sensor (single excitation spot). This particular fluorescence signal has a low SNR and could easily detect inaccurately the passing targets labeled with low amounts of fluorophores. The location of each particle within the channel as it passes the excitation beam causes differences in signal peak heights. The excitation beam is a Gaussian mode, so the particles which pass in the center of the mode tend to produce more fluorescence signal. The fluorescence peaks close to the noise floor could be counted as noise even if they came from a target molecule. Higher signal-to-noise ratios are needed to accurately and correctly identify passing biomarkers labeled with few fluorophores. Identification can be improved by adding multiple equally spaced excitation spots, creating a temporally encoded signal. Only the labeled molecule will produce the same time-dependent fluorescent signal as it passes through the multiple excitation beams at a constant velocity. This time-correlated signal can then be used to enhance SNR by using a simple signal processing method which takes the time-difference between spots, \( \Delta T \), into account.

There are many different ways to produce the multiple excitation spots, the simplest to understand being the Y-splitter device. Through its structure, the Y-splitter (Figure 7-2a) can directly guide the light to create equally spaced spots from a single optical source. We chose to keep the same rib waveguide structure (see Figure 7-2b) for the excitation and collection waveguides in the Y-splitter device. This enabled us to stay in close comparison with the basic sensor design [110]. Choosing this structure also keeps the fabrication process identical to those of the original design. The full fabrication process can be found in the “LC-ARROW Design and
Fabrication” section of this dissertation. The SU8 rib waveguide masking process needed to be modified slightly in order to successfully implement the splitter design. By increasing the baking and exposure times of the SU8 recipe, the desired resolution was achieved without wiggles in the structure, and reduced feature liftoff. The changes can be observed in Appendix B, which compares the original recipe with the optimized recipe.

![Fluorescence signal from a single excitation spot generated from exciting Alexa 546 fluorophores.](image)

Figure 7-1: Fluorescence signal from a single excitation spot generated from exciting Alexa 546 fluorophores.

We first designed the Y-splitters to stay in the 8x8 mm chip space constraint. With the liquid cores centered on the chip, we could follow the same dimensions of the basic design. This resulted in split angles of 2.865° and 3.82° for the 1 x 4 and 1 x 8 splitters respectively, reducing throughput and creating multi-spot patterns with unequal split-power ratios at the liquid core. The 1 x 2 splitter maximum split angle was 1.43° and was able to achieve desired spot patterns and throughput, which prompted an experiment to optimize the split angle within chip constraints. This led to a change in the design.
Figure 7-2: (a) Representation of microfluidic biosensor using ARROW Y-splitters for multispot detection. (b) A cross sectional view of the splitter rib structure with dimensions indicated. Reprinted with permission from reference [153].

### 7.2 Shift-Multiply Algorithm

The signal processing algorithm used to enhance SNR is dependent upon a time correlation within the fluorescent signal. The time correlation is created by the multi-spot excitation pattern. Each fluorescently labeled target that travels through the liquid core will pass $N$ excitation spots, giving off a burst of photons at each spot which is recorded within the time-dependent fluorescence signal $F(t)$. This signal will have $N$ peaks, one for each passing target, which are time-correlated to the $N$ excitation spots due to the particle’s velocity. The shift-multiply algorithm can then be applied to the signal, $F(t)$, as illustrated in Figure 7-3. The algorithm is mathematically defined as

$$S(t, \Delta T) = \prod_{i=0}^{N-1} F(t - i \cdot \Delta T)$$

(7-1)

for any $N$, where $S(t)$ is the processed signal and $\Delta T$ is the time difference between any of the two.

For a four-spot excitation pattern, the algorithm can be expanded for ease of understanding as

$$S(t) = F_0(t) \ast F_1(t - \Delta T) \ast F_2(t - 2\Delta T) \ast F_3(t - 3\Delta T).$$

(7-2)
In order to determine $\Delta T$, one can do a cross-correlation analysis on the signal, which is defined mathematically as

$$R(\tau) = \int F_0(t) * F_1(t - \tau) * F_2(t - 2\tau) * F_3(t - 3\tau) dt.$$  \hspace{1cm} (7-3)

The function $R(\tau)$ will maximize when the time difference between the two peaks equals $\tau$. An example graph of $R(\tau)$ for a cross-correlation analysis of an 8-spot peak pattern is shown in Figure 7-4. The maximum peak is the correlation between neighboring excitation spots and occurs at time $\Delta T_1$, which is the time difference between spots. The second tallest peak corresponds to the correlation of every other spot, the third tallest every three spots, and so on and so forth for each peak of $R(\tau)$. Another method of determining the time difference between the two peaks would
be to run the algorithm for all $\Delta T$. The processed signal $S(t)$ would be greatest at the correct $\Delta T$.

With both methods, the correct processed signal and time difference can be determined and used to correctly identify target molecules with enhanced SNR. However, using the cross-correlation method can automate the process and save time, as it can be acquired during the autocorrelation of the attained fluorescence signal.

![Graph of autocorrelation curve](image)

Figure 7-4: Graph of autocorrelation curve for an 8-spot excitation pattern with select time differences between spots labeled. Embedded in the graph is the fluorescence signal from the 8-spot excitation pattern, with corresponding time differences indicated.

Using the shift-multiply algorithm and optimally-designed Y-splitter waveguides, our collaborators at the University of California Santa Cruz were able to characterize the SNR enhancement for various $N$ spots [151]. They found experimentally that for an $N = 2$, $N = 4$, and $N = 8$ excitation spots a SNR enhancement of 4.72, 62, and 52,794 times were possible. Theoretical calculations predicted the enhancement to be much greater but on a similar scale. The discrepancies can be attributed to the non-idealities introduced by the Y-splitters causing non-uniform spots and propagation loss not accounted for in the calculations.
7.3 Split Angle Optimization Experiment

Many different splitter configurations have been developed to achieve the desired compactness. Some of these configurations include multimode interference couplers, photonic crystal, indexed tapered, and trench-based splitters [154, 155, 156, 157, 158]. Although the device performance and size can be improved with further design complexity, we chose to study the Y-splitter for its simplicity and spectral independence. The basic Y-splitter design relies on a linear divergence between two waveguides at a specific angle. Since the waveguide spacing \( d \) of each splitter is held constant to achieve the desired time difference, we can only vary the length of the splitter to obtain smaller angles. A trade-off must be made between chip size and loss. The split angle optimization experiment quantitatively analyzes this trade-off and optimizes the design.

7.3.1 Design and Fabrication

To achieve angle comparisons while limiting fabrication variances, a mask was created to pattern eighteen different angles from 0.5 to 9 degrees on the same 8 x 7.5 mm chip. A table with each Y-splitter design is shown in Table 7-1 and defines the designed dimensions shown in Figure 7-5. The length \( d \) was held constant as it provides the spatial distance between excitation spots, and subsequently, the desired time difference \( \Delta T \) needed to make the fluorescence time correlation. By holding \( d \) constant, the only way to adjust the angle was by adjusting the length \( L \) which caused the chip size to increase with each decreased angle.
Table 7-1: Design parameters for each of the 18 angles designed for the splitter experiment.

<table>
<thead>
<tr>
<th>θ (Degrees)</th>
<th>ϕ (Degrees)</th>
<th>L (μm)</th>
<th>d (μm)</th>
</tr>
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<td>2865</td>
<td>25</td>
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<td>843</td>
<td>25</td>
</tr>
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</tr>
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</tr>
<tr>
<td>9</td>
<td>175.5</td>
<td>159</td>
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</tr>
</tbody>
</table>

Figure 7-5: (a) Top view image of actual Y-splitter showing dimensions. The distance, R, is a fabrication non-ideality where the lithography process was unable to perfectly define the intended split angle. Reprinted from reference [153], with permission from SPIE.

A test wafer with numerous chips was fabricated using the following fabrication methods. We started with a <100> Si substrate which had commercially sputtered ARROW layers, alternating layers of SiO₂ and Ta₂O₅ (Figure 7-6a). To create the guiding layer of the waveguides, 6 μm of SiO₂ was deposited by plasma-enhanced chemical vapor deposition (Figure 7-6b). The oxide layer was then masked with SU8-10 and pattern aligned to the flat edge of the wafer to facilitate clean facets upon cleaving (Figure 7-6c). SU8 is a negative resist and can achieve good heights and aspect ratios [159]. The SU8 pattern served as an etch mask for dry etching. The Y-splitter rib waveguide structures were formed by etching 3 μm into the oxide with an inductively coupled
plasma (ICP) reactive ion etcher (RIE). After etching, residual SU8 was removed in a strong acid (Nanostrip) at 90° C, and the wafer was then placed in a clean oven at 150° C to evaporate any excess water molecules (Figure 7-6d). The SU8 lithography process; however, was not able to perfectly define the intended separations between the linear diverging waveguides. This is shown in Figure 7-5 by the blue lines which indicate the ideal designed splitter. The distance $R$ characterizes this fabrication limitation in which a small gap between the ideal and actual device is filled in. The nonideality $R$ effected the overall performance of the splitter. After fabrication and cleaving, the chips were placed in a dehydration bake oven to prevent aging due to moisture absorption in the SiO$_2$ film [125].

Figure 7-6: The fabrication process starts with (a) the initial ARROW layers on Si substrate, followed by (b) top 6 μm oxide growth, (c) SU8 mask patterning, and (d) SU8 mask removal. Reprinted from reference [153], with permission from SPIE. (e) An SEM image of a Y-splitter input facet.

7.3.2 Testing Setup and Procedures

Experimental testing was all conducted on our optical setup shown in Figure 7-7. A 635 nm laser (Thorlabs S1FC635) was coupled to the setup shown in Figure 7-7 via optical fiber, and all tests were done at this wavelength. The maximum throughput for each splitter leg was measured for each design angle. The chip with each of the design angles—eighteen in total—was mounted
to a five axis stage and aligned to bring the desired splitter’s output facets into the view of the charged coupled device (CCD) camera. The CCD was focused to the chip edge by an objective lens through 50/50 beam splitter backlit by a collimated white LED. The fiber was then coupled to the input facet of the Y-splitter, and the mode would be visible. A flip mirror and beam splitter were used to toggle between viewing the modes and the facet on the CCD and measuring throughput directly on the photodetector (Thorlabs SM1PD2A). With the mode focused directly on the photodetector, the fiber’s alignment was adjusted for best coupling (maximum throughput) and power documented.

Figure 7-7: Picture of optical setup with key components labeled.

Upon finding the optimal coupling, the mode image was taken by the CCD with the mirror replaced. These mode images were used to adjust the measured throughput, to account for only the light guiding in the waveguides, and to characterize the split ratio. An example as to why this is important can be illustrated with Figure 7-8. The mode image for the 9-degree split angle has power coupled to the photodetector between the splitter’s facets in the slab portion of the waveguide.
A MATLAB script was used to process each mode image. The optical power not contained in the output legs of the Y-splitter was discarded, and the properly guided light in the splitter rib was recorded. Larger angles tended to couple large portions of optical power through the oxide between the facets, whereas small angles maintained confinement in the splitter rib structure. The mode images were also used to determine if the splitter attained a 3dB split into the output legs. Top view images for each of the measured devices were also taken at a 10 dB laser attenuation to record scattering loss throughout the waveguide (Figure 7-8).

Figure 7-8: Select top view and mode images of different split angles under test. Reprinted from reference [153], with permission from SPIE.

7.3.3 Simulations

Simulations were also conducted using Photon Design’s FIMMWAVE software using a finite difference mode (FDM) solver. The total power transmitted from the input and out of the output legs for each Y-junction was simulated for various angles. The angle was adjusted by varying length as seen in Figure 7-9, which depicts the simulation run in FIMMWAVE. It is important to note that the simulations do not account for propagation loss and only account for the split angle loss at a given length. This discrepancy was accounted for when comparing experimental data to simulated data.
7.3.4 Optimization Experiment Data and Results

Seven Y-splitter waveguides for each angle were tested. Their throughput measurements were averaged, and error bars were added to the normalized transmission in Figure 7-10, which are displayed as dots with error bars. To determine the experimental error, as indicated by the error bars on the experimental data in Figure 7-10, a single device was measured ten times. We determined that the error from measuring was about 10% of the maximum throughput. FDM solvers were used to first simulate the ideal case where the split would happen as designed (where the split output legs are perfectly defined). The results of these simulations are indicated by triangles in Figure 7-10. As indicated before, the SU8 lithography process introduced non-idealities in the way the legs split off from the input. These fabrication non-idealities are described by the length $R$ in Figure 7-5. We ran addition simulations to account for the parameter $R$, which was measured for all angles using a high-resolution optical profilometer (ZETA-20). These measurements are plotted in Figure 7-11. The resulting throughputs for the non-ideal simulations are the squares in Figure 7-10, and they follow the trend of the experimental data. The limit to our fabrication process, described by $R$, appears to cause a deviation from the expected ideal in both the simulation and measured normalized transmission. This deviation happens because the ideal
split becomes multimode in nature as the waveguide diverges from it and optical power is lost in the higher order modes. The higher modes then scatter at the split and do not couple properly into the output waveguides. The simulated normalized-percent throughput and measured normalized-percent throughput increase with a decreased angle as theoretically expected [160]. We determined through the MATLAB script that lower angles achieved 3dB splits with 50% optical power in each leg, whereas the higher angles performed worse.

![Figure 7-10: Normalized transmission of experimental results (dots with error bars) and simulation data (ideal = triangles, non-ideal = squares) versus angle, in degrees, for various angles. Simulation data is not normalized as they do not take propagation loss into account. Each experimental point is a normalized average of seven measurements of seven different devices of the same split angle. Reprinted from reference [153], with permission from SPIE.](image)

As Figure 7-10 indicates, there is a significant amount of loss at the Y-junction for higher angles. The loss due to each split angle was calculated from

\[
\alpha_s = 1 - \frac{P_{out_n}}{P_{in_n(1-\alpha_f)e^{-\alpha_f L/c}}},
\]

(7-4)
where $\propto_f$ is the loss from all facets, $\propto_L$ is the propagation loss, $l_c$ is the total length of the Y-splitter chip, and $P_{out_n}$ and $P_{in_n}$ are the optical power out and power in for each device respectively. The facet and propagation losses were found experimentally by using the cutback method on multiple non-splitting rib waveguides [161]. These structures were also located on the chips with the eighteen waveguides of varying split angles for comparison. The average of three different chips on which cutback measurements were made found the average propagation loss to be 0.781 cm$^{-1}$, and the average coupling loss from the contributions of all three facets was 11.2%. With all variables now known, the angle-dependent loss at each split junction was calculated for each angle using Equation 7-1. The results of these calculations are shown in Figure 7-11. The split loss increases with larger angles as predicted. This loss can be accredited to the loss of mode confinement and the increased scatter at the junction of diverging arms. Important for the optimization purpose is to note how the length $L$ scales with the angle. This correlation is also plotted in Figure 7-11. The length is proportional to the inverse tangent of the angle, $L = \frac{d}{\tan(\theta)}$, with $d$ being held constant and $\theta$ increasing as $L$ decreases. Although the split loss is lowest when the angle is lower than 1°, the length also increases drastically, causing a 0.5° angle to take as much as 3 mm of chip space. This increase of length is undesirable, especially when multiple Y-splitters are linked together in order to produce a 1 to N splitter—drastically increasing chip size.

### 7.3.5 Experiment Conclusions

Both experimental and modeling results for power transmission through Y-splitters confirmed expectations that shallow angles have lower split losses. However, fabrication constraints dictate achievable split angles with 50/50 split ratios at the outputs. Based on the measured results, we concluded that using split angles less than 1° has little practical benefit in terms of performance
compared to the substantial increase of the overall splitter length. Optimization for Y-splitters relies heavily on chip size constraints, as splitters with lower split loss require longer waveguides. From this experiment we determined that future liquid core ARROW sensors built with Y-splitters—including linked Y-splitter elements—using an approximate 2° split angle would be a reasonable engineering compromise between transmission performance and chip size.

