An Integrated Model of Optofluidic Biosensor Function and Performance

Joel Greig Wright, Jr.
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An Integrated Model of Optofluidic Biosensor Function and Performance

Joel Greig Wright, Jr.

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

An Integrated Model of Optofluidic Biosensor Function and Performance

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Optofluidic flow-through biosensor devices have been in development for fast bio-target detection. Utilizing the fabrication processes developed by the microelectronics industry, these biosensors can be fabricated into lab-on-a-chip devices with a degree of platform portability. This biosensor technology can be used to detect a variety of targets, and is particularly useful for the detection of single molecules and nucleic acid strands. Microfabrication also offers the possibility of production at scale, and this will offer a fast detection method for a range of applications with promising economic viability.

The development of this technology has advanced to now warrant a descriptive model that will aid in the design of future iterations. The biosensor consists of multiple integrated waveguides and a microfluidic channel. This platform therefore incorporates multiple fields of study: fluorescence, optical waveguiding, microfluidics, and signal counting. This dissertation presents a model theory that integrates all these factors and predicts a biosensor design’s sensitivity. The model is validated by comparing simulated tests with physical tests done with fabricated devices. Additionally, the model is used to investigate and comment on designs that have not yet been allocated time and resources to fabricate. Tangentially, an improvement to the fabrication process is investigated and implemented.

Keywords: optofluidics, single molecule detection, integrated optics, ARROW, fluorescence, biosensor, lab-on-a-chip, microfluidics, model, FDTD
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I have not come to this mark on my own. I began my doctoral studies at Brigham Young University at the invitation of Dr. Aaron Hawkins, my advisor and committee chair, while I was concluding my undergraduate studies at Arizona State University. He has provided direction and mentorship invaluable to my work, and helped secure funding for my education and research. Dr. Hawkins’s interest and patronage made my doctoral studies possible. I would also like to acknowledge and thank my doctoral graduation committee for their insight and guidance.

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# TABLE OF CONTENTS

ABSTRACT .................................................................................................................................... ii  
ACKNOWLEDGEMENTS ........................................................................................................... iii  
TABLE OF CONTENTS ................................................................................................................ v  
LIST OF TABLES ....................................................................................................................... viii  
LIST OF FIGURES ....................................................................................................................... ix  
CHAPTER 1.   INTRODUCTION .............................................................................................. 1  
CHAPTER 2.   FLOW-THROUGH SENSING ORIGINS ......................................................... 4  
   2.1   A Brief History of Flow Cytometry ................................................................................. 4  
   2.2   Typical Flow Cytometry Operation .............................................................................. 6  
   2.3   Transition from Flow Cytometry to Optofluidic Flow-Through Biosensing .............. 8  
CHAPTER 3.   ARROW BIOSENSOR OPERATION ............................................................... 9  
   3.1   Overview of Biosensor Structures and Functions ........................................................ 9  
   3.2   Single-Mode vs. MMI Excitation and Signal Analyses ................................................ 12  
CHAPTER 4.   BIOSENSOR FABRICATION ........................................................................ 15  
   4.1   ARROW Layer Deposition ............................................................................................ 15  
   4.2   Sacrificial Channel Core ............................................................................................. 17  
   4.2.1 Fabrication for 2DHF Core Variation ....................................................................... 20  
   4.2.2 Fabrication for 3DHF Core Variation ....................................................................... 20  
   4.3   Pedestal Etch ................................................................................................................. 22  
   4.4   Silicon Dioxide Growth by PECVD ............................................................................. 24  
   4.5   Solid Ridge Waveguide Etching .................................................................................. 26  
   4.6   Silica Cladding Growth ................................................................................................ 26  
   4.7   ARROW Core Etch-Out ............................................................................................... 27  
CHAPTER 5.   SURFACE ROUGHNESS EFFECTS ON WAVEGUIDE LOSS ................... 29  
   5.1   Ridge Waveguide Etch Process Overview ................................................................. 29  
   5.2   Microstructure Residues Origins ................................................................................. 31  
   5.3   Wet Etch Supplement ................................................................................................... 32  
   5.4   Optical Characterization............................................................................................... 34  
   5.4.1 Optical Transmission Measurement ........................................................................ 34  
   5.4.2 Propagation of Uncertainty Calculation .................................................................... 37  
   5.5   Comparison of Waveguide Losses ............................................................................. 38
CHAPTER 6. EXCITATION REGION THEORY ................................................................. 43
6.1 Fluorescence and Signal Rates ............................................................................. 43
6.2 Fluorophore Absorption Cross-Section ............................................................... 47
6.3 Optical Intensity, Incident Photon Rate, and Maximum Fluorescence Photon Emission Rate 50
6.4 Exciting Optical Intensity Profile and Accumulating Incident Energy ............... 52
6.5 Flow Velocity and Time Profiles in the ARROW Channel .................................. 54
6.6 Fluorescence Generation and Collection ............................................................. 61
CHAPTER 7. MODEL CONSTRUCTION AND SIMULATION ......................................... 66
7.1 Fluorescing Particle Selection and Model Mesh Configuration ......................... 66
7.2 Optical Intensity Profile ..................................................................................... 67
  7.2.1 Side-Illuminated Modes .................................................................................. 67
  7.2.2 Top-Illuminated Windows ............................................................................. 69
  7.2.3 Summed-Power Vector ............................................................................... 70
7.3 Fluid Velocity Profile and Flow Time Profile ...................................................... 71
7.4 Incident Energy Profile ..................................................................................... 72
7.5 Collection Efficiency Profile ............................................................................. 73
7.6 Excitation Region Profile .................................................................................. 75
7.7 Excitation Region Sampling and Signal Simulation ........................................... 76
CHAPTER 8. VALIDATION: COMPARING THE MODEL TO EXPERIMENTS ............. 79
8.1 Three Biosensor Designs ................................................................................... 79
  8.1.1 Standard ARROW and Excitation Ridge ...................................................... 79
  8.1.2 “Three-Micron” ARROW and Excitation Ridge .......................................... 80
  8.1.3 “Sandwiched” Excitation Ridge ................................................................. 81
8.2 Testing and Simulating the Three Designs ......................................................... 83
  8.2.1 Standard Design ......................................................................................... 84
  8.2.2 Three-Micron Design ............................................................................... 87
  8.2.3 Sandwich Design ....................................................................................... 88
8.3 Discussion of Experiment/Simulation Comparisons ........................................... 90
CHAPTER 9. PROJECTING FUTURE DESIGNS ............................................................ 93
9.1 Validating the Top Illumination Format .............................................................. 95
9.2 Design Combinations ....................................................................................... 98
  9.2.1 Preliminary Information .............................................................................. 98
  9.2.2 Side-Illuminated Designs ........................................................................... 99
LIST OF TABLES