![Figure 7-11: Experimental split loss in percentage (dots) versus angle in degrees, the limiting distance, R, in micrometers (triangles with line), and length, L, in micrometers (diamond with line) versus angle in degrees. Error bars propagated from the uncertainties of all measured quantities including: throughput, facet loss, and propagation loss. Arrows point to which axis data corresponds to. Reprinted from reference [153], with permission from SPIE.]

7.4 Y-Splitter Sensor Fabrication and Results

Before the optimization experiment our initial Y-splitter biosensor design had split angles 2.865° and 3.82°, for the 1 x 4 and 1 x 8 splitters respectively, which caused poor performance for the sensor. The second design with angles and dimensions is represented in Figure 7-12. Only the 8-excitation spot configuration needed to increase chip length from 8 mm to 11.1 mm. As we can see the new design kept all split angles less than 2° and greater than 1° for each of the different
splitter configurations—1 x 2, 1 x 4, and 1 x 8—in order to keep good throughput and produce equal excitation spots.

Fabrication procedures, identical to those explained in the “LC-ARROW Design and Fabrication” section of this dissertation, were followed to build the Y-splitter excitation LC-ARROW platform. For the rib masking step the SU8 masking procedures were followed. SEM images are seen in Figure 7-13. The alignment of the Y-splitters to the liquid core during masking lithography is critical, as misalignment results in etching into the liquid core. A device with a slight misalignment which has not etched into the liquid core is indicated with a blue outline in Figure 7-13a. The SEM image in Figure 7-13b is a good indication of the scale of the Y-splitter.
Devices with the optimized design were used to characterize the achievable SNR enhancement and to detect individual H1N1 viruses. As seen in the top view images of Figure 7-14, the 1 x 8 and 1 x 4 Y-splitters were able to achieve good multi-spot excitation patterns. Results of these devices were published in “Signal-to-noise Enhancement in Optical Detection of Single Viruses with Multi-spot excitation” [151]. With 1 x 8 splitters SNR enhancements up to $5 \times 10^4$ were achievable and successfully demonstrated. Temporally encoded signal from H1N1 viruses were also successfully processed and identified with the signal processing algorithm. The
successful results from the spectral independent Y-splitter LC-ARROW design paved the way for a new design that capitalizes on spectral dependence.

Figure 7-14: Top view image of quantum-dot-filled liquid core showing (a) 8 excitation spots and (b) 4 excitation spots.
Selecting fluorescent labeling is a method commonly used to enable detection of multiple biomarkers simultaneously. In the case of detecting single nucleic acids, selective labeling can be accomplished with custom made oligo probes or molecular beacons which are designed to specifically attach to a desired target. These probes are made with fluorophores that can be excited to emit photons at a variety of wavelengths spanning the full visible spectrum; and are commercially available [162]. Thus, they are a simple method to allow selective detection and determination of genetic information [123]. This form of spectral multiplexing comes with a challenge caused by the spectral indifference of the light collection method. Detectors with single-photon sensitivity do not differentiate between the different wavelengths of light. Many methods have been employed to overcome this challenge, such as complex timing schemes and free space optical setups which involve multiple spectrally filtered detectors [163, 164, 165, 166]. The approach discussed here is simple and eliminates the need for multiple detectors by employing a simple photonic device, called the multimode interference (MMI) waveguide (Figure 8-1). This device forms multi-spot excitation patterns similar to those created by the Y-splitter waveguide but with wavelength dependence. Differing spot patterns which span the visible range can be formed at the liquid channel to simultaneously excite the wavelength-selective fluorescent markers at their corresponding wavelength (Figure 8-1c). As the biomarkers pass the spot pattern, they can be identified by the generated number of fluorescence peaks. The shift-multiply algorithm, as discussed in the previous chapter, can then enhance the signal and identify the biomarker by the
time between peaks $\Delta t$. In this way, different biomarkers or gene sequences can be identified in a single, one-shot test. To ensure high sensitivity detection of the passing targets, the spot pattern needs to have high fidelity. For the pattern to be considered of “high fidelity”, it should have a high peak-to-valley ratio, and each individual spot should be narrow to avoid overlap between fluorescence peaks that can confuse the algorithm. This is how we enable spectral multiplexing on the LC-ARROW biosensor (Figure 8-1b). This chapter overviews the MMI waveguide and its mathematical theory in the context of the design constraints posed by the optofluidic platform. Initial challenges in the design will be discussed followed by the experimental results used to optimize the design. This chapter will conclude with the biological detection results from both devices prior to and following optimization.

Figure 8-1: Multimode interference waveguide used to enable spectral multiplexing. (a) Schematic view of MMI waveguide intersecting a fluidic microchannel containing target particles. Targets are optically excited as they flow past well-defined excitation spots created by the MMI; fluorescence is collected by the liquid-core waveguide channel and routed into solid-core waveguides (red). (b) Scanning electron micrograph of MMI-based optofluidic chip. (Inset) Photograph of 8 x 8 mm chip, showing two fluidic reservoirs holding microliters of sample volume. (c) Top view images of multispot excitation patterns created in fluidic channel filled with fluorescent liquid. The entire visible spectrum is covered by independent channels (405 nm/11 spots, 453/10, 488/9, 553/8, 633/7, 745/6). (The original black and white color scale was rendered in the actual excitation colors.) Reprinted with permission from reference [109].
8.1 Multimode Interference Waveguide

The MMI coupler is a passive photonic device that was initially intended for use in telecommunications. It can split and/or combine signals and typically has generous fabrication tolerances, low loss, and a small footprint [167, 168, 169]. These advantages have made the MMI waveguide the focus of many studies, and its mathematical theory has been well developed [155, 170, 171, 172]. However, because of its usefulness in telecommunication, most papers on the subject are for devices made from materials with high refractive indices, such as silicon \((n = 3.5)\), for use in the infrared spectral range \((\lambda \text{ between 800 nm to 1700 nm})\) [173, 174, 175]. Using materials with high refractive indices provide high index contrasts between the core and cladding, which provides excellent resolution of the output pattern. The self-imaged mode resolution can be approximated by

\[
\rho \approx \frac{w}{m},
\]

where \(w\) is the effective width of the MMI waveguide and \(m\) is the waveguide’s number of guided modes [155, 176]. For example, a rib structure that uses silicon as the guiding layer would have a 45.8% index contrast, producing many higher order modes. A waveguide with the same dimensions but that uses SiO\(_2\) as the core \((n = 1.51)\) would reduce the index contrast to 28.1%. In order to attain the same resolution, the overall geometry of the waveguide would increase to support the same number of modes. This would require the MMI coupler to hold its fabrication tolerances over a larger area [170, 173]. Reduced resolution is undesirable because in order for the multiplexed fluorescent sensor to accurately detect with a high sensitivity, the excitation spots emerging from the MMI waveguide need to produce a high-fidelity spot pattern of \(N\) evenly spaced modes. The \(N\) modes, at the MMI waveguide output, are self-imaged from the input mode delivered by the single-mode excitation waveguide (Figure 8-2). The number of spots generated is
dependent on the wavelength and MMI waveguide geometry. The theory has been well established by Soldano and Pennings [155]. The governing equation is

\[ N \cdot \lambda = \frac{n_c w^2}{L}, \]  

(8-2)

where \( n_c \) is the effective index of the core, \( w \) is still the effective width, \( L \) is the length of the MMI waveguide, and \( \lambda \) is the wavelength of excitation light. From this equation, we can observe the dependence on \( w \) and \( L \). To achieve a desired even number of spots \( N \) at a wavelength \( \lambda \), a small increase in the width \( w \) triggers a corresponding larger increase in \( L \). This instills a formidable constraint on the MMI waveguide in optofluidic multiplexing. In order to ensure there is a large enough gap between spots the width must be kept relatively wide. With the width fixed, the length is the primary parameter that can be adjusted to obtain the desired spot number \( N \) for each wavelength. The wavelength dependency requires the length \( L \) to be a common multiple of the beat length for each color used [170]. For our design, the wavelength bands are far enough apart to avoid spectral overlap across the fluorescent labels. This again results in longer MMI waveguides, whereupon they must hold their tolerances. The sum total of all these constraints limit us to using relatively long MMI waveguides contradictory to the guideline provided by Soldano and Pennings, which is that “the multimode waveguide length must be made as short as possible in order to allow relaxed tolerance to the other parameters” which are the wavelength, width, and index [155]. Their guideline comes from

\[ \frac{\delta L}{L} = 2 \frac{\delta w}{w} \simeq \frac{|\delta \lambda|}{\lambda} \simeq \frac{\delta n_c}{n_c}, \]  

(8-3)

which is derived in reference [170].
Further tolerance restrictions on the MMI waveguide are imposed by the fluorescent dyes (Integrated DNA Technologies) and the penta-bandpass filter (FF01-440/521/607/694/809-25, Semrock) used to filter excess noise generated from the platform. This constraint is best illustrated by the filter’s spectrum (light blue) with an overlay of the biomarker fluorescence spectra for excitation (dark blue) and emission (magenta) (Figure 8-3). Excitation and emission spectra do not follow the y-axis, but they are placed to correctly follow the x-axis of the graph. Here we can observe which colors will provide highest signal and which may be cut off by the filter. The dotted arrows represent the wavelength range for optimal excitation in which the sensor can achieve reasonable detection without filtering out the signal. It is at these wavelengths that the MMI waveguide must maintain its $\delta\lambda$ tolerance. For example, if the MMI waveguide is made wider than the design, the color at which it produces the cleanest pattern will shift. To provide high fidelity patterns and maintain the wavelength at maximum absorption for the chosen fluorescent dye and the available filter, we can only afford to tune the white laser source’s (NKT Photonics) wavelength approximately 10 nm, which is a wavelength tolerance $\frac{|\delta\lambda|}{\lambda}$ of 1.6%. This translates to
tighter fabrication tolerances (~0.8%) on the required width of the MMI waveguide. For example, a 75 μm wide waveguide can only vary in width approximately +/- 0.6 μm and approximately +/- 0.8 μm for a 100 μm wide waveguide.

![Diagram](image)

Figure 8-3: Penta-bandpass filter response with overlay of the fluorescence response spectra for four fluorescent dyes typically used for biomarker detection. The filter’s spectrum was taken from the datasheet in reference [177] and the x and y axis corresponds to it (light blue spectra). The spectra of each dye are superimposed on the filter’s graph following the x axis. Although they do no follow the y axis it can be assumed that the maximum response occurs at their peaks. Each dye’s spectra are labeled according to its type and were taken from their corresponding data sheets, A488 [178], A546 [179], TYE665 [180], A745 [181]. Circled are the peak emission wavelengths for each dye. The excitation spectrum is dark blue and the emission spectrum is magenta for each dye. Dotted arrows represent the excitation wavelength range whereupon maximum collected emission may be attained with the filter pass bands.

Each of the aforementioned constraints were taken into account while designing the MMI waveguide. MMI waveguide dimensions were calculated using Equation 8-2 and then further modeled using Photon Design’s FIMMWAVE software to find the length at which the spot pattern was best resolved over the desired wavelengths. The sensor design is meant to give N = 6 spots at 738 nm, N = 7 spots at 633 nm, N = 8 spots at 554 nm, and N = 9 at 492 nm, which will excite the molecules within the acceptable band. MMI waveguide dimensions suitable at these wavelengths
and a core index of $n_c = 1.51$ were designed to be 75 μm wide by 1,932 μm long or 100 μm wide by 3,442 μm long. The first mask set also contained waveguides that were designed 50 μm wide. However, at this width, the pattern’s spots were too close together to fully resolve the pattern in the fluorescence signal and the design was quickly discarded. Both the 75 μm wide and the 100 μm MMI waveguides performed well and were used in various single-molecule detection studies. It is important to note that the initial design did not take into account a buried waveguide structure. Instead, it consisted of the same rib structure (etched 3 μm into the top oxide) as the basic design. Sensors with MMI waveguides could follow the same fabrication procedures as the basic design. Excitation waveguides (Figure 8-2) initially remained 4 μm wide as they worked well for the basic design. However, to fully optimize the MMI waveguide the excitation waveguides were eventually changed and a buried waveguide structure was implemented to improve the fidelity of the excitation spot pattern. For reference, the buried MMI waveguide was designed with the same core index covered with an oxide cladding ($n_c = 1.45$). The reduced index contrast between the core and cladding required a change the MMI waveguide design, we kept the widths 75 μm and 100 μm wide and increased the lengths to 1,975 μm and 3,475 μm long.

### 8.2 Challenge with Spot Patterns

Many of the initial devices produced irregular spot patterns, which caused poor performance in the overall sensor. For example, the MMI patterns in Figure 8-4 were taken from a device which had superior SNR when excited only from the excitation waveguide. The MMI patterns however were far from desirable, having low peak-to-valley ratios and variably sized peaks.
Poor MMI spot patterns cause poor peaks in the signal and degrade the sensitivity of the sensor. The ability to identify a biomarker relies heavily on the autocorrelation $G(\tau)$ of the signal (Figure 8-5c and d). The autocorrelation of the peak pattern in the fluorescence trace determines the $\Delta t$ between the spots which is used to distinguish between target molecules labeled with different colored dye. This phenomenon is best illustrated in Figure 8-5. The graphs on the left (Figure 8-5a and c) were generated from an optimized MMI device capable of properly distinguishing targets, which is evident in the well-defined peaks of the autocorrelation curve with the tallest peak being the time between neighboring spots $\Delta t$. The graphs on the right (Figure 8-5b and d) are processed from a pattern that is not capable of properly identifying the passing biomarkers. The poorly defined autocorrelation curve could easily confuse $\Delta t$ and the sensor would then inaccurately determine the concentration of the analyte solution. Producing spot patterns with the highest fidelity is thus essential to the operation of the optofluidic sensor. Many attempts were made to improve the spots. The initial experiments that failed will be presented first and then followed by the optimized experiment that ultimately corrected the poor fidelity problem.
Figure 8-5: Zoomed in view of a fluorescent trace used to quantify the fidelity of the MMI waveguides spot pattern. The autocorrelation curve corresponding to the trace is found directly below. (a and c) Represent a pattern with “high fidelity” and (b and d) represent fluorescent trace and autocorrelation curve of a poor spot pattern.

8.2.1 Preliminary Experiments

The first hypothesis for the poor spot fidelity was that the deposition method (PECVD) used to grow the film incorporated several defects in the film [137]. It was predicted that these defects negatively distorted the phase of the propagating modes and thus resulted in a skewed interference pattern. To test this hypothesis MMI waveguides were built on ARROW layers with a different material that had less impurities. We chose SU8 as it was readily available and had recently been shown to guide light in the visible regime under proper processing conditions [182]. Then we built a wafer with various chips that incorporated five SU8 MMI waveguides of differing excitation waveguide geometries. Devices were then cleaved at the marked length and optically tested. The MMI waveguide output pattern was captured by scanning a white light laser source (NKT Photonics) coupled via optical fiber into the ARROW chip. As we can see in Figure 8-6, the SU8
did not improve the performance of the MMI spot patterns, which is evident in the extra lobes between spots and the inconsistent size.

Figure 8-6: Side view images of the MMI spot patterns taken from the output facet (left). Normalized intensity profiles which correspond to the images on the left.

A less likely second hypothesis was that the interference effect from the ARROW layers interfered with multimode interference in the waveguide. Devices with and without ARROW layers were created. The devices without ARROW layers were made on top of a thick, $n = 1.45$, cladding layer in order to guide light in the rib waveguides. The guiding in the non-ARROW device was so poor compared to the ARROW device. The hypothesis was discarded because the two interference effects are orthogonal to each other. The ARROW layers are only below the waveguide and do not provide any confinement on the rib sidewall. The multimode interference occurs because of total internal reflection at the side walls.

Many other experiments also failed, such as roughening the side walls, annealing the waveguides to eliminate water absorption prior to testing, varying the width of the MMI waveguide to adjust for fabrication tolerances, etc., but it wasn’t until after the Ni Lift-off step was developed that the MMI waveguides started to produce high-fidelity spot patterns. For many of the experiments a lithography mask was designed and used to produce varied excitation waveguide
widths, the mask also had various MMI waveguide widths but the width was maintained on a single chip (Figure 8-7). This lithography mask was also used in the successful optimization experiment discussed below.