Table 5-1: Plasma Etch Process Parameters ................................................................. 31
Table 5-2: Loss and Loss Error Results for each Waveguide Category .................... 39
Table 7-1: List of Model Parameters used in Chapter Continuing Example ............ 77
Table 8-1: Design Simulation and Test Parameters ..................................................... 84
Table 8-2: Signal Statistics of Simulations and Physical Test Results ...................... 90
Table 9-1: Excitation Region Cross-Sections and Estimated Test Times ............... 99
Table 9-2: General Model Parameters ..................................................................... 99
Table 9-3: Signal Statistics of Pre-Optimized Biosensor Design Combinations .... 103
Table 9-4: Signal Statistics of Side/3DHF Optimization Parameter Sweeps ............ 108
LIST OF FIGURES

Figure 2-1: Operation of a flow cytometer. The cell sample passes through an exciting laser on the left. On the right are multiple photomultiplier tubes functioning as optical detectors. The PMTs are dedicated to detecting either forward scatter (FSC), fluorescence (FL), or side scatter (SSC) signals. The signals are then compiled and used to identify the cell sample. (Image is in the public domain, https://commons.wikimedia.org/wiki/File:Cytometer.svg).. 7

Figure 3-1: Illustration of biosensor operation. A fluid sample flows from one reservoir to another in the channel shaded blue. An exciting optical mode (x-axis) intersects the channel, generating fluorescence as a signal. This signal is then guided down the channel and couples into the collection ridge waveguide (z-axis) for off-chip measuring and analysis. (© IEEE/OSA 2021 [8]) ........................................................................................................ 10

Figure 3-2: Photograph overview of the integrated components of the biosensor chip. The ARROW channel runs diagonal, briefly running horizontal in the center. The collection ridge waveguide couples with the ends of the horizontal section of the ARROW. Vertically, the single-mode waveguide (left) and multi-mode interferometer (MMI) (right) intersect the ARROW. The single-mode waveguide provides a single excitation region, and the MMI allows multiple excitation regions for signal multiplexing. .......................................................................... 10

Figure 3-3: Example of (a) simulated signals across test time and (b) the distribution of these signals. (© IEEE/OSA 2021 [8]) ................................................................................................................ 12

Figure 4-1: Illustrated demonstration of anti-resonance reflection in the ARROW layers. (Image is in the public domain, https://commons.wikimedia.org/wiki/File:ARROW_waveguide_example.png)................ 16

Figure 4-2: First stage of the biosensor’s fabrication has ARROW layers sputtered onto bare silicon. (© 2021 MDPI [9]). .......................................................................................................................... 17

Figure 4-3: SU8 sacrificial core on the ARROW layers after patterning and development. (© 2021 MDPI [9]) .............................................................................................................................. 19

Figure 4-4: Sacrificial core for 2DHF-designed devices. Two branches intersect the main line at angles, which form inlet channels for buffer fluid to enter the main channel. (© 2021 MDPI [9]) ................................................................................................................................ 20
Figure 4-5: An initial trench is etched into the wafer ARROW layers and silicon substrate prior to SU-8 application. (© 2021 MDPI [9]) ................................................................. 21

Figure 4-6: Two SU-8 steps are done to form a channel where buffer fluid will eventually focus the sample stream horizontally and vertically. (© 2021 MDPI [9]) ......................................................... 22

Figure 4-7: The pedestal for pending waveguides is etched into the ARROW layers and silicon via RIE and DRIE, respectively. (© 2021 MDPI [9]) ................................................................. 24

Figure 4-8: A silica layer is deposited on the biosensor device via PECVD. (© 2021 MDPI [9]) ........................................................................................................ 25

Figure 4-9: Ridge waveguides are etched via RIE into the silica layer previously deposited. (© 2021 MDPI [9]) ........................................................................................................ 26

Figure 4-10: Completed biosensor with the silica cladding layer deposited and SU-8 sacrificial core etched out. (© 2021 MDPI [9]) .......................................................................................... 28

Figure 5-1: Summary illustration of the ridge etch process. (a) The process begins with ARROW layers on a silicon substrate. (b) Silica with a 1.51 refractive index is grown over the ARROW layers. (c) A nickel mask is applied to the silica by photolithography. (d) The silica is etched by RIE. (e) The nickel mask is stripped. (f) A silica cladding with a 1.46 refractive index is grown over the ridge. (© 2020 IEEE/OSA [7]) .............................. 30

Figure 5-2: Microstructure formations under study. (a) Nanotubes, (b) “grass”, and (c) “stalks”. (© 2020 IEEE/OSA [7]) ........................................................................................................ 32

Figure 5-3: Microstructure residue after being treated with 5% HF. (a) Nanotubes, (b) grass, and (c) stalks. (© 2020 IEEE/OSA [7]) .......................................................................................... 33

Figure 5-4: (a) Cross section schematic of etched ridge waveguide with microstructures in the field and cladding grown over. (b) SEM image of ridge cross section with microstructures present under cladding. (c) SEM image of ridge cross section that has been treated with 5% HF prior to cladding and featuring a clean interface between ridge core and cladding. (© 2020 IEEE/OSA [7]) ...................................................................................... 34

Figure 5-5: Mode image of a 10µm ridge waveguide that has been aligned in the objective lens and captured with a CCD camera. ............................................................................. 36

Figure 5-6: Top-down image of a fiber aligned with a ridge waveguide on a sample chip, guiding light from the laser source to the chip facet. ........................................................................ 36
Figure 5-7: An example of a semilogarithmic plot of waveguide transmissions with respect to waveguide length. The transmissions are plotted as red Xs on a logarithmic y-scale and a linear x-scale. An exponential curve is fitted to the plotted points, which appears as a straight line on the semi-log plot. The exponential slope is taken to be the waveguide loss.