![Figure 8-7: Schematic of the chip design used for the various MMI waveguide experiments.](image)

### 8.3 Optimization Experiments

The optimization experiments focused not solely on the MMI device but rather on optimizing the mode used to excite the MMI waveguide. The excitation waveguide’s geometry determines the shape of the input mode and thus the fidelity of the self-imaged modes at the MMI waveguide’s output. The concept of improving the input’s geometry was obscured by the FIMMWAVE simulations which ideally showed that the MMI waveguide would continue to work with the pre-proven excitation waveguide geometries of the LC-ARROW platform. However, the simulations do not account for poor fiber coupling to the chip, bad facets, or other non-idealities incorporated during fabrication. Thus, to produce high fidelity excitation spots at the MMI waveguides output, the input mode must be narrow and well confined in the rib structure. The experiments concentrated on the excitation waveguide’s width and etch depth for the excitation waveguides and demonstrated how these parameters directly affected the resulting spot patterns. To accomplish...
this result, a photolithography mask was made with excitation waveguides of varying widths that
coupled into same-sized MMI waveguides (Figure 8-7). Devices could then be made with little
variance in the fabrication steps. After optimizing the basic rib structure, the buried rib waveguide
(BRW) structure was developed and incorporated into the platform [128]. Another optimization
experiment was conducted for the buried structure due to the reduced index contrast 3.9%
compared to the rib structure’s contrast 28.1%. The added cladding (6 μm of SiO₂, \( n = 1.45 \)) widens
the mode of the excitation waveguide. Both experiments for the rib and BRW will be described
together as the experimental procedures were the same.

Rib waveguides with varying widths and depths were fabricated on ARROW layers,
following the Ni Lift-off step described in Chapter 5. Those waveguides used for the buried
waveguide experiment had a 6 μm layer deposited over top of the rib structures (Figure 8-8). The
waveguides were characterized by capturing mode images for each geometry. The waveguide’s
widths were designed to be 2, 4, 6, 8, and 10 μm wide. However, the 8 μm and 10 μm wide
waveguides were too multimodal to obtain useful data and were therefore discarded. Each chip
was fixed to a chip mount, attached to a five-axis stage, and aligned to a 633 nm laser (Thorlabs
S1FC635) which was used to couple light to excite the waveguide’s fundamental mode. The output
at the chip edge was captured by an objective lens and imaged onto a CCD camera, and then the
mode images were recorded. We then calculated the horizontal FWHM for each mode from the
images using a MATLAB script (Appendix C). Simulations of the mode profiles expected for the
various widths and depths were also run using Photon Design’s FIMMWAVE software. The
modes from these single-mode excitation waveguides are self-imaged in the MMI waveguide to
produce \( N \) spots. It is expected that well confined modes with small FWHMs would produce high
fidelity spot patterns, reducing spot overlap and noise in the overall sensor.
8.3.1 Single-Mode Results

Low lithography tolerances inherent in the manual fabrication methods result in variances in the waveguide dimensions. SEM images revealed that for the rib structures, the measured widths $w_e$ were 2.8, 4.9, and 6.7 µm. However, for the $h = 6$ µm etch depth devices, which were constructed on a separate wafer, resulted in narrower widths (2.7, 4.6, and 6.3 µm). This accounts for the narrower data points on the graph at this etch depth (Figure 8-9a). For the rib waveguide structures the data showed that the etch depth plays a large role in the lateral confinement independent of waveguide width. The width expectedly showed that narrower waveguides produced tightly confined modes evident in the narrower waveguides. This statement begins to break down at depths shallower than 3 µm. At these depths, the narrower waveguides do not provide sufficient confinement, resulting in much of the light guiding in the oxide on the side of the rib structure (evident in the top images of Figure 8-9b). Light guiding in the side oxide adds noise between the peaks, killing the spot pattern fidelity.
As the etch depth \( h \) increases, the optical mode’s shape becomes more elliptical and more confined laterally within the area of the rib itself. Increasing the etch depth past 4.5 \( \mu m \) does not show much reduction in the FWHM. Thus, we concluded from this experiment that the optimal etch depth for a rib structure made with 6 \( \mu m \) of oxide is more than 4.5 \( \mu m \), as long as the etching does not reach the ARROW layers. Etching through the SiO\(_2\) to the silicon substrate results in large micropillars, which makes a rough surface that can break off and land on the uncovered waveguide, adding to the scattering loss. Also, etching further increases the plasma etch time substantially. Thus, to improve process time, deeper etches should be avoided. We also confirmed that the optimum width for the excitation waveguides (in order to get narrow, high-fidelity spot patterns) is between 2 \( \mu m \) and 3 \( \mu m \) with our facilities, which is as narrow as the lithography and etching process resolution can provide.

The buried rib results were similar but also interesting. We again concluded that narrow waveguides were prerequisite to producing high-fidelity spot patterns and that there was an etch depth for which one could achieve optimal performance. Interestingly, the gap between high and
little to no confinement is much smaller in the buried structures. The window is somewhere between 3.3 \( \mu m \) and 3.9 \( \mu m \) evident in the mode images for the 2.8 \( \mu m \) wide waveguides and experimental data (Figure 8-10). The experimental and simulated data for the BRWs begins to diverge at heights less than 3.5 \( \mu m \). We attribute this to the reduced index contrast and the microbubbles formed in the cladding over the micropillars. The pillars, formed from the reactive ion etch, cause air pockets between the core oxide and the cladding (Figure 8-8). The roughness in the field on either side of the rib structure could weaken mode confinement for shallower etch depths.

![Figure 8-10](image)

**Figure 8-10**: Experimental and simulated data for BRW structured excitation waveguides FWHM for various width waveguides versus etch depth. (Blue dots = 2.8 \( \mu m \) wide, Black triangles = 4.5 \( \mu m \) wide, Gray squares = 6.5 \( \mu m \) wide, Lines with corresponding shapes and colors are the simulated data of the waveguides with matching widths). Insets – Mode images captured for 2.75 \( \mu m \) wide waveguides of various etch depths, with rib profiles sketched and rib height \( h \) indicated. All data acquired with a semiconductor laser at a wavelength of 633 nm.

In order to confirm that water absorption in the buried rib structure prevented the FWHM of the excitation mode from increasing, an experiment was also performed to monitor the waveguide’s FWHM over time in a controlled environment kept at 60° C and 99% humidity. The
data from this experiment is shown in Figure 8-11. Data points represent an average of 5 FWHMs measured each day. Error bars were experimentally determined to be ~20% by testing the same chip over a period of 10 days while being kept in a “dry” environment. Both the 2 and 4 μm wide waveguides which were monitored do not show an increase in FWHM, confirming that the cladding layer keeps the buried rib waveguides environmentally stable.

Figure 8-11: FWHM of 2 μm (dots) and 4 μm (squares) wide buried rib waveguides monitored over time in a high humidity environment.

8.3.2 Correlation Between Input and Output

In order to characterize the fidelity of the MMI waveguides output pattern, a figure of merit which we call Peak-Valley Difference (a type of normalized Peak-Valley Ratio defined as the normalized intensity difference between a detected peak and an adjoining valley) was calculated for each imaged MMI spot pattern (Figure 8-12). We also measured the FWHM for each individual spot in the pattern and took their average to compare the output’s average FWHM to that of the single-mode inlets. These metrics are used to quantify the fidelity of the MMI waveguide’s interference pattern and verify the input mode is properly self-imaged at the liquid core. Wavelength scans between 430 nm and 670 nm were made to generate different MMI waveguide
spot patterns and find those of highest fidelity. Images were taken at the optimal wavelength, and then the single-mode waveguide was excited and imaged at the same wavelength.

![Image](image_url)

Figure 8-12: Spot pattern intensity plot showing how we calculate the Peak-Valley Difference (P2VD). Dotted blue line represents the average height of the peaks and the dotted pink line represents the value of the averaged valley heights. $P2VD = \text{average(peaks)} - \text{average(valleys)}$

Images of single excitation modes and their corresponding interference patterns at the MMI waveguide output are shown in Figure 8-13a. Each image shows that the self-imaged modes correspond well to the modes at the input. The top two images were taken from rib excitation structures that were 2.8 μm wide with different etch depths, and the bottom image was taken from a BRW 3 μm wide and 5 μm deep. As we can see, the fidelity of the 3 μm etched ribs is worse compared to that of the 5 μm etch depth, and the BRW appears to outperform both rib structures. The average FWHM for the MMI spot pattern of the 3 μm etch depth was 4.5 μm, the 5 μm etch depth FWHM was 2.2 μm, and the BRW’s FWHM was 1.8 μm. The poorly confined mode resulted in a P2VD of 0.76, while the well confined mode (middle image) resulted in a 0.81 P2VD. The BRW (bottom image) resulted in a P2VD of 0.86, truly outperforming the rest. The poorly confined rib (which uses the original etch depth of the basic LC-ARROW) makes it obvious that the wide peak and randomness of the extra light between spots cause the distance between spots to be inconsistent. This inconsistency negatively influences the autocorrelation and the ability to identify
the fluorescently labeled biomarkers, indicating the reason behind the poor patterns of the first few devices constructed.

Further, the synergetic relationship between the mode confinement of the excitation waveguide and the fidelity of the spot patterns can be illustrated with the graphs in Figure 8-13b and c. Figure 8-13b is a graph of the average FWHM of the output peaks across multiple wavelengths, and Figure 8-13c is the P2VD of the same. The 3 μm deep waveguides produce larger FWHMs, which results in poorer spot fidelity evidenced in reduced P2VD. These results conclude that well-confined modes with low water susceptibility will create the highest fidelity spot patterns for highly sensitive diagnostic applications utilizing single-molecule detection.

Figure 8-13: High fidelity patterns correlate to a well confined mode. (a) Comparison of a poorly confined rib excitation mode (top left) and spot pattern (top right) to a well confined rib excitation mode (middle left) and spot pattern (middle right). Also an optimized BRW excitation waveguide (bottom left) and spot pattern (bottom right). Rib excitation waveguides are 2.8 μm wide and 3 μm or 5 μm deep. BRW rib structures are 3 μm wide and 5 μm deep. All MMI waveguides were 100 μm wide. Some of the modes were taken with red LED backlighting which is reflected by the Si substrate. Excitation rib waveguide images were also taken at a higher magnification than the other images. Reprinted with permission from [183, 184]. (b) Average FWHM versus number of spots (which corresponds to wavelength) and (c) P2VD versus number of spots for BRWs with etch depths $h = 3$ μm (orange) or $h = 5$ μm deep (blue).
8.4 Tapered Inputs

The optimized width $w_e$ for excitation waveguides are as narrow as the lithography and etching processes will allow (2 μm - 3 μm). The narrow waveguides pose a constraint on the maximum achievable throughput (Figure 5-2) due to the fiber-to-facet mode mismatch which reduces coupling efficiency [139, 185]. The fiber’s mode is larger than that of the waveguide’s across all the desired wavelengths. For example, a 2.8 μm wide waveguide etched to $h = 5$ μm produces a FWHM of ~1.45 μm where the fiber’s FWHM at the same wavelength produces a FWHM of ~2.25 μm. To overcome this setback, we incorporated tapers into the excitation waveguide design. An experiment was conducted to determine the appropriate taper length $L_T$ and starting width $y$. A mask was made with 6 different taper lengths $L_T$: 10, 50, 100, 500, 1000, and 5000 μm. These lengths were chosen to span a large range with a minimum number of waveguides. Again, the experiment was conducted initially for the rib structured waveguides and then again for the buried rib devices. Rib waveguides and BRWs began at the chip edge with various widths (6, 8, 10, and 12 μm) and then reduced linearly in width down to a 2 μm width along the length of the taper section $L_T$ (Figure 8-14). The design also included non-tapered waveguides varying in width from 2 μm to 12 μm in 1 μm increments to provide a control which compared waveguides that experienced the same fabrication procedures. Wafers with the various tapers, for the rib and BRW variety, were created and throughput measurements conducted. In comparing non-tapered rib waveguides of the same length, waveguides wider than 4 μm were able to achieve throughputs around 30%, whereas the narrow (less than 3 μm) waveguide’s throughput dropped to ~13%. Due to differences in fabrication, the BRW non-tapered comparison resulted in throughputs around 25% for larger waveguides and less than 1% throughput for narrow waveguides. We can expect throughputs somewhere between this range for both the rib and buried rib tapered waveguides.
The buried rib waveguides were expected to require longer taper lengths (which decreases the angle of the taper) in order to obtain decent throughputs because of the lower index contrast between core and cladding [186]. The lower contrast will result in more loss into the cladding due to the reduced mode confinement. The results for the different taper experiments are shown in the graphs of Figures 8-14 and 8-15. Both rib and buried tapers used in these experiments were made with optimized etch depths.

![Figure 8-14: Rib waveguide taper experiment results. Percent throughput versus the log scale of the taper length for various starting widths, ending with 2 μm wide waveguides (gray diamond: y = 12 μm, orange triangle: y = 10 μm, yellow square: y = 6 μm). Taper length $L_T$ is defined as the length between the wider waveguide and the 2 μm waveguide. A larger length gives a smaller taper angle and less scattering loss. Error bars are the experimental error and were found to ~10%. Reprinted with permission from [183].](image)

The rib waveguides with optimal etch depths provide good mode confinement thanks to the oxide-air index contrast. The confinement provided low loss through the taper (Figure 8-14). A tapered 2 μm wide waveguide attains on average a total throughput of 30% compared to the non-tapered ~13%. The data suggests that taper lengths above 100 μm improve the coupling efficiency while still providing the desired narrow FWHM at the MMI waveguide input. Furthermore, the experiment showed that wider input facets allow for improved fiber-to-chip alignment tolerances. From the results of this experiment, new lithography mask designs were created to incorporate
eight-to-two and six-to-two tapers for the rib excitation waveguides used to excite the MMI waveguide.

![Figure 8-15: Buried rib waveguide taper experimental results. Percent throughput verses the log scale of the taper length for various starting widths, ending with 2 μm wide waveguides (gray diamond: y = 12 μm, orange triangle: y = 10 μm, blue dot: y = 8 μm, yellow square: y = 6 μm). Error bars are the experimental error and were found to ~10%. The above image is a top view image of a BRW taper under test, evident is the scattered loss from the taper. Inset – SEM image of the 2 μm facet from a buried tapered waveguide.](image)

The buried rib waveguides have reduced mode confinement due to the low oxide-oxide index contrast. The lower contrast increased taper loss when compared to the rib taper results. We found that the throughput increases exponentially as the length increases, providing for a shallower bend angle (Figure 8-15). The 5000 μm long tapers that were tested only increased throughput to 12 %, which is about half of the maximum throughput attained by the tested non-tapered waveguides on the same chip. This length is too long to incorporate onto the LC-ARROW platform without drastically increasing the current chip’s size. After weighing the cost of chip size constraints and the improvements to be made with increased throughput, it was determined that tapers ~1000 μm in length could be incorporated in the MMI waveguide excitation platform. The improvements in optical throughput from incorporating the tapered excitation waveguides far outweighs the cost of
increased design complexity. Future buried MMI waveguides will incorporate ~1000 μm long tapers to improve the sensitivity of the system.

### 8.5 Optimized MMI Waveguide

After determining the optimized geometries for the excitation waveguides, the devices were finally capable of producing multi-spot patterns across multiple wavelengths with high fidelity (Figure 8-16). With optimized rib MMI waveguides we verified the dependency of length on the resolution of the MMI waveguide—as described by Soldano—as well as the dependency of the number of spots on the fidelity of the pattern [155]. Better P2VD were expected in patterns with fewer spots because they are distributed across the width of the waveguide, so larger numbers of spots tend to have more overlap, decreasing the P2VD. This prediction proved true in the data of Figure 8-16a. Also, the experiment showed improved spot-pattern fidelity for shorter MMI waveguides, as evidenced in the improved P2VD for shorter waveguides with the same number of excitation spots. To obtain the data in Figure 8-16, the same MMI waveguide was tested at different cleaved lengths. After each cleave, the facet was checked to make sure it was clean, free of cracks and debris. Excitation wavelengths were adjusted to produce integer numbers of spot patterns with the highest fidelity at the corresponding length. The excitation waveguides used to excite the MMI waveguide were 4.9 μm wide and etched ~5 μm deep.