Figure 5-8: Waveguide transmission plot with error accounted. The solid line is the transmission with the fitted loss slope, and the dashed lines are the transmissions with the error added and subtracted from the fitted slope. (© 2020 IEEE/OSA [7])

Figure 5-9: Comparison of waveguide modes in (a) a 3-µm wide waveguide that was not treated with 5% HF and (b) a 3-µm wide waveguide that was treated with 5% HF. These ridges were etched by the O2/CHF3 “Half Power” process. (© 2020 IEEE/OSA [7])

Figure 6-1: Jablonski diagram of the fluorescence process. An exciting photon is absorbed, exciting an electron from a ground state to a higher energy band. The electron then relaxes back across the bandgap and returns to a ground state. This relaxation emits another photon of slightly lower energy than the exciting photon. (Image is in the public domain, https://commons.wikimedia.org/wiki/File:Jablonski_Diagram_of_Fluorescence_Only.png)

Figure 6-2: Illustration of the relation between the incident photons (a) on a fluorophore, Cy5 in this example, and the fluorescence photons (b) emitted by the fluorophore. Knowing the maximum emission rate, we can calculate if the incidence rate approaches that limit when we calculate the statistically-derived absorption cross-sectional area, represented by the dashed line (c). (This figured is a modified version of an image in the public domain, https://commons.wikimedia.org/wiki/File:Cy3_Cy5_dyes.gif)

Figure 6-3: Illustration of the Beer-Lambert Law. As light with an intensity I₀ passes through a medium of thickness d, the intensity will reduce to I(x=d).

Figure 6-4: An illustration of how optical intensity profiles, shown as large red circles, relate to the fluorescing particle’s cross-section, shown as small black circles. (a) If an intensity profile is uniform, the rate photons incident on the particle is constant regardless of the particle’s position within the illuminated region. (b) If an intensity profile is nonuniform, the rate of photons incident on the particle will vary with the particle’s position. Area
resolutions are constructed to be the smallest rectangular area that encapsulates particle
cross-section. ............................................................................................................................. 51

Figure 6-5: Illustration of the excitation region orientation to follow for the remainder of this
work. The width of the ARROW channel is oriented with the x-axis, and the height with the
y-axis. The fluorescing particles will travel down channel along the positive z-axis and
across the optical intensity profile. (© 2021 MDPI [9])......................................................... 53

Figure 6-6: Illustration of a parabolic flow velocity profile developing in a laminar flow regime
across the distance of a fluid duct. The arrows represent fluid flow velocity, and they
gradually increase in magnitude closer to the center as the fluid develops. The fluid
eventually becomes fully developed at which point the velocity profile attains a steady state;
the distance for this profile to fully develop is the entrance length, $L_H$........................... 55

Figure 6-7: Cross-section and orientation of the ARROW channel. ............................................. 58

Figure 6-8: Illustration of a calculated fully developed flow velocity profile................................. 59

Figure 6-9: Illustration of the amount of time a fluorescing particle passes through the excitation
region as a function of its x-y position within the ARROW. ................................................ 60

Figure 6-10: Illustration of the amount of fluorescence energy generated by x-y position........ 62

Figure 6-11: Example of ARROW geometry construction for FDTD calculation, built within
Lumerical® FDTD Solutions™. ........................................................................................... 64

Figure 6-12: Collection efficiency profile done by FDTD sweep across the ARROW cross-
section.................................................................................................................................. 64

Figure 6-13: Excitation Region profile of collectible fluorescence energy according to x-y
position within the ARROW channel. ......................................................................................... 65

Figure 7-1: (a) Example of an excitation ridge waveguide cross-section, color-coded according to
refractive index. (b) Example optical mode intensity profile with a 200nm mesh size. ...... 68

Figure 7-2: (a) Optical mode intensity profile exported to MATLAB with a peak value of 1. (b)
Profile of the laser power distributed over the intensity profile. ......................................... 69

Figure 7-3: Summed power values across the mode profile width, i.e. the ER’s depth. ......... 70

Figure 7-4: Flow velocity profile with a 2 cm/s mean velocity. ................................................. 71

Figure 7-5: Time profile of an excitation region with a 2µm depth. ............................................ 72

Figure 7-6: (a) Profile of excitation energy incident on a passing particle as a function of the
particle’s x-y position. (b) Profile of the amount of energy a fluorescing particle will emit.73
Figure 9-4: (a) Excitation region profile of the side-illuminated, parabolic flow regime design. (b) Signal distribution of the side/parabolic design. (© 2021 MDPI [9]) ................................. 100

Figure 9-5: (a) Excitation region profile of the side/2DHF design. (b) Signal distribution of the side/2DHF design. (© 2021 MDPI [9]) ............................................................................... 100

Figure 9-6: (a) Excitation region profile of the side/3DHF design. (b) Signal distribution of the side/3DHF design. (© 2021 MDPI [9]) ............................................................................... 101

Figure 9-7: (a) Excitation region profile of the top-illuminated, parabolic flow regime design. (b) Signal distribution of the top/parabolic design. (© 2021 MDPI [9]) .................................. 102

Figure 9-8: (a) Excitation region profile of the top/2DHF design. (b) Signal distribution of the top/2DHF design. (© 2021 MDPI [9]) ................................................................................ 102

Figure 9-9: (a) Excitation region profile of the top/3DHF design. (b) Signal distribution of the top/3DHF design. (© 2021 MDPI [9]) ................................................................................ 103

Figure 9-10: The ER profiles and signal distributions of the side/3DHF optimization sweep of HDF cross-section heights. (a) 4µm, (b) 3µm, (c) 2µm, and (d) 1µm. (© 2021 MDPI [9]) 106