We can attribute the improved P2VD for shorter waveguides to the refractive index variations in the guiding layer [173]. Due to the PECVD growth process, the refractive index can vary throughout the film. This is especially true for thicker films, ours being 6 μm [187]. Because the longer MMI waveguides must hold the index tolerance over more material, the fidelity of the spot pattern weakens. From the results in Figure 8-16, we can conclude that shorter MMI
waveguides can produce higher fidelity spot patterns. Unfortunately, shorter MMI waveguide designs are difficult to obtain for the LC-ARROW platform due to the aforementioned constraints (see previous section “Multimode Interference Waveguide”).

![Figure 8-16: Experimental data collected from exciting MMI waveguides at differing wavelengths. (a) Normalized Peak-Valley distance versus number of spots for different MMI waveguide lengths, L, and wavelengths. (b) Left- Side view images taken from the same 75 μm wide MMI waveguide cleaved to L = 1.855 mm. Right- Intensity plots for each corresponding image. Reprinted with permission from [183].](image)

### 8.6 Biomarker Detection Results

The MMI waveguide has proven useful for multiplexed diagnostic tests such as Ebola and Zika virus detection, cancer biomarkers, and short DNA strands. These results have demonstrated the ability to detect multiple targets simultaneously, to increase multiplexing capacity through the use of color combinatorics, and to enhance diagnostic methods through the simultaneous screening of multiple biomarkers generated from the same disease. These results have been published in various journals, but only summaries are presented here [109, 111, 188]. These results were obtained before the spot pattern fidelity of the devices was fully optimized. Preliminary results form the recently optimized MMI waveguides show great promise for applications where higher sensitivity is prerequisite, as is the case in the diagnosis of antibiotic resistance in bacteria where DNA strands are labeled with trace amounts of fluorophores.
8.6.1 Results Prior to Optimization

Single-virus detection assays have been demonstrated in “Optofluidic wavelength division multiplexing for single-virus detection” [109] and “Dual Detection of Zika Virus Nucleic Acid and Protein Using a Multi-mode Interference Waveguide Platform” [188]. In [109], we demonstrated the MMI waveguide’s ability to simultaneously detect differently labeled single-viruses by both individually labeling the target and by labeling with multiple colors for enhanced multiplexed capacity. In [188], we show a dual detection scheme utilizing different biomarkers of the Zika virus, demonstrating the versatility of the MMI waveguide detection scheme. The multiplexed assay results of the single-label experiment of reference [109] are shown in Figure 8-17. For this experiment, three inactivate, whole influenza virus samples were used—β-Propiolactone-inactivated A/PR/8/34 (H1N1), UV-inactivated A/2/Japan/305/57 (H2N2) (Advanced Biotechnologies Inc.), Formalin-inactivated A/Aichi/68 (H3N2) (Charles River). The viruses were labeled individually with three fluorescent labels (NHS-activated Dylight by Thermo Scientific) that fluoresce when excited by 488, 633, and 745 nm wavelengths respectively. The individual, pre-labeled virus solutions were then mixed together and injected into the chip’s liquid core. The MMI waveguide was excited continuously at all three wavelengths to excite the viruses as they passed through the specific number of spots \(N = 9 \@ 488\text{ nm}, N = 7 \@ 633\text{ nm} \text{ and } N = 6 \@ 745\text{ nm}\) to collect the fluorescence trace (Figure 8-17d). The interference pattern generated by the MMI waveguide produced different times between the peaks. Those times could be used to identify the specific viruses as they passed. Events were identified using single-particle autocorrelations, and all but one of the fluorescence burst events (99.2%) agreed with the manual inspection of the signal. The sensor showed the ability to detect three targets simultaneously without the need for fancy timing schemes or multiple detectors. Another experiment was
conducted which showed the ability to increase multiplexing capacity through the use of color combinatorics, which involve labeling one target with multiple colors. This technique can be used to overcome multiplexing limits from the restricted choice of fluorescent dyes and number of achievable MMI spot patterns of varied spot number $N$. The result of adding colors overlays the different spot pattern signals together in the fluorescence trace (Figure 8-17e). The color combinatorics approach demonstrated that the MMI waveguide LC-ARROW biosensor can successfully identify three viruses with two colors.

Figure 8-17: Three-color multiplex virus detection. (a) Representative fluorescence signal emitted by single H1N1 virus excited at 488 nm showing nine distinct peaks spaced by $\Delta t_B$. (b) Corresponding single-particle autocorrelation signal. Multiple peaks are observed at multiples of $\Delta t_B$; dashed line: fit to underlying flow-limited correlations. (c) Single-particle autocorrelation for three virus types after subtraction of flow-based contribution (arrows mark the fifth peak for each virus type). (d) Segment of fluorescence trace from virus mixture excited at all three colors. (e) Two-color combinatorial detection of single viruses. Schematic view of labeling scheme for the three influenza types and their resulting single-virus fluorescence signals; the H2N2 virus shows a mixture of six and nine peaks upon blue and dark red excitation. Reprinted with permission from reference [109].

The flexibility of the MMI waveguide detection scheme is further demonstrated from the results of reference [188]. Here we tackled a unique challenge in early detection of the Zika Virus, namely that antibody detection is insufficient due to cross-reactivity of Zika antibodies with other
faviruses and that different biomarkers are detectable at different stages [189, 190]. To overcome these challenges, we used the MMI waveguide to simultaneously detect for multiple biomarkers like the Zika Virus nucleic acids and protein antibodies which are detectable at different stages of the infection [191]. These different types of biomarkers were labeled with specific fluorescent dyes utilizing a bead-based solid phase extraction method. Once targets were properly isolated, the analyte solution was pipetted into the liquid core and accurately detected in the fluorescence trace. Over 98% of the target biomarkers were identified with high accuracy. These results demonstrated the robustness of the MMI waveguide through the detection of different classes of biomarkers down to a single antigen.

In addition to infectious virus detection we further demonstrated the applicability to disease diagnostics through the early detection of cell-free nucleic acid (CNA) cancer biomarkers. High concentrations of the mutated CNAs, BRAFV600E and NRAS, can be used for early detection of cancer [192, 193]. The BRAF and NRAS single-stranded DNA sequences were labeled with molecular beacons that excite at 633 nm and 750 nm wavelengths, and simultaneously detected with a 96% accuracy. This showed the relevancy of the LC-ARROW platform to blood-based diagnostics and again demonstrated the multiplexing abilities of the MMI waveguide.

Although the aforementioned results are impressive, they were achieved with low fidelity spot patterns. The optimized spot patterns have the potential for even higher sensitivities and more applications. We have attained preliminary results, such as the early detection of antibiotic resistance genes, for such applications when using the optimized MMI patterns (Figure 8-18). Two antibiotic resistant genes NDM and VIM [194] were detected in separate assays utilizing the high fidelity MMI waveguide. These DNA strands and plasmids are challenging to detect with high sensitivity for various reasons, and until now, we have been unable to detect them with high
sensitivity. The improved sensitivity is credited to the optimization of the MMI waveguide device. These results were obtained by implementing a buried rib MMI waveguide with ~ 3 μm wide excitation inputs prior to the incorporation of the tapers. Higher sensitivities are expected with the tapers’ incorporation because there will be less optical loss across the waveguides.

Figure 8-18: The preliminary results acquired from an optimized MMI waveguide. (a) Single DNA detection of 500bp VIM DNA labeled with trace amounts of SYBR gold dye. (b) Single DNA detection of 45kbp NDM (300pM) plasmid stained with 1μM TOPRO. Both fluorescence traces have inserted a zoomed in view of the multi-peak patterns generated by the MMI waveguide.

In conclusion, the MMI waveguide has proven to be a versatile way to improve the multiplexing ability perfect for on-chip bioanalysis, turning the LC-ARROW platform with single-molecule detection sensitivity into a versatile diagnostic instrument. The high sensitivity and selectivity of the platform makes it suitable as a disease diagnostic tool. Further on-chip sample preparation done in less than an hour can be achieved through the integration of a simple microfluidic system previously demonstrated [52].
9 SPATIAL MULTIPLEXING

There are limitations to the target capacity when implementing spectral multiplexing provided by the MMI couplers. The limit is defined by the finite absorption bandwidth of the fluorescence dye labels. With increasing number of targets, labeled with different colored dye, the absorption spectrum begins to overlap, leading to channel cross-talk and, consequently, reduced specificity. Combinatorial multi-dye labeling is one approach to increase the multiplexing capacity and has been proven viable by Ozcelik et. al. in reference [109]. However, it faces similar limits when attempting to scale to increased target numbers. To enhance the ability to detect multiple markers simultaneously, we implemented spatial multiplexing [195].

Spatial multiplexing is the use of the space dimension to transmit independent, separately encoded signals, and it has been widely implemented in multiple-input, multiple-output (MIMO) wireless communication [196]. For our optofluidic system, spatial multiplexing is implemented by adding multiple spatially separated liquid cores connected to individual reservoirs (Figure 9-1). With separate liquid channels, the reservoirs can be filled with diverse biomarkers labeled with the same color fluorophore. Their fluorescence signal can then be detected separately off-chip by individual detectors. Theoretically, an infinite amount of targets can be identified using this method. In practice, however, this arrangement is limited by the cost to implement and by size constraints, as each channel would need its own detector and optical equipment to guide the light off the chip. In order to overcome the added costs of multiple detectors, we integrated spectral and
spatial multiplexing together in what was called the “Triple-Core design”. In this way we improved the multiplexing capacity of the biosensor.

![Illustrative representation of spatial multiplexing.](image)

**Figure 9-1:** Illustrative representation of spatial multiplexing.

### 9.1 Triple-Core Design

The Triple-Core design is relatively complex as it incorporates an MMI waveguide for the target excitation beams with S-curved 3 x 1 Y-couplers for signal collection. We chose to incorporate only three microfluidic channels in order to reduce the overall complexity of the system. Also, more channels would increase the length of the MMI and splitter waveguides, resulting in added optical propagation loss and reduced sensitivity. The additional design complexity is a small cost compared to the improvements it brings in the multiplexing capacity as well as eliminating the need for multiple detectors.
9.1.1 Design Considerations

The MMI waveguide is the key to allowing detection from a single collection waveguide as it supports numerous modes with different propagation constants, allowing them to interfere as they propagate along the structure [155]. At specific distances where the phases of these modes match up constructively, well-defined spot patterns are generated. The equally spaced spot patterns created by the MMI coupler encode all spatial information in the fluorescence signal in the same way it encoded the spectral information. This effect is accomplished through the number of spots \( N \) and through the time-dependence of the signal, as the labeled particles pass. To review, the dependence of the number of spots \( N \) and distance \( L \) is given by:

\[
N \cdot \lambda = \frac{n_c w^2}{L}
\]

where \( w \) is the effective width, \( \lambda \) the excitation wavelength, and \( n_c \) the effective refractive index of the core. The number of spots depends upon the length of the MMI waveguide, which can be utilized to encode the signal so it can be collected at a single detector without confusion. To guide the signal to the detector, a 3 x 1 coupler combines the signals from the three channels into a single collection waveguide, which then guides the light off the chip to a single avalanche photo diode. The efficient collection of signals from all three channels is another important aspect of the design. The S-curve coupler should have optimized dimensions in order to achieve high and equal signal collection efficiency from all three channels. In order to accomplish equal signal collection from the collection coupler, we must consider the intensity of the excitation beam arriving at the core. The intensity will be affected by both the propagation loss across the MMI waveguide and the power spread across the differing number of spots. For example, the summed total of the intensity peaks in channel one will be higher as the light arriving has undergone less propagation loss. However, the peak intensity is spread across the total number of spots. In our design, we made the first
channel have a larger number of spots to equalize the intensity in channel one as compared to channel three.

Other considerations include fabrication constraints, liquid core lengths, reservoir placement, and chip size constraints. The time to etch the liquid cores increases dramatically as the length increases [136]. The added number of bends to the liquid cores also adds more points of stress, increasing the probability of cracking the hollow cores. The reservoirs are placed by hand in our research environment using crystal wax. Unfortunately, this crude technique can be difficult to achieve high precision. Larger chip sizes result in higher propagation loss and lower sensitivity. All of these considerations were addressed as we developed the design.

Initially, we designed two options for the Triple-Core as we were concerned about how the MMI waveguide’s interference pattern would be affected as it crossed the liquid channel. The first design, as seen in Figure 9-2a, avoided having the MMI coupler cross through multiple liquid cores by connecting a 1 x 3 coupler to three separate MMI waveguides of various lengths with a width of \( w = 75 \) μm. The splitter offset the MMI waveguides such that each would intersect the liquid core only at a specific channel inlet. The fluorescence emitted by the target molecule would then be collected by another splitter waveguide. The second design (Figure 9-2b) was a simpler approach that designed a single MMI waveguide to produce a differing number of excitation spots at each liquid channel inlet based on the distance \( L \). This design would require the MMI waveguide to pass through all three channels and to excite the target molecule and its fluorescence signal would then be collected by a Y-coupler. We placed both designs on a single wafer layout so that both could be built and tested in equivalent processing conditions. The initial designs were fabricated with the old PECVD machine with SiO2, \( n_c = 1.46 \). The initial fabricated devices were made here and then sent to UCSC for biological testing and optical characterization. The initial
results were all but desirable. Although both initial designs failed, we learned what was required to fully implement spatial multiplexing on-chip.

![Figure 9-2: Top view images of the initial Triple-Core designs with important features labeled. (a) Design with 1x3 splitter connected to 3 separate MMI waveguides for excitations. (b) Single MMI excitation with intersecting cores design.](image)

### Three Separate Excitation MMI Waveguides

The design, which combined three separate MMI excitation waveguides, incorporated too much loss with the dual Y-splitters. In order to avoid having the MMI waveguide cross through multiple liquid cores, trades in chip size and reservoir placements had to be made but doing so crippled the design. It required placing the reservoirs for the sample inlets between the excitation 1 x 3 splitter waveguide. This required the splitter to incorporate larger bends, which reduced the overall optical throughput of the device. The simulated data shown in Figure 9-3 illustrates that the larger the bend of the S-curve waveguide, the less optical throughput is collected. Light coupled into the splitter excitation waveguide attenuates drastically in the lower and upper legs which have a larger bend. We discontinued this design for future implementations because of the increased
optical loss in the first and third leg and the challenge of accurately placing the reservoirs in between the splitter waveguide.

![Graph showing simulated percent throughput versus the bend of the S-shaped waveguide. The lines are linear fits to the data. Red circles are for curves with 10 mm lengths and blue squares are for 5.8 mm lengths. (Inset) The excitation splitter of the 3 MMI excitation design under test at high optical power.]

9.1.3 Single Excitation MMI Waveguide

The simplicity of the single MMI waveguide made it superior to the first design. The reservoirs were located farther from the waveguides, making it easier to use and allowing the S-curves to have reduced bend offset. The initial fear was that the MMI interference pattern would be negatively affected as the modes propagated through the liquid core. However, initial testing and further simulations proved this to not be the case. We kept this design for the final iteration and optimized its optics and fabrication methods in order to attain desired results.
9.1.4 Final Design

The final design, pictorially illustrated in Figure 9-4 with its channels numbered and components labeled, followed the single excitation MMI design. It was redesigned based on the experiments of the initial design, and simulations were run to optimize its performance. Beam propagation software (FIMMWAVE) was used to design the S-curve waveguide sections to determine their size for least bend loss. Based on these simulations, the split angles of the outmost collection arms were kept small while the waveguide remained short with a minimum offset. The offset was determined by simulating the MMI waveguide such that it could produce $N$ distinct spot patterns at the determined length. These dimensions are included in Table 9-1 for the two spectral designs implemented. Both designs were based off of 75 μm wide MMI waveguides.

Figure 9-4: Schematic of the Triple-Core design under test.

In order to balance the signal strength across all three channels, and by doing so increase detection sensitivity, we underwent thorough analysis of the MMI waveguide and Y-coupler to balance excitation propagation loss and bend loss of the splitter. The propagation loss of the MMI waveguide results in a smaller excitation power in the third channel, as it is the last channel the excitation beam sees. However, as seen in Table 9-1, the number of spots in Channels 2 and 3 are
less than that of Channel 1. The emitted intensity from the fluorescent marker depends on each individual spot’s excitation power. This is determined by dividing the total excitation power at that liquid core by $N$ spots. Since the total power in the third channel is divided amongst fewer spots than that of the first channel, the signal intensity is equal, which compensates for the increased propagation loss through the MMI waveguide. For this reason, the S-curved Y-coupler branches 1 and 3 are kept the same. The second branch is centralized so the S-curve offset is minimal, making bend loss the least for this waveguide. Again, the division of the spots helps to equalize power distribution, making the collected signal balanced across all three channels.

Table 9-1: Design specifications for the final Triple-Core designs implemented.

<table>
<thead>
<tr>
<th>Channel</th>
<th>$L$ (µm)</th>
<th>$\lambda$ (nm)</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1682</td>
<td>745</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>633</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>553</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2261</td>
<td>633</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3380</td>
<td>633</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Results from the initial investigation into the S-curved Y-couplers on the first design showed that uneven collection efficiencies were produced from the different branches. This was unexpected as the signal collected from Channel 1, which receives the least attenuated excitation beam, was producing the lowest intensity. To further investigate, throughput measurements for the Y-couplers were collected after cleaving through the liquid core. Light was coupled via optical
fiber to the single end of the device and collected from the multi-branched side. Branch 1 on average collected about 0.17% of the optical beam whereas Branches 2 and 3 collected 3% and 1.15% respectfully. Branches 1 and 3 were designed with the same split angles and S-curve shape, so similar throughputs were expected. SEM images of the coupler showed that the arms of the split did not diverge in unison, but one arm branched off before the other two (Figure 9-5). This was attributed to the resolution of the SU8 mask used to etch the rib waveguides. To adjust for the poor split divergence in the redesign, the split no longer occurred at a single point. Rather, the design purposely added two split points to controllably resolve the junction. For the final structure, we also stopped using SU8 as the mask for the etch step. Instead the Ni mask as described in the fabrication section of this dissertation was used. Using the Ni mask improved the resolution of the junction and provided the equal collection efficiencies desired from the S-curved Y-coupler in the final design.

Figure 9-5: Top view image of the collection waveguide split junction in the SU8 pattern (Left) and SEM image of the waveguide after etching and removal of the SU8 (Right) for the initial single MMI excitation design prior to optimization.
9.2 Biological Results

After the design and fabrication procedures were optimized for the final design, multiple wafers with 52 devices, 10 x 10 mm in size, were built and sent to our Californian collaborators for biological testing. These devices were built using the un-buried rib structure for the excitation and collection waveguides as described in Chapter 8. To further improve the design, one could redesign and build the devices using the optimized buried MMI waveguide structure to create high fidelity spot patterns and improve the overall sensitivity of the platform. However, with the non-buried rib waveguides, we were able to demonstrate spatial and spatial-spectral multiplexing for a variety of biological targets [188, 195]. For these tests the spot patterns were characterized across all three channels by taking top view images of the MMI excitation pattern as it illuminated quantum dots which filled the liquid core. The patterns with the highest fidelity were found at \( \lambda = 520 \text{ nm} \) and \( \lambda = 633 \text{ nm} \), which are visible in Figure 9-6.

9.2.1 Spatial Multiplexing Demonstration

The ability to spatially multiplex was demonstrated using H2N2 viruses labeled with red fluorophores (Dylight 633). The solution was pipetted into each of the reservoirs and then pulled towards the outlet through negative pressure. As the labeled single viruses traveled through their respective channels and passed the MMI excitation beam, their spatially encoded signals were collected by the Y-coupler and recorded off chip by the APD. Figure 9-7 shows the collected signal. Passing targets were identified by the number of peaks present in the signal: four for Channel 3, six for Channel 2, and eight for Channel 1.

The spatial multiplexing ability opens the door to a variety of new tests panels on a single chip. For example, different biological samples could be placed in the three different channels and
selectively identified using only one excitation wavelength. Another method would be to split a single sample and process it faster by running it in the three channels simultaneously. Or one could simply test three different patients in parallel. The ability to spatially multiplex on a chip is clearly advantageous for single-molecule analysis systems used in disease diagnostics.

Figure 9-6: Images of the excitation patterns in the three different channels across two wavelengths with normalized intensity plot for each image. Reprinted with permission from Scientific Reports (Copyright Springer Nature 2017) [195].

Figure 9-7: Fluorescence signal collected from single H2N2 viruses excited with laser light at $\lambda = 633$ nm. Reprinted with permission from Scientific Reports (Copyright Springer Nature 2017) [195].
9.2.2 Spatial-Spectral Multiplexing

In this dissertation, I have shown the increased capacity to detect biomarkers simultaneously, both by spectral multiplexing using solely the MMI waveguide across multiple wavelengths and by spatial multiplexing across multiple channels. Coupling these together like we intended to in the design is extremely advantageous, as it scales the multiplexing capacity greatly. For example, in our design with three channels and two wavelengths, we could theoretically test for nine pathogens simultaneously, one for each channel and one for each wavelength with an additional three using color combinatorics [109]. This aligns well with current virus infection diagnostic test panels [26, 25]. To demonstrate, we used the Triple-Core devices to simultaneously detect six different influenza strains. Six deactivated virus solutions were prepared: H1N1, H2N2, and H3N2. Each virus strain was labeled with Cy3 fluorescent dye (green), while adenovirus (AV) and Parainfluenz PIV1 and PIV3 viruses were labeled with Cy5 fluorescent dye (red). Three individual solutions were prepared with one green labeled virus and one red labeled virus. The solutions were then separately pipetted into the three channels and pulled to the outlet via negative pressure. Again, as the samples passed the MMI waveguide’s spot pattern, the signal was properly encoded based on the passing, selectively labeled virus. We were again then able to determine which virus had passed based upon the number of peaks and upon the signal processing method as described in Chapter 7 of this dissertation. The results of this test are reprinted in Figure 9-8. The full discussion and experiment can be found in “Scalable Spatial-Spectral Multiplexing of Single-Virus Detection Using Multimode Interference Waveguides” [195].
Figure 9-8: (a) Fluorescence signal trace collected from viruses in the three intersecting channels with each channel containing one virus strain labeled with a green (Cy3) dye, and one virus strain labeled with red (Cy5) dye, and simultaneously excited with wavelengths $\lambda = 525$ nm and $\lambda = 633$ nm. They are subsequently identified uniquely by determining the number of spots in each individual peak. (b) Peak patterns observed for each type of virus strain with the green excitation ($\lambda = 525$ nm) generating $N = 10$, 7 and 5 spots and red excitation ($\lambda = 633$ nm) generating $N = 8$, 6 and 4 spots at channels 1, 2 and 3 respectively. Reprinted with permission from Scientific Reports (Copyright Springer Nature 2017) [195].
9.3 Summary

The Triple-Core design brought spatial multiplexing ability to the basic optofluidic platform as well as the ability to combine spatial and spectral multiplexing. This brings the LC-ARROW optofluidic platform that much closer to becoming a disease diagnostic system based on single-molecule analysis. As shown in Figure 9-8, there is still room to improve the fidelity of the spot patterns in the Triple-Core design. This can be easily done by applying the principles discussed in Chapter 8 of this dissertation to produce high-fidelity spot patterns across multiple colors. This quick optimization will increase the LC-ARROW platform’s multiplexing capacity as three and four colors could be used across multiple channels.
10 ABEL TRAP

At this point the focus of this dissertation switches from optofluidic multiplexing to single-molecule trapping in solution, another important aspect to true single-molecule analysis. The single-molecule trap which is to be discussed for implementation on the LC-ARROW platform is the on-chip Anti-Brownian Electrokinetic (ABEL) trap. Brownian motion is the random movement of particles suspended in a fluid that is the result of their collision with smaller faster moving molecules around them [197]. This trap couples an optical feedback loop to monitor a particle’s location to an electrokinetic force that cancels the Brownian and other drift motion of the molecule in solution [10]. The original idea of the trap can be dated back to as early as 1828 [198]; however, it was not until more recently that ABEL traps have been more widely studied and implemented [9, 199, 200, 201, 202]. In many cases, however, these systems tend to need bulky microscopy equipment to function [203]. Thus, the focus here was not solely on implementing the ABEL trap, but in its integration on-chip with a nanopore gate on a single micro scale optofluidic system. This would enable particle selection and trapping on a single easy to use optofluidic system for rapid analysis of single molecules. My focus has been in the implementation of a two-dimensional version of the ABEL trap based on the LC-ARROW platform. In order to accomplish this, an understanding of the one-dimensional trap had to take place. The on-chip 1D trap had already proved feasible [27], which we redesigned and integrated with the nanopore gate [204]. This chapter briefly overviews optical trapping techniques and specifically those used for single-
molecule analysis, followed by detailed discussion of the integration of the ABEL trap on the LC-ARROW platform for both 1D and 2D trapping. The focus of the work in this dissertation was in the on chip incorporation and optimization of the ABEL trap LC-ARROW optofluidic device. The optical feedback and electrode circuitry used for the trap and the nanopore were developed by our collaborators at the University of California, Santa Cruz (UCSC). Extensive research into the micropore integration for the nanopore functionality was also accomplished by my colleague John Stout. Only an overview of these components will be given as they are important for understanding the design and material constraints on the integration of the 1D and 2D ABEL traps which were my main focus.

10.1 Optical Trapping’s Role in Single-Molecule Analysis

Optical particle traps gained traction in 1970 when gradient forces on micro particles were first reported by Arthur Ashkin [5]. Traps that use the inherent momentum of light for trapping particles are termed direct optical force traps. One such direct force trap that is commonly known and commercially available is the optical tweezers [205, 206]. Optical tweezers utilize radiation pressure from the optical beam which is composed of two forces; scattering which pushes the particle in the beam-propagation direction and a gradient force that pulls the particle into higher intensity beams [5]. In order to trap a particle the system must overcome the scattering force which typically requires high power beams, either with dual divergent beams or with a single laser beam focused by an objective lens with high numerical aperture (NA) [5, 6]. This direct force method typically requires higher optical powers which tend to damage the trapped biological particle [207]. To avoid this indirect optical methods can be used. Indirect methods utilize an optical feedback loop to apply some other external force, such as electric or magnetic forces, to the particle. This allows the trap to work at low optical powers and at the cost of added circuitry. Some
of these traps include optoelectronic tweezers, optomagnetic tweezers and ABEL traps [8, 208, 209]. These traps enable the ability to manipulate single cells and for some, single molecules [7, 210, 211]. For example, Fields and Cohen utilized an ABEL trap to manipulate single 200 nm fluorescent polystyrene nanoshperes to draw a smiley face. With the same trap they held labeled λ-DNA, GroEL and B-phycoerythrin single molecules in place over various periods of time [212].

The standard configuration of such a trap (Figure 10-1) uses a high speed confocal microscope to provide ultrafast scans in real time which are analyzed by custom digital hardware to locate the molecule and provide high precision feedback voltages to the sample which controls the position of the target. Such a system can be expensive and bulky to use requiring experienced operators.

Direct optical trapping techniques and an ABEL trap have previously been demonstrated on the LC-ARROW optofluidic platform [27, 213, 214]. The novelty in the LC-ARROW platform based traps was in their scale and use of ultralow optical power [27]. Low operation power enables the ability to trap a myriad of targets in the nanoscale with less photodamage affects to single molecules. This system also has the advantage that targets do not accumulate in the trapping

![Figure 10-1: Optical Layout of the ABEL trap in a standard configuration. Reprinted with permission from ScienceDirect Methods in Enzymology [212].](image-url)
region. The reduced device-footprint enabled by the on-chip design allows for a compact, economical and portable system. The previously developed LC-ARROW ABEL trap, however, was limited to 1D trapping and it was only capable of analyzing molecules at low throughput rates. Here we worked towards adding a dimension for 2D molecule trapping and integrating a nanopore which enables rapid analysis.

A LC-ARROW based optofluidic trapping platform with integrated nanopore is a high throughput single-molecule approach to analyzing molecular properties [119]. The nanopore is a high-throughput single-molecule detection tool which has also been previously shown without the ability to trap on the LC-ARROW platform [215]. Its integration with the ABEL trap will allow for high-throughput and “true” single-molecule studies [216]. The nanopore will monitor the analyte solution and allow only one select molecule to enter the fluid channel at a time. This molecule will then be trapped and observed while the environment can be altered and controlled for ultimate analysis. This will enable new and exciting discoveries in the biosciences, physiology and analytical chemistry fields thus providing a deeper understanding of molecular functions [217, 218].

10.2 1D ABEL Trap

The original LC-ARROW 1D ABEL trap maintained most of the same structural dimensions as the basic LC-ARROW used for detection. Slight modifications in the platform’s design enable the ability to trap. The 1D ABEL trap adiabatically tapers the collection waveguide from 4 μm at the chip edge to 12 μm at the LC junction [27]. The taper purpose is to suppress mode beating to ensure a well-defined transverse profile which doubles the detection efficiency [214]. Rather than exciting from one side, the ABEL trap excites with two 4 μm wide 3 μm deep rib waveguides.
offset by 2 μm which intersect the liquid core (6 x 12 μm). This provides spatial-temporal light modulation to track the particle as it flows into the ~100 fL excitation volume, providing feedback to the electrodes that secure the particle in the trap (Figure 10-2). This active trapping scheme can provide trapping at low optical powers, ~1μW, and eliminates the need for bulky and expensive high speed cameras which are typically used for ABEL traps [219, 220]. Another advantage is that the trapping regions are precisely defined during the fabrication of the device rather than mid operation of the trap.

A single laser source is used to couple light via optical fibers to both excitation waveguides routed via standard bench top optics. An optical chopper is positioned such that light can be coupled periodically to one fiber at a time depending upon the blade position. When desired the chopper can be replaced with a beam splitter to excite both fibers simultaneously — this is the process for capturing the beams fluorescence profile. The chopper wheel optical setup allows for the fluorescence signal to be temporally modulated, where the speed of the chopper wheel determines the modulation time. The 2 μm offset allows for the temporally modulated excitation
beam to encode spatial information of the particles location. The offset was chosen as it keeps the excited analyte volume small, target molecule’s excitation efficiency high and maximizes the gradient in the position signal. A top view representation of where the excitation beams intersect at the liquid core and the fluorescent profile of the ideal excitation overlap is shown in Figure 10-3a-d. The laser provides a Gaussian beam to the waveguide, as we can see in the profile the beams intersect at half the beam waist, their points of steepest descent. The timing and intensity of the fluorescence event tells the feedback loop where the particle is located and how much voltage is required to push the particle back to the center of the trap. This arrangement cancels drift (from residual pressure flow) and diffusion (Brownian) motion in only the z direction, making it a 1D trap. The timing sequence of Figure 10-3e can be understood as follows: light from only one of the excitation waveguides, a or b, is on at any given time. The fluorescence signal is collected simultaneously as the light is modulated and counts from both beams indicate the location of the particle within the excitation pattern. The signal intensity and time determines the amount of corrective voltage to apply to the electrode in order to maintain the molecule in the center of the trap. This happens in rapid succession and the particle can be maintained at the center of the two beams.