Figure 9-11: ER profiles and signal distributions of the side/3DHF optimization sweep of HDF stream x-position. (a) 6µm, (b) 7µm, (c) 8µm, (d) 9µm, and (e) 10µm. (© 2021 MDPI [9]) ......................................................................................................................................... 107

Figure A-1: (a) Define the object dimensions in MODE. (b) Define the object refractive index by either selecting a material from the MODE database or define it manually. ...................... 120

Figure A-2: The simulation region (green box) is defined by dimensions and mesh size........... 120

Figure A-3: Eigenmode Analysis window................................................................................. 121

Figure A-4: Tool for exporting the intensity profile to MATLAB ........................................ 122

Figure B-1: Construction of the ARROW geometry. Define each component’s dimensions and refractive indices.................................................................................................. 134

Figure B-2: Define the dipole parameters and monitor dimensions and position. ................. 135

Figure B-3: Define the FDTD simulation region...................................................................... 136

Figure B-4: Create sweep of the dipole’s y-position............................................................ 137

Figure B-5: Create sweep of the dipole’s x-position............................................................ 138

Figure B-6: Visualization window of the concatenated FDTD calculation sweep.................. 139
CHAPTER 1. INTRODUCTION

Optofluidic flow-through biosensors are a technology that have been studied and developed since the early 2000s. The research group I am a part of developed a platform for the optofluidic fast detection of pathogens and other potential biological targets. Integrating several technologies, this platform utilizes a lab-on-a-chip system to pass a fluorescing target through an exciting light and collects the fluorescence for signal analysis. This integration of technologies offers sensing applications for a variety of biological targets. It can be used for the detection of a variety of targets which effects a diagnosis of disease. This technology can detect a variety of targets, such as antigens, cancer biomarkers, liposomes, nucleic acids, proteins, ribosomes, and virions. Utilizing these targets, the sensor has demonstrated the detection of a variety of disease-causing pathogens, including *E. coli*, *E. aerogenes*, *K. pneumonia*, and of great current note, SARS-CoV-2 [1]-[6]. As antibiotic-resisting bacteria are a concern for future anticipation, some work was done to develop applications to detect bacterial genes characteristic of such resistance, such as VIM, NDM, KPC, and IMP [5].

Detection and diagnosis procedures to ascertain the presence or absence of a given target increases in value when they are both accurate and fast. Established biosensing methods include culture growths and polymerase chain reaction (PCR) processes. Culture growths take a biosample and provide nutrients for present microbes to multiply until noticeable; this process can take days for a positive detection to be determined. The PCR method is another amplification method that induces fast multiplication of the pathogen’s nucleic acids, which can
be detected *en masse*; this process can take approximately 18 hours for a positive result to be acquired. The biosensor technology described in this dissertation can provide a signal in tens to hundreds of seconds.

The core purpose of this dissertation is to present a theory and model that explains how the optofluidic flow-through biosensor’s sensitivity behaves as a function of its many design parameters. This model will be explained in its constituent factors. After the theory description, the model will be constructed for demonstration as a simulation tool. This simulation tool will then be compared to physical devices to validate the model theory with real results. After the model is validated, its value will be demonstrated further by projecting the behavior of previously unexplored biosensor design variations. To give proper context for this stated purpose, this dissertation will also explain predecessor technologies, current biosensor function, and the device fabrication process.

This dissertation is based on my original works, published in the following academic journal articles:

This dissertation will describe the background of this biosensor technology, the development of its behavior theory, the construction of a computer model, and the use of this model to accurately simulate biosensor operation. Chapter 2 will describe the origins of flow-through biosensing technology. Flow cytometry was an important technological predecessor, and its development and subsequent evolution into lab-on-a-chip biosensors will be described. Chapter 3 outlines the biosensor structure and its operation. Chapter 4 describes the fabrication process of a standard biosensor device, and some notable differences are highlighted for certain design variations. In Chapter 5, I discuss how residue microstructures from the biosensor fabrication process affects optical transmittance in the biosensor’s waveguides, and I study three different reactive ion etching (RIE) processes that cause this residue formation. I present a hydrofluoric acid treatment that can be used to resolve the microstructure issue.

Beginning with Chapter 6, I discuss my core work in my doctoral studies. I formulated a model that describes the excitation region of the biosensor devices, and in Chapter 6, I describe the various factors of this model theory. In Chapter 7, I rehearse the model theory presented in Chapter 6 but with the purpose of describing how to construct the computer model and how to simulate a given design’s sensitivity. In Chapter 8, three design variations were both constructed in the computer model and fabricated; the model was then compared to the physical device tests for validation of the model. With this validation completed, I describe in Chapter 9 future designs to be fabricated and use the computer model to compare their sensitivities. Chapter 10 outlines future work needed to make the model into a robust designer’s tool, and I give my thoughts on the future development of this biosensor technology.
CHAPTER 2.  FLOW-THROUGH SENSING ORIGINS

2.1 A Brief History of Flow Cytometry

A precursor to optofluidic flow-through biosensing is the practice of flow cytometry. In this practice, whole cells are flowed through an intersecting laser for detection of a target and quantification of the target. Modern flow cytometry has several component concepts, each of which have their own points of origin.

The first published description of cells flowing in alignment, one after another, in a sheath fluid was written by Andrew Moldavan in 1934 [10,11]. The first known demonstration of detecting aerial particles by means of injections of air into a fluid sheath stream was done by Frank Gucker and Chester O’Konski [10,12]. In their study, they demonstrated a detection of 0.5µm particles by the light scattered by the particles as the sample flowed through a dark-field microscope focal point. In 1942, Albert Coons and his associates demonstrated fluorescence detection of Streptococcus pneumoniae [10,13].