A silver-chloride electrode is placed in each of the reservoirs to provide the electrical voltage which induces the electrokinetic force that manipulates the molecule in solution. Both classes of electrokinetic forces, electrophoretic and electroosmotic, play a role in the trap’s ability to manipulate the target molecules. The total velocity of the particle will be the sum of the electrophoretic drift and electroosmotic flow velocities. This understanding and factors which depend on the target (diameter, charge, mobility) and experimental environment (flow velocity, solution pH level and etc.) unique to each experiment determines the calibration of voltage levels
used to cancel the Brownian motion of the target. To avoid shorting the applied voltage from the liquid to the p-doped silicon substrate, the pre-core step in our fabrication process is used to protect the dielectric ARROW layers under and around the reservoir. This provides an insulator $\sim 1.3 \, \mu m$ thick between the electrode and substrate, which is thick enough to withstand dielectric breakdown at the required voltages for trapping, 1-100V. The voltage applied depends on all the above mentioned experimental variables, but keep in mind that the voltage scales up for decreased target size. For example, a relatively large 1 $\mu m$ bead, only requires between two and five volts.

Figure 10-3: Top view representation of excitation beams and their desired fluorescence profile. (a-c) Excitation beam illustration with top, bottom, and both beams activated. (d) The ideal fluorescence profile to achieve decent trapping, labeled to match excitation beams. (e) Timing diagram example of the top and bottom beam periods with representation of fluorescent signal (FS) used for particle tracking.

10.2.1 Initial 1D Results

The on-chip 1D ABEL trap, as previously described, achieved trapping of single microbeads, for 1 minute, and the DNA of Escherichia coli bacteria (E. coli), 20 seconds [27]. There are biomolecular processes that evolve at these timescales, including large-amplitude conformational changes, protein folding, and complex formations. The trap demonstrated that it was capable of monitoring such processes, and successfully observed the photobleaching process of the labeled E. coli DNA. In addition, the traps’ stiffness was calculated to be 35-140 times
higher than many optical near-field traps at that time. Limitations were also apparent in the trap. The particles, although confined in the \( z \)-direction, freely moved in the \( x \)- and \( y \)-directions to sample the entire cross-section of the waveguide. As the target approaches the channel walls its fluorescence collection efficiency lowers to the point of vanishing and it can no longer be tracked. To overcome the escape routes of the particle without the help of higher powered guiding beams [213], 2- and eventually 3-dimensional versions of the trap should be implemented.

10.2.2 1D Learning Curve

Before attempting to implement a 2D version of this trap, I first attempted to recreate the already proven 1D structure. Lithography masks were redesigned with the same dimensions and some design optimizations, such as lengthened liquid channels for additional reservoir placement and a wider core protection mask during the rib etching step. The optimized design was used to recreate the 1D trapping devices, which were used to test the integration of the nanopore with the trap (Figure 10-4). The addition of the nanopore adds dual modalities for single-molecule identification, which allows for selective particle analysis based on correlations between optical and electrical properties [221, 222, 223]. The confidently identified target analyte will then be trapped, for prolonged observation without compromising its biological function.

Nanopores add an additional voltage/current source (\( V_{NP} \)) to the liquid core channel, for detection and selective gating of the nano/micro particles. Our collaborators from UCSC developed the custom circuitry to control both the nanopore and ABEL trap concurrently with the optical feedback [204]. The nanopore for this device was cut into the oxide by our collaborators at UCSC. First a FEI Quanta 3D FEG DualBeam SEM/FIB milled most of the way through the 6 \( \mu \)m of the top oxide layer leaving a \( \sim 150 \) nm membrane intact. Then nanopores, of diameters \( \sim 20 \) nm
and 1.4 μm, were milled into the membranes on separate chips. This crude but precise method of creating the nanopore can be an excessively long process. In order to provide for large scale fabrication a micropore will be implemented with standard microfabrication steps on future devices. The chips were successful in both matching trapping capabilities of the previous devices and allowing integration with the nanopore [204]. The 1D trap incorporation of the nanopore provided a stepping stone for me to fully understand the development of the 2D chip based version of the ABEL trap as part of the LC-ARROW optofluidic platform.

![Figure 10-4: Schematic of the 1D ABEL trap with nanopore integrated. Top right inset: nanopore SEM image.](image)

### 10.3 2D Trapping Overview

To overcome the challenges inherent in the chip based 1D ABEL trap, namely escape holes within the trap due to lack of confinement in more than one direction, a 2D version was we designed and implemented [224]. The 2D chip-based ABEL trap operates similarly to that of the 1D trap. A single laser beam is coupled via optical fibers to the chip edge, except for the 2D trap there are four fibers with four excitation waveguides (Figure 10-5a) to provide the temporal
excitation sequence. The excitation beams for both vertical and horizontal waveguides offset design follow the same guidelines as the 1D trap, to keep the excitation volume small, target molecule’s excitation efficiency high and maximize the gradient in the positioning signal (Figure 10-5c&d). The offset is again at half the beam waist of the mode, Δx and Δz. The bench top chopper periodically rotates through exciting the four spatially distinct waveguides to track the particle’s location (Figure 10-5e). The fluorescent signal collected at a given time indicates to the microcontroller which of the electrodes to turn on and at what voltages to keep the target confined to the center of the trap.

Although the operation procedures and concepts are the same between the 1D and 2D versions, there are a variety of design and fabrication challenges inherent in implementing the on-chip 2D ABEL trap. Light and fluid must interact in the same excitation volume in such a way to provide accurate tracking and efficient electro-fluidic control. This requires that the single-mode intensity profiles be created with correct lateral offsets at a fluid junction of four separate channels (Figure 10-5c&d). In addition the use of an orthogonal separate collection waveguide is no longer available and one of the “single-mode” excitation waveguides must also be utilized as the collection waveguide, which lowers the collection efficiency. Further four liquid channels, four reservoirs and four electrodes are required. The end goal is to add a nanopore, which adds an additional reservoir, requiring longer liquid channels than that of the 1D version. The increased chip size and waveguide length incorporates more propagation loss and reduces collection and excitation efficiencies. Adjustments, e.g. increased optical power or structural changes for improved throughput, must be made in the design to account for the loss of efficiency. Mechanical stability of the central excitation region must also be kept intact while addressing the optical concerns.
Figure 10-5: Top view representation of excitation beams and the desired fluorescence profile. (a-b) Excitation beam illustration with top, bottom, and both beams activated. (c-d) The ideal fluorescence profiles of both horizontal and vertical excitation beams. (e) Timing diagram example of beam on/off period and how the fluorescent signal will scale at certain periods.

Three different designs were considered for the 2D trap in the LC-ARROW platform (Figure 10-6). The first (Figure 10-6a) utilizes a square shape for the excitation region with both solid core waveguides and liquid channels entering in parallel. This requires liquid and solid core separation wide enough for the excitation waveguides not to prematurely couple light into the liquid core, ~5 μm. For the original 4 μm wide excitation rib waveguides and a reduced 6 μm wide liquid core the minimum square would be 28x28 μm in size. Liquid cores much smaller than 6 μm would provide too much resistance for the trap and severely increase the etch time of the channels. Quickly the idea of a square design was discarded as a square of that size would be difficult for both coupling efficiencies and the structural integrity of the hollow-core. The second idea (Figure 10-6b) utilizes liquid core waveguides to provide the offset excitation beam profile. Initial simulations of this design showed that 6 μm wide LC waveguides at offsets of 2.4 μm would provide the ideal fluorescence profile to track the target. With these dimensions the center square is only 8.4 x 8.4 μm, providing both structural integrity and the potential for good fluorescence
tracking. For these reasons it was the first design attempted. For the purposes of this dissertation it will be referred to as the 2DAT for 2D ABEL trap. The third design (Figure 10-6c) was developed after the 2DAT design showed some major challenges. This design utilized an octagon shape for the center region, thus avoiding the parallel alignment of liquid channels and excitation waveguides. With the liquid inlets coming in on their own edge, or corner of the square, the overall size of the center region could be reduced. This design was called OAT for octagon shaped ABEL trap. This was the second design implemented and it eventually was able to trap target microbeads efficiently. The rest of this chapter will explain the microfabrication and optical challenges of each design. The pros and cons of each will be weighed and discussed followed by the results attained from each 2D design. All designs followed the microfabrication procedures found in Appendix B.

![Figure 10-6: Schematics of three different chip-based 2D ABEL trap designs.](image)

**10.3.1 First 2D ABEL design**

The first design attempted to couple light and liquid into four liquid-core (LC) waveguides which formed a junction at the center (Figure 10-7). In attempts to keep the center region small the liquid core waveguides were chosen to be 6 μm wide. At this size the fluid would maintain laminar flow and the optical mode could be well defined. Simulations showed that an offset of 2.4 μm in the liquid cores would provide the desired fluorescent profile (Figure 10-5) in both the horizontal and vertical directions. Further simulations were conducted to determine the solid core
(SC) dimensions which provided optimal mode matching to the liquid core. From these simulations it was concluded that for LC waveguides 5.7 μm tall by 6 μm wide the best mode match would occur with SC waveguides 5.6 μm tall by 5.6 μm wide. These simulations were initially run for rib waveguide structures (Figure 10-8a) and later for ridge (aka. “through etch”) waveguides (Figure 10-8b), for both structures the 5.6 μm height proved optimal.

Figure 10-7: Schematic of the 2D ABEL Trap (2DAT) design with top view image of the center trapping region. The nanopore reservoir is not shown in this schematic.

To keep optical loss from the LC waveguide low the length of the liquid core was designed 100 μm long, from the center region to the SC intersection. If the LC was kept 6 μm wide from the reservoir to the center region the etch time of the SU8 sacrificial cores would excessively increase due to the resistance of the proximal sidewalls [136]. To avoid exhaustive etch times the LCs were tapered from 12 μm at the reservoir to 6 μm just before the bend into the light confinement arms. To facilitate the placement of all five 2 mm diameter reservoirs the total chip size was designed to be 1 cm² in size. This would give enough room for each reservoir and provide room for cleaving error. A broken facet causes poor mode coupling which ruins the systems tracking capabilities.
The first constructed devices were made with the basic rib structure etched only 3 μm into the guiding oxide. However the problem of water absorption in the guiding layer caused the mode to be pulled up and to the sides which caused the target analyte to fluoresce prematurely, limiting tracking capabilities (Figure 10-9) [125]. This was the first challenge to overcome in producing a decent trap. To eliminate the problem temporarily, the oxide was through etched forming a ridge waveguide structure (Figure 10-8b). This structure maintained the mode confined to the waveguide allowing for light to couple into the liquid core waveguides.

The second and more detrimental challenge we had in creating the 2D trap was in the alignment of the SC to LC (Figure 10-10a). The rib alignment step is manually performed in BYU’s cleanroom facility. This alignment is especially challenging due to the tall features. The high features covered in oxide play tricks on the operator’s perception of the alignment marks used.
to align the features. Further the mask set used for this design was created during heavy construction near the mask writer. This could have caused some of the features to have shifted slightly. Overall even if some devices were “perfectly” aligned many others would not be which reduced the overall microfabrication yield. Misalignment causes discrepancies in the fluorescence profile used to track the target (Figure 10-10b). The extra lobe confuses the tracking feedback system lowering the trap’s stiffness. Misalignment also weakens the mode’s offset which provides the ability to track.

Figure 10-10: (a) Poor SC to LC alignment. (b) Top view image of the negative effect the poor alignment has on the fluorescence profile.

In an attempt to improve the alignment tolerances a new mask was created to allow creating the LC and SC waveguides simultaneously, eliminating the alignment step. With this design SU8 would be used as both the sacrificial core and the SC waveguides (Figure 10-11). The SU8 creates a buried channel waveguide with oxide as the cladding. The SU8 waveguide concept was proven by Giraud-Carrier et. al. for a hollow-core waveguide design which was used for atomic spectroscopy and gas sensing [225]. A gap between the LC and SC provides space for the top oxide to fill in and provide a barrier during sacrificial core etching. In order for the SU8 to be transparent in the visible regime it must be cured at a lower max temperature, less than 200° C [182, 226]. The subsequent oxide deposition also must be kept at a lower temperature. Devices utilizing this design were created following the procedures described by Giraud-Carrier in the
“New Design” chapter of his dissertation [182]. Although they fixed the alignment issue, the design was discarded after initial testing. The guiding capabilities of the SU8 were less than desirable and the structures did not allow for chip reuse. Nanostrip is typically used to clean out the liquid core after a biological test in the research lab. This was no longer possible as the strong acid would etch the waveguides that were now exposed at the cleaved facets. Alignment still remains an issue in the 2DAT design.

To date the 2DAT design has only accomplished trapping in 1-dimension. The fluorescence profiles shown in Figure 10-12 illustrate the challenge in achieving 2D trapping. Only the vertical excitation waveguides provided a suitable offset in the fluorescence profile for target tracking. The horizontal excitation profile had no offset. There are a variety of explanations as to why this is the case: the SC could be poorly aligned to the LC causing coupling to a higher order mode that is not centered in the LC, the facets for the horizontal waveguides could be damaged, the fibers could be misaligned, etc. Whatever the reasons this particular device had a decent vertical fluorescence profile and no horizontal. This device (Figure 10-12) was used in a 1D trapping experiment and the results will follow.

Figure 10-11: (a) Top view image of 2DAT design after SU8 patterning at 5x magnification. (b) Zoomed in (100x) view of the gap between the LC and SC which shows perfect alignment created by the lithography mask.
10.3.2 OAT Design

After the failed attempts of the 2DAT design the OAT design was developed (Figure 10-13), which followed the same fabrication procedures as that of the 2DAT design. This design utilizes an octagon center such that the SC and LC waveguides can intersect at 45° angles to each other. To eliminate the multimode nature of the wider waveguides the excitation waveguides were designed to taper from 5.6 μm at the chip edge to 4 μm at the LC junction (5.6 μm tall). The 4 μm width allowed reuse of the previous 1D design offset, 2 μm (Figure 10-13). Ridge structures were initially implemented but later transitioned to a buried rib structure (Figure 10-8c) to overcome the negative effects of water absorption [128]. Concerns for the future integration with the nanopore arise with the buried waveguide structure, because there is 12 μm of oxide above the liquid core which increases etch time and could reduce the functionality of the nanopore. LC structures used the previously simulated and proven dimensions, 5.7 μm tall by 6 μm wide with taper from 12 μm
starting at the reservoir. The chip size was further enlarged to provide space for multiple re-cleaves in hopes to produce cleaner facets, 13 x 13 mm.

Figure 10-13: Schematic of the octagon shaped 2D ABEL Trap (OAT) design with top view image of the center trapping region built with a buried rib structure.

The main challenge in implementing this design comes from the SU8 lithography process for the sacrificial core. The channels entering the octagon center region cause curvature in walls where the excitation rib waveguides intersect. The added curvature adds additional coupling loss at the juncture, and reduces the collection efficiencies at the cores. Further the LC branches cause the excitation waveguides to couple into the center excitation region from further away, due to the angle of the LC side walls (visible in the top view image of Figure 10-13), this also reduces coupling and excitation efficiencies. To determine if an optimal exposure time would remove the curvature at the junction, an experiment was conducted with the SU8 lithography step on plain silicon wafers. The full SU8 core process, as described in Appendix B, was followed with varying exposure times between 15 and 30 seconds. Under exposed SU8 (~15 or 16 seconds) produced features with smooth curvature and thinner features (Figure 10-14a). Over exposed (more than 18 seconds) SU8, however, produced jagged walls and wider features (Figure 10-14b), which is as expected for negative resists. Many exposure times were used and the optimal time (~17 seconds)
did produce a flatter side wall and the desired LC width (6 μm), however widening still occurred as the branches entered the core (Figure 10-14c).

OAT devices utilizing the buried rib structure produced the best optical results for two dimensional trapping. The optical offset and mode parameters for the same device which was used in the 2D trapping experiment are also shown in Figure 10-15. The experimental offset varied from that of the simulated because of the non-idealities introduced from the LC branch sidewalls and the slight curvature at the junction. Although the offsets do not follow the simulated data they were able to track the particle for a short period of time. The non-idealities resulted in a reduced stiffness of the optical trap.