Critical to the advancement of flow cytometry technology was the development of the Coulter counter in 1945. Developed by Wallace Coulter and described by DeBlois and Bean [14], the Coulter counter works on the principle that passing cells will have a measurable effect on nearby electric currents. The Coulter counter components consist of a tube that conducts a flowing saline solution, an aperture that conducts an electric current, and a system to measure real-time voltage and current. When biological cells are conducted through the tube, they will pass through the electrified aperture. The cell’s displacement of saline solution will cause a
change in impedance. Changes in the measured current indicate the presence of a cell passing through the aperture. In fact, Coulter was able to correlate the degree of change in the aperture’s impedance with the volume of the passing cell [10,14]. DeBlois and Bean mathematically describe this relationship in (2-1) and (2-2).

\[ R = \rho \int \frac{dz}{A(z)} \]  

(2-1)

\[ \Delta R = \frac{4\rho}{\pi D} \left[ \frac{\sin^{-1}\left(\frac{d}{D}\right)}{\frac{d}{D}} - \frac{d}{D} \right] \]  

(2-2)

After the development of the laser in 1960, Katmentsky studied how the optical properties of a cell forward-scatters light, particularly when the cell passes through a laser beam [15]. Katmentsky would later found Bio/Physics Systems in New York, USA in 1970 to manufacture and sell cytograph and cytofluorograph products for cell counting and analysis [10]. In the 1970s, further work was done by adding measuring optical scattering at multiple angles simultaneously [16]. Multiple exciting wavelengths were added to cytofluorograph analyzers, enabling multiple fluorescence wavelengths to be emitted and detected. Curbelo et al. did this by using an arc lamp to use several beams in 1976, whereas Shapiro et al. illuminated the passing sample with three lasers [17,18]. These analyzers could detect cells at a rate of 30,000 events/s.

Over the next few decades, flow cytometry technology advanced in terms of increased cell count rate, number of simultaneous fluorescence capacities, greater fluorescent marker control, and general compactness [10]. Nanocrystalline fluorochromes named Qdots™ became an option for fluorescent marking; the semiconductor nature of these crystals allows for more wavelengths to be used for emission and detection. Perfetto et al. reported up to seventeen
simultaneous fluorescence wavelengths utilizing Qdots in 2004 [19]. Cell count rates at up to 200,000 events/s are commonplace today, and cells can be sorted at a rate of 100,000 cells/s.

Cytometers are more compact today as lasers used in cell detection are increasingly solid-state diodes rather than gas media, and photomultiplier tubes are now replaced with avalanche photodiodes (APD) [10]. Modern flow cytometers have multiple simultaneous functionalities. They are able to count cells on a scale of $10^5$/s through multiple lasers, calculating each cell volume by the Coulter Principle, measuring optical scattering at multiple simultaneous angles at multiple simultaneous fluorescence wavelengths.

2.2 Typical Flow Cytometry Operation

While flow cytometers have multiple working parts, a cytometric system is made of three components, namely a hydrodynamic focusing system within the fluid channel, an optical excitation system for fluorescent emission and light scattering, and an electronic signal analysis system to interpret the signal into comprehensible information. The first component for consideration is a fluidic system that will hydrodynamically focus the sample stream to a constrained cross section within the larger fluid channel. This is critical to make accurate cell counts and to avoid clogging apertures. The sample fluid is injected into a flowing sheath, creating a coaxial laminar condition in which the sample is hydrodynamically focused, conducting the sample one cell at a time through the channel.

The cell stream passes through an intersecting laser, or perhaps multiple lasers with different wavelengths, and typically three different optical signals are detected: forward scatter, side scatter, and fluorescence. The forward scatter signal informs the signal analysis computer on the passing cell’s volume, the side scatter signal informs on the internal complexity of the
passing cell, and fluorescence intensity describes the number of contained nucleic acids [20,21]. These various signals are correlated to identify the cell. Using multiple fluorescent labels to bond with different nucleic acid targets will allow for multiple simultaneous species detections if multiple laser wavelengths are used. An overview of a flow cytometer’s operation is illustrated in Figure 2-1.

Figure 2-1: Operation of a flow cytometer. The cell sample passes through an exciting laser on the left. On the right are multiple photomultiplier tubes functioning as optical detectors. The PMTs are dedicated to detecting either forward scatter (FSC), fluorescence (FL), or side scatter (SSC) signals. The signals are then compiled and used to identify the cell sample. (Image is in the public domain, https://commons.wikimedia.org/wiki/File:Cytometer.svg)
2.3 Transition from Flow Cytometry to Optofluidic Flow-Through Biosensing

Flow cytometry has developed to be very powerful when analyzing whole cells. Advancing the biosensing technology, however, drives the need for accurate detection of ever smaller biosample sizes. With single particles becoming acceptable detection targets, a smaller apparatus for detection became necessary. To that end, the micro-scale channel intersecting single-mode waveguides were developed.

These micro-scale biosensors have the ability to detect single particles and single nucleic acid strands, whereas established flow cytometers detect whole cells. This difference in scale also prevents the flow cytometer to detect viruses. Because the targets for a micro-scale biosensor are molecular, fewer fluorophore markers can attach to the target molecules than can be attached to a whole cell and its contents. The size needed to accommodate a whole cell renders the flow cytometer unable to detect such small fluorescence intensities. The biosensors described in this dissertation are capable of collecting enough of the molecular-scale fluorescence to register a target presence. The following chapter will describe the basic operation of this biosensor.
CHAPTER 3. ARROW BIOSENSOR OPERATION

3.1 Overview of Biosensor Structures and Functions

Prior to the detection process, the biosample is processed to cause fluorescent markers to bond to present targets. These targets can be viral or bacterial nucleic acids, and they can even possibly be cancer cells. These fluorescent markers will fluoresce to indicate the presence of a test target. Some markers have a feature that causes them to be unquenched until they bond to a target, which would cause them to be unquenched. The quenched state would cause them to not fluoresce when the biosample is examined, indicating the lack of a pathogen. The unquenched state would give an indication of the pathogen’s presence, and it is the fluorescence photons that comprise the signal in photon counts per unit time.

When the biosample is processed to bind fluorescent markers to present targets, the sample is then conducted to the biosensor chip, typically 1 cm² in area, as shown in Figure 3-1. The sensor chip consists of a silicon substrate, Anti-Resonant Reflecting Optical Waveguide (ARROW) layers on top of the substrate, several integrated silicon dioxide (silica) structures fabricated on top of the ARROW layers, and two reservoirs for the biosample. All of these structures also usually have a 6-μm thick overcoat of silica with a lower refractive index than the underlying structures. Figure 3-2 shows a photograph taken from a microscope image of a fabricated biosensor chip.
Figure 3-1: Illustration of biosensor operation. A fluid sample flows from one reservoir to another in the channel shaded blue. An exciting optical mode (x-axis) intersects the channel, generating fluorescence as a signal. This signal is then guided down the channel and couples into the collection ridge waveguide (z-axis) for off-chip measuring and analysis. (© IEEE/OSA 2021 [8])

Figure 3-2: Photograph overview of the integrated components of the biosensor chip. The ARROW channel runs diagonal, briefly running horizontal in the center. The collection ridge waveguide couples with the ends of the horizontal section of the ARROW. Vertically, the single-mode waveguide (left) and multi-mode interferometer (MMI) (right) intersect the ARROW. The single-mode waveguide provides a single excitation region, and the MMI allows multiple excitation regions for signal multiplexing.
The core of these integrated structures is a hollow channel through which the sample flows. The channel is typically 12µm wide and 5-6µm tall, but these dimensions can change if called for by the designer. The walls and ceiling of the channel are made of silica, and the top surface of the top ARROW layer forms the channel floor. This channel connects the two reservoirs. The biosample first collects in one reservoir after its fluorescent-marker processing, then it flows down the channel, and finally collects in a second reservoir. Often, this second reservoir is connected to a vacuum source to conduct the sample along its path in the channel.

The critical portion of the channel is a 300µm straight segment that interacts with the other silica structures on the sensor chip. Optical modes intersect this segment for the purpose of illuminating the sample. As the sample flows past the exciting modes, the fluorescent markers on the targets will fluoresce their own light. Part of this light will then be conducted down the 300 µm segment. As the channel fluid has a lower refractive index than the surrounding silica, the guiding is done by way of anti-resonant reflection, provided by the ARROW layers below.

Intersecting this 300 µm segment are single-mode ridge waveguides and commonly multi-mode interferometers. These optical structures are responsible for guiding the excitation modes to the sample. Typically, an optical fiber is butt-coupled to one of the facets that are flush with the chip’s edge. This optical fiber conducts a laser-generated light source to the excitation ridge/MMI structures. The mode at the end of the excitation structures form the excitation region in the ARROW channel, through which the fluorophore-tagged targets pass. This excitation region shall be investigated in-depth later in this work.

The remaining silica structures are large ridge waveguides that couple with the ends of the 300-µm segment of the channel. The fluorescent light that is guided down the ARROW channel
will couple into these waveguides for collection, and they will be guided off-chip to an objective lens and avalanche photodiode for photon counting.

### 3.2 Single-Mode vs. MMI Excitation and Signal Analyses

The presence of both a single-mode excitation ridge and a multi-mode interferometer (MMI) allow for different excitation methods. The single-mode excitation method allows for simple photon counting, where a positive signal is characterized by a count rate significantly higher than the noise level. The MMI excitation option allows for several optical multiplexing schemes, including spectral and spatial multiplexing.

![Example of (a) simulated signals across test time and (b) the distribution of these signals.](image)

**Figure 3-3:** Example of (a) simulated signals across test time and (b) the distribution of these signals. (© IEEE/OSA 2021 [8])

In single-mode excitation, the laser light forms a tight-radius mode that intersects a sub-picoliter volume in the ARROW channel and extends across the channel width. As targets pass through the intersecting excitation region, a record of the signal peaks can be taken across the sample test time (Figure 3-3a). When these peaks are sufficiently greater than the noise level, it can be qualified as a detected target. These signals can also have some statistics drawn from them, such as a maximum signal, mean signal, median signal, and signal variance to give information
about the biosensor’s performance. When these signals are arranged in a histogram distribution (Figure 3-4b), further design modifications can be better informed.

MMI excitation begins with the laser-sourced optical fiber aligning with a ridge waveguide as with single-mode excitation. After some length, the ridge couples into a wide structure, the MMI. The mode then divides into multiple modes, and these modes then create several interference patterns as they propagate down the MMI length. The MMI width and length are designed to have a pre-selected number of well-defined constructive interference spots that intersect with the ARROW channel; the number of spots is calculated by (3-1) where \( N_C \) is the number of defined optical spots, \( n \) is the MMI refractive index, \( W \) is the MMI width, \( L \) is the MMI length, and \( \lambda \) is the mode wavelength [5].

\[
N_C = \frac{nW^2}{L\lambda} \quad (3-1)
\]

A single target will then pass through multiple excitation regions, emitting fluorescence down the ARROW at each point. As each illumination spot has a fraction of the original power, these several signal peaks for a single target can then be analyzed with a shift-multiply algorithm to amplify the signal detected. This algorithm is given in (3-2), where \( S \) is the shift-multiplied signal, \( F \) is the component fluorescent signal, \( t \) is the time at which a component signal is detected, \( \delta t_C \) is the characteristic time for a particle to flow between two spots, and \( i \) is the \( i \)th spot the flowing particle has passed [5].

\[
S(t, \delta t_c) = \left\{ \prod_{i=0}^{N_C-1} F(t - i \cdot \delta t_c) \right\}^{1/N_C} \quad (3-2)
\]

The fluorescence signal detected in a given spot, single mode or MMI, is in part a function of the optical intensity profile of each spot, the dwell time of a particle in a spot, and the collection
efficiency of generated fluorescence. Chapters 4 and 5 will describe the fabrication process of a biosensor chip, including the guiding of an excitation mode to the ARROW. The remaining chapters will describe how the excitation spot, a.k.a. excitation region, will determine the quality of the fluorescence signal detected.
CHAPTER 4. BIOSENSOR FABRICATION

As shown in the previous chapter, the optofluidic biosensor consists of several integrated components. The fabrication of these components involves a series of photolithographic patterning, material growth/deposition, and selective material reduction. The core technology of this biosensor chip is the anti-resonant reflecting optical waveguide (ARROW) which allows for optical guiding in a core medium of low refractive index, bypassing the need for total internal reflection (TIR). As the fluorescent signal needs to be generated and guided in this component, all other components are integrated around it. The order of fabrication processes follows as:

- Deposit ARROW layers onto a silicon wafer substrate
- Form a sacrificial core around which the channel is constructed
- Etch a pedestal on which the channel and solid waveguides will stand
- Grow a high-index silicon dioxide layer
- Etch solid waveguides into the high-index silicon dioxide material
- Grow a low-index silicon dioxide layer as a cladding
- Etch out the sacrificial core to clear the channel.