Figure 10-14: SU8 octagon sacrificial LC structure formed on silicon substrates from (a) underexposure, (b) overexposure (c) optimal exposure.
Figure 10-15: Fluorescence profiles of an OAT device for the horizontal (left) and vertical (right) excitations. (Bottom) Top view image of the OAT device with experimentally determined fluorescence parameters.

10.4 Trapping Results

To prove and compare the functionality of each design (1D ABEL trap, 2DAT and OAT) microbeads were used as a target and trapped under similar conditions for each on-chip ABEL trap. Each design was tested separately with the appropriate feedback loop and control system. To provide optical tracking of the beads, excitation waveguides were excited with $\lambda = 633$ nm from a single HeNe laser (Newport). Laser light was split by multiple 50:50 beam splitters (Thorlabs) and directed to four separate optical fibers which were butt coupled to each waveguide facet. Spatial modulation (on-off) was accomplished using a chopper wheel at an optimal frequency determined by the chip design and number of spatial dimensions trapped (e.g. for the 1D trap a frequency of 1 kHz was used). Fluorescent signal was collected in-plane by one of the four coupled fibers and guided off chip through a one way mirror and spectral filter (Edmund Optics) to the detector. The mirror allowed for simultaneous collection and excitation as the reflected signal would be directed
to the detector while passing the excitation beam to the chip. The filter removes some reflected noise from photoluminescence and the excitation wavelength allowing for efficient photon collection at the single-photon avalanche photodiode (Excelitas). In order to quantify the trap a camera (EM-CCD Andor) was focused on the center excitation region and recorded the trajectory of each microbead while it was trapped. The four silver chloride electrodes were controlled by a feedback amplifier circuit that generates the correction voltage $V_F$ to counteract the microbeads movement. Further electronics were used to synchronize the chopper frequency and corresponding photon counts to extract the spatial information from the counts.

![Figure 10-16](image)

Figure 10-16: Time-dependent x-z trajectory of the of each microbead experiment extracted from the recorded video of the bead.

Solutions of 1 μm microbeads (with electrokinetic mobility of $\mu_e \approx 5.4 \times 10^{-2} \mu$m s$^{-1}$m V$^{-1}$) were injected into one of the channels via its reservoir. Each solution was pre-concentrated to avoid having multiple particles enter the trap simultaneously. Single microbeads diffused into the trapping region and then were trapped for a prolonged time for each device. The results of these experiments are depicted in the x-z trajectory of Figure 10-16. The OAT design was the only
device capable of trapping in two dimensions. However the 1D experiment of the 2DAT design appears to trap two dimensionally, because pressure equilibration was applied in the x direction maintaining confinement while 1D trapping was attempted. The trap stiffness can be calculated by binning the frequency of the particle being located at a position in x or z and then fitting the generated histogram to a Gaussian. The calculated stiffness and time the microbead spent trapped for each device and experiment is shown in Table 10-1. This data reveals the potential of the chip based 2D ABEL traps. Although the performance is still not optimal, the OAT design shows the ability to trap in two dimensions and the 2DAT design can achieve pseudo 2D trapping while maintaining pressure equilibration across the non-trapping axis. The off-chip components still needs fine tuning as evidenced in comparing the 1D 2DAT experimental data with the OAT experimental data. While using the 1D setup (timing sequence and hardware calibrations) higher stiffness was attained for the 2DAT design in both directions when compared to the 2D OAT stiffness. The improved stiffness across both directions can only be attributed to the need for improvement in the trapping setup because optically the OAT designed chip far outperforms the 2DAT chip. After further improvements in the setup it is predicted that the OAT design will achieve superior trapping abilities that can be used for single-molecule analysis.

Table 10-1: Experimental ABEL trap results.

<table>
<thead>
<tr>
<th></th>
<th>1D Basic</th>
<th>1D 2DAT</th>
<th>2D OAT</th>
<th>1D OAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xstiffness (nN/m)</td>
<td>-</td>
<td>27</td>
<td>10.5</td>
<td>-</td>
</tr>
<tr>
<td>Zstiffness (nN/m)</td>
<td>25</td>
<td>26</td>
<td>12.6</td>
<td>-</td>
</tr>
<tr>
<td>Trap Time (seconds)</td>
<td>87</td>
<td>34</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>
11 CONCLUSIONS AND FUTURE WORK

The major contribution in this dissertation was turning the LC-ARROW optofluidic sensor, already capable of single-molecule detection, into a versatile diagnostic instrument. This work brought the ability to multiplex to the sensor, and has proven its ability to detect multiple pathogens simultaneously with high sensitivity. This was accomplished through the implementation of new excitation waveguide designs. The Y-splitter demonstrated the ability to improve SNR by incorporating multi-spot excitation and through the use of a simple signal processing algorithm. The MMI waveguide brought spectral and spatial multiplexing to the sensor by its crossing of one or more liquid cores (Triple-Core design). This work incorporated the new designs and optimized them to achieve highest sensitivity on chip, making them suitable for clinical diagnostics.

Further, this work further demonstrates the LC-ARROW platforms versatility through the design and incorporation of a 2D ABEL trap. Which has the potential to improve single-molecule trapping on a single easy to use optofluidic system for rapid analysis of single molecules. Preliminary 2-dimensional trap results have been demonstrated, and further optimization is needed in order for it to be useful as a rapid analysis system.

11.1 Future Work

Although the work in this dissertation has turned the LC-ARROW sensor into a versatile diagnostic tool there is still more work to be accomplished before it is ready for clinical use. The
optimized MMI waveguides have yet to be implemented on the Triple-Core design. The optimized MMI waveguides will allow for more wavelengths to be used in conjunction with the increased channels and will further the device’s multiplexing capacity, providing for even more targets to be detected simultaneously. In order to reach the multiplexing limits of the LC-ARROW sensor the optimized waveguides need to be implemented with multiple liquid channels.

The current methods for aligning the excitation fibers to the chip are crude and not optimal for a simple to use bench top system. A simple method to overcome these challenges would be to excite the analyte solution from the top rather than the side. This would allow for a “plug and play” system in which the chip could be freely placed in view of a wide area excitation beam. The excitation beam could be generated by an optical grating that produces long and narrow evenly spaced lines. The grating can be designed such that it produces different number of lines dependent on wavelength or multiple color specific gratings could be used. There are a number of companies that make optical lenses that incorporate such gratings, however the cost is rather high for small order quantities which would be required initially to test the concept [#]. The high cost for custom gratings come from the initial cost to design the grating. In order for the top down excitation method to work, a “black out layer” must first be implemented. This layer would prevent the wide area excitation beams from inducing higher background noise into the system through photoluminescence. A higher noise floor can be prevented, by blocking undesired material excitation from the large area beam. Research on such a layer has already commenced, and preliminary results are promising.
REFERENCES


APPENDIX A. LC-ARROW TIMELINE

This appendix gives a timeline overview of the progress made on the LC-ARROW platform in our group between the years of 2002 to 2017. The information comes from a review of the publications during this time frame and from my own experiences after 2013. The goal is to outline design dimension changes, and provide a chronological list of publications to provide future researchers an overview and where to find pertinent information.

2002

• Dr. Hawkins starts working at BYU and PhD Student John Barber started to develop the ARROW fabrication process.

2003 (First conference paper)

   • First ARROW simulations, proposed for on-chip spectroscopy.

2004 (First Publications)

   • ARROW layers are comprised of SiN and SiO2, they are incorporated both on the bottom and top of the liquid core
   • SU8 sacrificial cores are 3.5 μm thick, varying widths 6-50 μm to find optimal dimensions, they are 2 cm long
Hollow cores are covered with ARROW layers and 3 μm thick oxide top capping layer
SU8 core etching is accomplished by a strong acid mixture, H₂O₂ and H₂SO₄ at 85° C
Liquid core optimizations were the focus, solid cores were not added yet
Design principles were outlined and the first report of low-loss propagation in the hollow cores

2005

   - First Al cores are 700 nm thick by 10 to 300 μm wide, used (HCL:HNO₃ aqua regia 55-85C) to etch Al
   - Al cores are patterned using a lift-off step
   - Al etched liquid core topped with ARROW layers and 2 μm thick top cladding layer
   - Al sacrificial cores have deficiencies in side wall roughness and max achievable thickness but etch faster than SU8
   - SU8 cores are now 5 μm thick.
   - Proposed idea of intersecting the hollow and solid cores
   - First demonstration of fluorescence detection on the LC-ARROW platform
   - Introduction of the pedestal to have air on all sides of hollow core
   - Optimization of ARROW layers thicknesses to account for thinner arrow layers on side walls
   - Reflow resist arch shaped cores are also presented (max height ~5 μm use of AZ3330, 9 μm to 15 μm wide)
   - Arch shaped core are optically better and more mechanically stable but not ideal for solid to liquid intersections
• Causes for hollow core structural faults are modeled, simulated and determined experimentally. The optimal width/thickness (w/t) ratios were determined. The ratio should be below 35 to achieve high yield with minimum core cracking. (Analysis conducted for Al cores)

2006


• More published results on arch shaped core
• Proposed planar single-molecule detection on LC-ARROW platform
• Solid cores are 1 μm deep etched into 3 μm ribs that are 15 μm wide, hollow core is 5.8 μm x 12 μm
• The solid core waveguide design causes the rib etch to happen over the solid core

2007


• Experimental demonstration of a hollow core ARROW’s ability to filter the excitation wavelength from the emitted fluorescence wavelength
• Nanopores first incorporated with a nitride membrane
• Top ARROW layers are removed and replaced with a single overcoat (SOC) oxide layer, this improved transmission at liquid to solid interfaces
• Typical hollow core dimensions 10-50 μm wide by 3-6 μm tall
• Nitride was pinpointed as a main background noise contributor
• A hybrid sacrificial core design was proposed with a fast etch and arched sidewalls using aluminum and reflow resist

2008


• Liquid core dimensions given as 5 μm tall x 12 μm wide also 3.5 μm tall × 24 μm wide and 4 μm tall x 10 μm wide
• Still use 1 μm ribs for solid cores
• Equation developed for critical failure pressure of the liquid cores, determined by finite-element analysis
• For optimal channel stability determined ratios for deferent shaped micro channels rectangle w/t = 10, trapezoidal w/t = 25 and arch w/t = 35
• Developed equation for etch length as a function of time for sacrificial cores
• SOC was published again and again, SOC works!
• Top oxide thickness 3 μm for overcoat with ARROW layers and 5 μm tall for SOC

2009


- Detection and analysis of the Qbeta bacteriophage on the single virus level using LC-ARROW
- SC collection waveguide tapered from 4 μm to 12 μm. Improves fundamental mode coupling, doubles detection efficiency of fluorescent particles. It was demonstrated in an all optical particle trap. (This was not maintained in future designs, which begs the question: Why not?)

2010


- 1D planar ABEL trap demonstrated
- Core Exposure step uses reactive ion etching rather than BHF
- Nanopore fully implemented on ARROW platform
- Nanopore utilizes a nitride membrane, micropore etched through ARROW layers thus not implemented with SOC
- Used SU8 3005 for the hollow cores
- Investigated 6 different etch stop materials, chrome, titanium, aluminum, Ni, AZ3330, and SU8 3005 for nanopore membrane
- TC1 recipe of 10:1 (RS6:H2O2) at 40° C, Bosch and Si Isotropic recipes also explained
- Self-aligned pedestal (SAP) recipe was introduced
- SU8 cores reported 5.8 μm tall and 9 μm, 12 μm, and 15 μm wide
- Fabrication yield was over 90% for the SAP ARROWs for waveguides up to 15 mm long for all three above mentioned widths
- ARROW hollow core is 4 mm long
- SU8 core fabrication processes was explained in detail
- Piranha optimized for a SU8 core 4 mm long 5 μm x 12 μm took 120 hours to etch
• Optimized piranha solution 1:1 (H₂O₂:H₂SO₄) at 100° C when changing acid daily
• Hints that previous use of commercial Nanostrip took 6-8 weeks to completely etch out the core

2011


• Spectral filtering of ARROWs demonstrated by tailoring the bottom ARROW layers, a complex frequency response was implemented without complicating the fabrication process
• Ta₂O₅ introduced for ARROW layers replacing SiN
• ARROW layers attempted by sputtering and PECVD
• Dual color FCCS presented on LC-ARROW platform
• Epoxy was used to attach reservoirs
• Ta₂O₅ decreases background noise by a factor of 10 and improve SNR by a factor of 12
• 2 μm deep ribs are etched into 3 μm of oxide, core size 5 μm x 12 μm
• The hollow core loss compared for SiN/SiO₂ samples for Ta₂O₅/SiO₂ samples
• Waveguide loss calculation method introduced using scattered light imaging
• Demonstrated that low temp (< 100° C) SiN could also be used to improve photoluminescence (PL) when compared to earlier ARROW structures made by depositing high-temperature (HT) SiN (250° C), PL background decreases by a factor of 10 when low-temperature (LT) SiN (100° C) films are used
• Some devices still don’t utilize SAP
• SU8 10 used for core
• Core expose procedure still utilize a RIE dry etch process rather than a wet etch
• Selective deposition of dielectrics using lift-off steps
To get the best of both the top cladding layers and SOC transmission at the solid-liquid core interface a hybrid overcoat (HOC) device was proposed. Polymethylglutarimide (PMGI) was used for lift-off mask and SU8 3005 as a cap-on mask. HOC device provides superior transmission to the SOC and TC (top ARROW cladding) devices for hollow-core waveguide lengths longer than 1 mm. Difficulties in fabrication weighed more than the improvement and we no longer make them this way.

2012


Top oxide thickness optimization shows oxide 6-6.5 μm thick is far superior to the old 3 μm thick cladding. The coupling efficiency was improved by a factor of 3.7 and the overall throughput was enhanced by a factor of 17.1 for devices with optimized oxide thickness. Simulations that took real film profiles into account match experimental trends and illustrate the need for good mode alignment at waveguide interfaces. Thickness optimization was conducted on devices with top ARROW layers. Thickness optimization paper points out that SOC device improvement could have been due to the 5 μm thick oxide and not removal of the ARROW layers. Hollow core still 5 μm x 12 μm. Demonstration of how annealing Ta2O5 affects photoluminescence. On-chip spectral filtering can be achieved by selective layer design in the solid core waveguides or by careful design of the liquid-core cladding layer thicknesses.

2013


I started research as an undergraduate in 2013. The rest of this timeline comes from my notes and experiences. Fabrication yields had been low for a couple years. This was mainly due to the SU8 2025 ridge step not working properly. My first summer researching, I ran an SU8 experiments to determine how to improve the process. Longer PEB was a solution and it was determined that
agitation while developing had nothing to do with features floating off. Yields improved after fixing this step.

- As of 2013 we were commercially receiving ARROW layers made with Ta₂O₅
- Core thicknesses are now standard 5-6 μm tall x 12 μm wide
- Ribs are etched 3 μm deep utilizing a somewhat isotropic etch recipe
- We use a wet etch core expose versus the previously reported RIE etch process
- Piranha recipe is (60 mL H₂O₂:40 mL H₂SO₄) @ 130° C rather than the recipe reported previously
- The Self-Aligned Pedestal is considered a must have on all devices
- Many unnecessary or detrimental artifacts were found in the ARROW fabrication recipe from previous student assumptions
- The published work for this year was based solely on integration of a fluidic sample processing device with LC-ARROW chips
- Attempts were made to use 12 alternating dielectrics as the bottom layers to improve throughput and device sensitivity, they showed no significant improvement
- The first splitters and MMI waveguides were implemented with little success
- Devices I made that spring and summer, showed first decent results from UCSC
- These initial results showed less than ideal spot patterns
- MAD series devices were designed and lithography masks were created. The idea of this design was to determine where the best placement of excitation beams and to slightly adjust the MMI widths plus or minus a couple microns to see if spot patterns would improve. It was determined that having the excitation beam closer to the collection waveguide was better. Also it was determined that fabrication tolerances must not be the cause of poor spot pattern fidelity.

2014


My first year as a PhD student was in 2014. The rest of this time line will be quick bullets as most of my dissertation covers the material for the following years.

- We came to the conclusion that water absorption in the top oxide guiding layer is a major contributor to poor performance of the waveguides
• I first attempted to help with water absorption by using silicate SOG
• First designs of 2D ABEL trap
• First Triple-Core devices were made
• We determined that the splitters would work better if we decreased the split angle. I redesigned the splitter mask correcting previous inconsistencies in the design
• I spent some time eliminating redundancies in the recipe and clarifying steps in the recipe, such as bake times for core expose step

2015


• We published bio detection results from “MA13” made in 2013
• Optimization experiment was conducted to show how the split angle affects throughput
• I continued working with SOG, by attempting a siloxane-silicate hybrid overcoat to hopefully show that a filled crevice would improve throughput
• It was determined that SOG did not help with the water absorption problem, and complicated the process too much so scrapped the idea
• Redesigned the Triple-Core mask set with improvements on the S-curve collection Y-couplers
• Redesigned the 2D ABEL traps with better simulation data. Determined that a through etch could eliminate premature fluorescence in ABEL trap.
• Developed a new ridge masking process for the purpose of etching through the full 6 μm of oxide
• Multiple designs and materials were attempted, Ni or Cr with SU8 top, Cr, Ni in varying thickness
• Determined that a sonication lift-off step with just Ni was the best. This also improved side wall roughness and simplified the fabrication process so it was eventually implemented kept for all future devices
• PECVD3 was installed as well as the 3D profilometer. Now we could measure stress in oxide
• Buried channel waveguide (BCW) idea proposed to mitigate water absorption

2016


- Good Triple-Core device (TC7) fabricated with good results
- Finally we started understanding how to improve MMI waveguides
- MMI waveguides without ARROWs and different materials were tested
- Lots of results from un-optimized MMIs waveguides
- First BCW results showed could fix the water absorption issue
- Low stress oxide from PECVD3 enable many of the new designs

2017

- BCWs are good but BRWs (Buried rib waveguides) are better
- Triple-Core device successfully detect 6 targets simultaneously
- MMI waveguide optimized by changing excitation inlet waveguide dimensions
- Redesigned 2D ABEL trap and 8 seconds of trapping achieved with a buried version of the design

It is important to realize that this Timeline is not fully comprehensive nor was it meant to be, but hopefully it helps researchers to find information as they study LC-ARROWs.
APPENDIX B. LC-ARROW FABRICATION PARTICULARS

B.1 2013 Recipe

**Pre-Core:**
PECVD: Deposit 20 nm oxide
E-beam: 120 nm Cr
AZ3330: 5000 rpm, 60 sec
Softbake: 90° C, 60 sec
Expose: 8 sec, no Filter
Develop: 300MIF, ~ 60 sec
Hardbake: 110° C, 2 min
Descum: 100 W, 60 sec
Etch Cr
Clean of resist with Acetone & IPA
Descum: 150 W, 4 min
Dehybake oven

**Core:**
SU8 10: 500 rpm, 6s – 4400 rpm, 60s – 6000 rpm, 2s
Softbake: 65° C, 8 min → 95° C, 8 min (~9 min) → 65° C (~7 min)
Expose: 20.5 sec, with Filter
PEB: 65° C, 6 min → 95° C, 6 min (~7 min) → 65° C (~7 min)
Develop: ~1 min (SU8 Developer)
1st Hardbake: 65° C → 200° C, 15 min (~17 min) → 65° C (~20 min)
Descum: 45 W, 60 sec
2nd Hardbake: 65° C → 250° C, 5 min (~8 min) → 65° C (~23 min)
Check Height: ~5-6 μm
Descum: 45 W, 60 sec

**Pedestal Protection:**
(if necessary) HMDS: 3000 rpm, 60 sec
AZP4620: 2800 rpm, 30 sec – 5000 rpm, 2 sec
Softbake: 70° C, 60 sec; 90° C, 60 sec; 120° C, 20 sec
Expose: 55 sec (normal) + 5 sec (Flood Expose)
Develop: 400k:Water =1:4(25 mL:100 mL)
Check height over core: 2.3 μm or more
Descum: 40 W, 30 sec
Hydrochloric acid dip: HCl:Water = 1:2, (15 mL:30 mL) few seconds
E-beam: Ni, 75 nm
Lift off with Acetone, rinse with IPA
Descum: 30 sec, 50 W

**Pedestal Etch:**
SAP Oxide: target: 1.3 μm
TC1: (RS-6:H2O2 (Hydrogen Peroxide) = 10:1) @55°C, 10 min
Dummy Step
Bosch, target: 6 μm
Si_isotropic, 60 sec
TC1 @55°C, 10 min
Ni off with Ni etchant (~3 min)
TC1@55°C, 10 min
Descum: 50 W, 30 sec
Dehydration bake

**Top oxide coat:** 6000 nm (6 μm)
STD PECVD2 procedure,
Check rate w/ test wafer
then with calc. rate run for calc. needed time
Index: ~1.46
**Ridge:**
SU8 2025: 500 rpm, 6 sec – 3000 rpm
60 sec – 6000 rpm, 2 sec
Softbake: 65°C, 8 min → 95°C, 8 min (~9 min) → 65°C (~7 min)
Expose: 15 sec, no Filter
PEB: 65°C, 6 min → 95°C, 6 min (~7 min) → 65°C (~7 min)
Develop: 5 min (do not agitate!)
Hardbake: 65°C → 180°C, 10 min (~12 min) → 65°C (~18 min)
Descum: 100 W, 45 sec
Check height of core: target < 18.5 μm

**Ridge Etch (Trion):**
SiO₂-Anisotropic, target 3000 nm (half of top oxide)
Rate (~7.21 nm/sec)
Etch ~416 sec
Nanostrip: 30 min, 90°C (to strip SU8) rinse w/ water
Descum: 100 W, 60 sec
Dehydration bake

**Core Expose:**
AZP4620: 1500 rpm, 60 sec
Softbake: 80°C, 20 min
Wait for 30 min @ room temperature
Expose: 30 sec, soft contact 0 sec, no Filter
Develop: 400K:water = 1:4 (~2 min 20 sec)
Hardbake: 150°C, 2 hr
Descum: 150 W, 60 sec
BHF etching usually do for ~490 sec
then check with microscope to make sure oxide is gone around the tip of core

**SU-8 Core Etching:**
Piranha: 60 mL H₂O₂ + 40 mL Sulfuric Acid @ 130°C
After 1 hr, check for defects
Change piranha everyday
check cores in microscope
When it is done etching:
Water (1 day) → Nanostrip (1 day) → Water (1 day) @ room temp

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**Figure B-1:** Example of what to look for when checking the sacrificial cores during the etching process.

My first year of research we experienced many issues with successful competition of the SU8 Ridge step. Experimentally I determined that the recipe should follow the below steps rather than those listed on the previous column. Following the below procedure resulted in less feature distortion and liftoff. The fixed step provides desired crisp waveguides without residue in the field.

**Ridge Fixed:**
SU8 2025: 500 rpm, 6 sec – 3800 rpm
60 sec – 6000 rpm, 2 sec
Softbake: 65°C, 6 min → 95°C, 8 min → 65°C (~7 min)
Expose: 17 sec, no Filter
Adjust exposure time to get correct feature widths after measuring in 3D profilometer.
PEB: 65°C, 7 min → 95°C, 9 min (~10 min) → 65°C (~7 min)
Develop: ~1.5 min (agitare properly)
Hardbake: 65°C → 180°C, 10 min (~12 min) → 65°C (~18 min)
Descum: 100 W, 120 sec
Check height of core: target < 18.5 μm
B.2 2018 Recipe

**Pre-Core:**
PECVD: Deposit 20 nm oxide  
E-beam: 70 nm Cr  
AZ3330: 5000 rpm, 60 sec  
Softbake: 90º C, 60 sec  
Expose: 8 sec, no Filter  
Develop: 300MIF, ~ 60 sec  
Hardbake: 110º C, 2 min  
Descum: 100 W, 60 sec  
Etch Cr  
Clean of resist with Acetone & IPA  
Descum: 150 W, 60 sec  
Dehybake ~15 min

**Core:**
SU8: 500 rpm @ 100 rpm/sec, 6sec – ~4000 rpm @ 1200 rpm/sec, 60sec – 6000 rpm @ 6000rpm/s, 2 sec  
Softbake: 65º C, 8 min → 95º C, 8 min (~9 min) → 65º C (~7 min)  
Expose: ~17 sec, with Filter  
PEB: 65º C, 6 min → 95º C, 6 min (~7 min) → 65º C (~7 min)  
Develop: ~1 min (SU8 Developer)  
Check Height: ~6 μm  
1st Hardbake: 65º C → 200º C, 10 min (~12 min) → 65º C (~20 min)  
Descum: 50 W, 60 sec  
2nd Hardbake: 65º C → 250º C, 5 min (~8 min) → 65º C (~23 min)  
Check Height: ~6 μm  
Descum: 50 W, 60 sec

**Pedestal Protection:**
AZP4620: 2800 rpm, 30 sec – 5000 rpm, 2 sec  
Softbake: 70º C, 60 sec; 90º C, 60 sec; 110º C, 30 sec  
Expose: 55 sec (normal) + 7 sec(FloodExpose)  
Develop:400k:Water =1:4 (25 mL:100 mL)  
Check height over core: between 1.8 μm and 2.7 μm  
Descum: 50 W, 30 sec  
HCL dip: (HCl:Water = 1:2) quick dip  
E-beam: Ni, 75 nm  
Lift off with Acetone, rinse with IPA  
Descum: 30 sec, 50 W

**Pedestal Etch:**
SAP Oxide: target: 1.3 μm  
TC1: (RS-6:H₂O₂, 10:1) @55º C, 10 min  
Dummy Step  
Bosch, target: 5-6 μm  
Si_isotropic, 60 sec  
TC1 @55º C, 10 min  
Ni off with Ni etchant (~3 min)  
TC1@55º C, 10 min  
Descum: 50 W, 30 sec  
Dehydration bake

**Core Oxide coat:**
~6000 nm (6 μm) SiO₂  
PECVD3: 0 Stress 1.51 index  
Temp 250 ºC  
Index: ~1.51  
Placed in Dehybake
**Ridge:**  
AZ3330: Poor ~3 in circle of resist on wafer then spin  
1000 rpm @ 1200 rpm/sec, 60 sec → 6000 rpm @ 6000 rpm/sec 1 sec  
Softbake: 90° C, 7 min  
Expose: 30s (without filter)  
Develop: 300K: ~1 min  
Checked under scope in lithography room  
Flood Expose: 1 sec  
Develop: 300k: ~15 sec  
Checked under scope and then in 3D profilometer  
Descum 100 W, 60 sec  
HCL dip  
Ebeam: 100 nm Ni  
Sonicate in acetone ~1 min then rinse with IPA  
Descum 100 W, 60 sec  

**Rib Etch (Trion):**  
SAP-Oxide, target between 4 μm and 5 μm (usually ~2200 sec of etch time)  
Dummy Step  
Bosch: 3-5 steps  
TC1: 10 min  
Ni Etch ~10 min (check to make sure Ni removed)  
TC1: 10 min  
Descum: 50 W, 30 sec  
Dehybake or Begin the Bake Out step  

**Rib Bake out:**  
Clean Oven 1: Place in at 70° C and ramp up to 300° C @ 4° C/min  
Hold at 300° C for at least 12 hours  
Ramp back down to 150° C  
Do this step immediately before cladding growth, remove wafer from oven and place directly into PECVD3  

**Cladding Oxide coat:**  
~6000 nm (6 μm) SiO2  
PECVD3: Steve Low Stress Low Index  
Temp 250 °C  
Index: ~1.45  
Placed in Dehybake  

---  

**Core Expose:**  
AZP4620: 1500 rpm, 60 sec  
Softbake: 80° C, 10-15 min  
Expose: 30 sec, soft contact 0 sec, no Filter  
Develop: 400K:water = 1:4 (until features look clean)  
Hardbake: 100° C, 1-2 hr  
Descum: 150 W, 60 sec  
BHF etching usually for ~700 sec  
Check under microscope to make sure oxide is gone around the tip of core  

**SU-8 Core Etching:**  
Piranhna: 60 mL H₂O₂ + 40 mL Sulfuric Acid @ 130° C  
Change acid every day, remove wafer from old acid and place immediately in new acid (do not let dry)  
Check cores in microscope, if need to place back in acid, fill hollow cores with DI water first to reduce core cracking  
When the wafer is done etching:  
Water (a few hours) → Nanostrip (1 day) → Water (a few hours) @ room temp  

---  

Figure B-2: Picture of a wafer mid process.
APPENDIX C. MATLAB CODE

All of the MATLAB functions below could be called from another script to automate the process by running calculations for multiple images.

C.1 Splitter Throughput Analysis

This function was used to calculate total optical power in the waveguide. This calculates the total percentage of light in the facet as compared to the light guiding in the underlying oxide or scattered away from the waveguide. The percentage calculated was then uses to adjust the measured throughput of that waveguide.

```matlab
function [PercentInFacet] = ThroughputinFacetAnalysis(picName)
close all;
clic;
t=12;
I = imread(picName);

I = rgb2gray(I);
%   imtool(I);

Is = size(I);
roww = Is(1);
colw = Is(2);

[K, rect] = imcrop(I);
rect(1) = rect(1) + 670;

J = imcrop(I, rect);
imshow(I);

figure;
subplot(2,1,1);
imshow(K);
imshow(J);
```

```matlab
subplot(2,1,2);
imshow(J);
```
Ks = size(K);
row = Ks(1);
col = Ks(2);

% convert uint8 to a double
K = double(K);
J = double(J);
I = double(I);
%

% Sum of intensities in left image filtering out noise pixels
countl = 0;
leftI = 0;
for i=1:row
    for x=1:col
        if K(i,x) > t
            leftI = leftI + K(i,x);
countl = countl + 1;
        end
    end
end

% Sum of intensities in right image filtering out noise pixels
rightI = 0;
countr = 0;
for i=1:row
    for x=1:col
        if J(i,x) > t
            rightI = rightI + J(i,x);
countr = countr + 1;
        end
    end
end

% Sum of intensities in whole image filtering out noise pixels
wholeI = 0;
countw = 0;
for i=1:roww
    for x=1:colw
        if I(i,x) > t
            wholeI = wholeI + I(i,x);
countw = countw + 1;
        end
    end
end

subtracted = wholeI-leftI-rightI;
PercentInFacet = (leftI+rightI)/wholeI;
C.2 Splitter Split Ratio Calculator

Function used to determine the split-ratio of the Y-splitter waveguides based off of the captured image of the modes from both legs.

```
function [PercentThroughputLeft, PercentThroughputRight] = SplitterThroughputAnalysis(picName)
    close all;
    clc;

    I = imread(picName);

    I = rgb2gray(I);
    % imtool(I);

    [K, rect] = imcrop(I);
    rect(1) = rect(1) + 610;
    J = imcrop(I, rect);

    subplot(2,1,1);
    imshow(K);
    subplot(2,1,2);
    imshow(J);

    Ks = size(K);
    row = Ks(1);
    col = Ks(2);

    % convert uint8 to a double
    K = double(K);
    J = double(J);

    % Sum of intensities in left image filtering out noise pixels
    countl = 0;
    leftI = 0;
    for i=1:row
        for x=1:col
            if K(i,x) > 8
                leftI = leftI + K(i,x);
                countl = countl + 1;
            end
        end
    end

    % Sum of intensities in left image filtering out noise pixels
    countr = 0;
    rightI = 0;
    for i=1:row
```
for x=1:col
    if J(i,x) > 8
        rightI = rightI + J(i,x);
        countr = countr + 1;
    end
end
end

PercentThroughputLeft = leftI/(leftI+rightI)*100;
PercentThroughputRight = rightI/(leftI+rightI)*100;

C.3 FWHM Calculator

This function calculates the horizontal and vertical FWHM of a waveguide from a side view image of the confined mode.

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

% Calculates the horizontal and vertical FWHM of a waveguide based on a side view image
conversion = .039041;  %um/px
% determined by SEM measurements and side view image of facet
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;
end

% 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)]);