4.1 ARROW Layer Deposition

The ARROW utilizes a series of alternating dielectric layers to create a Fabry-Perot reflector. Fabry-Perot etalons commonly optimize the alternating dielectrics’ thicknesses to
create a resonant condition that will maximize constructive interference; this is done to increase optical transmission between media. Instead, the ARROW application of the Fabry-Perot etalon requires the thicknesses to be optimized to create an anti-resonant condition that will maximize destructive interference, as illustrated in Figure 4-1. This creates a reflector similar to a Bragg mirror but for propagating a wave in parallel with the reflector’s surface [22].

![Illustrated demonstration of anti-resonance reflection in the ARROW layers.](https://commons.wikimedia.org/wiki/File:ARROW_waveguide_example.png)

The mathematical condition for selecting the dielectric layers’ thicknesses is given in the following equation:

\[
d_i \approx \left( \frac{\lambda}{4 n_i} \right) (2N - 1) \left[ 1 - \frac{n_c^2}{n_i^2} + \frac{\lambda^2}{4 n_i^2 d_c^2} \right]^{-\frac{1}{2}}. \tag{4-1}
\]

The variable \(d_i\) represents the calculated thickness of each dielectric with respect to given inherent refractive index (\(n_i\)), core (channel) refractive index (\(n_c\)), core thickness (\(d_c\)), and wavelength to be propagated (\(\lambda\)) [23]. The dielectric materials typically used in our biosensor
ARROWs are three pairs of silicon dioxide (silica or SiO$_2$, n=1.47) and tantalum pentoxide (Ta$_2$O$_5$, n=2.107). For a fluorescence signal with a wavelength of 667 nm to be propagated down the ARROW, the thicknesses of the silica and Ta$_2$O$_5$ layers calculate to be 265 nm and 102 nm, respectively. These layers are sputtered onto the silicon wafer substrate, silica first such that they alternate with Ta$_2$O$_5$ is topmost. Nova Electronic Materials, a private company based in Texas, sputters ARROW layers onto silicon wafers for the devices in this project. At this point, the wafer appears as shown in Figure 4-2.

![Figure 4-2: First stage of the biosensor’s fabrication has ARROW layers sputtered onto bare silicon. (© 2021 MDPI [9]).](image)

### 4.2 Sacrificial Channel Core

After the ARROW layers are deposited onto the silicon substrate, a sacrificial core is deposited around which the ARROW channel may be built. The sacrificial core is made of a hardened SU-8 photoresist that has been spun on, exposed by photolithographic process, and developed to leave a pattern of the later channel.

SU-8 is a family of negative photoresists [24]. There are many variations used for different layer thickness ranges. SU-8 10 is used for these biosensor cores as it can create layers as thin as 5 µm, a typical height. Adjusting the spin speed of the spinner used to coat the wafer
with the resist has some fine effect on the post-spin thickness. For example, one of our devices (MABRW42) with a 5-µm high core had SU-8 10 spun on in a three-step process:

1. 500 rpm speed, 500 rpm/s acceleration, 6 seconds duration
2. 5300 rpm speed, 1000 rpm/s acceleration, 60 seconds duration
3. 6000 rpm speed, 6000 rpm/s acceleration, 2 seconds.

As an alternative example, another of our devices (MABRW39) was designed with a 3-µm high core. A different SU-8 resist was required to get a core height that thin; in this case SU-8 2002 was used. This resist layer was spun on in a two-step process:

1. 540 rpm speed, 176 rpm/s acceleration, 60 seconds duration
2. 4000 rpm speed, 2064 rpm/s acceleration, 6 seconds duration.

After the SU-8 has been spun on, each wafer is placed on a hotplate to “soft bake” the photoresist. This is to slightly harden the resist that it may physically withstand the later lithographic alignment process. After the soft bake is done, the wafer is then patterned by photolithography. SU-8 is a negative photoresist, meaning portions of it that have been exposed to a UV light will form molecular cross-links to bind its molecules more strongly together. When later placed in a developing solution, the unexposed SU-8 will be cleared away; the exposed, cross-linked SU-8 will remain.

The pattern of the core is made by selective exposure. A photolithographic mask with the core pattern is placed over the wafer. Using a SUSS MicroTec® (formerly Karl Suss®) MA 150 CC™ aligner, the mask is aligned to the desired position, and a 405-nm light then illuminates the mask and exposed SU8 beneath. To get core features 12µm wide, an exposure dose of 250 mJ/cm² is needed. After exposure, the wafer is given a post-exposure bake to slightly solidify the exposed SU-8 prior to chemical development.
The wafer is then placed in an SU-8 developer solution (from Kayakli™ Advanced Materials) for 60 seconds. This clears the unexposed SU-8 and leaves the core structure to remain. After this photoresist development, the wafer is “hard baked” on the hotplate to harden the SU-8 core structure. At this point, the SU-8 cores are measured under the Zeta Instruments® Zeta 20 3D Profilometer to verify they are 12 µm wide. A ±0.5 µm error is considered tolerable. After this measurement, the wafer is given a second hard bake.

After a brief descum (50W, 60s), the height of the SU8 cores are then measured by the Tencore Instruments Alpha-step® 200 profilometer to confirm they meet the designed parameter. Figure 4-3 illustrates the state of the biosensor this far in the fabrication process. This outlines the SU-8 core fabrication process for a typical biosensor design with a parabolic flow regime. However, devices with hydrodynamic focusing (HDF) integrated into the design require different process variations for both two-dimensional and three-dimensional hydrodynamic focusing (2DHF & 3DHF).

Figure 4-3: SU8 sacrificial core on the ARROW layers after patterning and development. (© 2021 MDPI [9])
4.2.1 Fabrication for 2DHF Core Variation

The 2DHF core variation is the simpler deviation from the typical fabrication process. SU-8 is spun on to the wafer as before, but the photolithography requires a different photomask with a different pattern. Rather than a simple rectangular structure, the core has two other branches that intersect the main line. These branches form the inlet channels through which the buffer fluid enters the main channel, compressing the sample stream horizontally. This core variation is shown in Figure 4-4. The remaining fabrication steps require changes to the photolithography patterns to accommodate these additional SU-8 branches.

![Figure 4-4: Sacrificial core for 2DHF-designed devices. Two branches intersect the main line at angles, which form inlet channels for buffer fluid to enter the main channel. (© 2021 MDPI [9])](image)

4.2.2 Fabrication for 3DHF Core Variation

The 3DHF core variation requires the most additional steps to the overall fabrication process. To achieve 3DHF, buffer fluid must compress the sample stream both horizontally and vertically. The vertical focusing aspect requires some of the buffer fluid to flow from above and below the sample stream as the sample and buffer channels intersect, an added complication to an otherwise planar operation. This will require that the biosensor wafer be etched before SU-8 deposition and two steps of SU-8 coating, patterning, and development.
While other potential methods for integrating 3DHF in a design have been reported in this biosensor project [25,26], one has been chosen for discussion later in this work [25]. This biosensor design requires an etched trench after the ARROW layers have been sputtered but before the SU-8 photoresist is applied. A layer of nickel is deposited on the wafer in the Denton Vacuum E-Beam Evaporator (~100nm thick). Then, a positive photoresist, AZ3330, is spun onto the wafer. As a positive resist, portions exposed to near-UV light will clear away in chemical development. A photoresist mask is patterned by photolithography and developed such that the whole wafer is covered in photoresist except for the trench positions. The wafer is placed in a nickel etchant, and the trench locations are stripped of their nickel covering. The photoresist is cleaned off, and the remaining nickel forms a protective mask in the dry etch.

The trench is etched by both reactive ion etching (RIE) and deep reactive ion etching (DRIE) in the Trion Technology® Minilock Phantom III RIE/ICP until a depth of 5-6µm is achieved. The ARROW layers are etched away by a simple isotropic RIE. The silicon is then etched by DRIE in a bosch cycle. This cycle is an alternating series of passivation and etching steps. This is done to etch deeply without undercutting the desired features. When the trench is completed, the biosensor at this stage appears as illustrated in Figure 4-5.

Figure 4-5: An initial trench is etched into the wafer ARROW layers and silicon substrate prior to SU-8 application. (© 2021 MDPI [9])
After the trench is etched, two steps of SU-8 deposition, patterning, and development are done. In the first step, the spun-on SU-8 will fill the trench and form a film of some thickness above the ARROW layer surface. The photoresist is exposed and developed such that SU8 still fills the trench, forms a main channel intersecting the trench orthogonally, and have some overlap extending beyond the trench width. This SU-8 is hard-baked until the SU-8 above the wafer surface forms the designed height, 5-6µm.

A second SU-8 application is done, similar to the first. The spin speed is selected such that the applied SU-8 covers the whole wafer topography and forms a planar film with a top height 5-6µm above the first SU-8. The new film is patterned, developed, and baked such that a rectangular structure intersecting the main channel line lies above the trench. The final 3DHF core structure appears as shown in Figure 4-6. The remainder of this chapter follows the fabrication process of a standard biosensor without HDF, but each step’s respective photolithography patterns would be appropriately adjusted to accommodate the 3DHF design.

![Figure 4-6: Two SU-8 steps are done to form a channel where buffer fluid will eventually focus the sample stream horizontally and vertically. (© 2021 MDPI [9])](image)

4.3 Pedestal Etch

With the sacrificial core in place, a pedestal is etched into the wafer on which the ARROW and eventual solid waveguides stand. The pedestal’s purpose is to strengthen the
structural integrity of the ARROW channel. When silicon dioxide is grown over the sacrificial core (which will be covered thoroughly in the next section), a crevice forms between the oxide growing from the side of the core and the wafer surface in immediate proximity to the core. This crevice is a structural weakness that can be an origin point for cracking in the oxide. The pedestal etch puts some distance between the crevice and the ARROW channel walls, decreasing the frequency of broken devices and improving device yield per wafer. To create the pedestal in the etch process, a protective metal mask, e.g. nickel, is applied to the surface of the wafer. This nickel mask is patterned to create the selective area to preserve from the etch process.

The pedestal is patterned by photolithography. The wafer is coated with a spun-on positive photoresist, AZ4620. After the resist is spun-on, the wafer is soft baked. The wafer is then aligned with the pedestal photolithography mask on the aligner and exposed to near-UV light for about 55s. This exposure patterns the pedestals for the solid waveguides. A second “flood” exposure is done (exposing the whole wafer without a mask) for about seven seconds. The flood exposure’s purpose is to expose the photoresist sufficiently that the top surface of the photoresist will be lowered to just below the SU-8 core’s top’s height in the developing process. The wafer is placed in a developer solution (80% water, 20% 400K developer) until the pedestal pattern is cleared and the top of the remaining photoresist is ~2µm below the core height.

After the photoresist has been spun on and patterned, the wafer is descumed (50W, 30s) and submerged in a hydrochloric acid solution (66.7% water, 33.3% HCl) for a few seconds. This promotes adhesion between the surface of the exposed ARROW layers surface and SU-8 core top and the nickel material. Nickel is then evaporated in the Denton Vacuum E-beam Evaporator and deposited onto the wafer. This deposition runs until the metal layer is 120nm
After the nickel is deposited on the wafer, it is placed in acetone to clear the AZ4620 photoresist and lift off the nickel; the pedestal pattern remains.

After the undesired nickel is removed, the wafer is placed in the Trion Technology® Minilock Phantom III RIE/ICP for three dry-etch processes. The first process is an isotropic reactive ion etch (RIE) that etches through the ARROW layers but leaves the masked portions intact. Second, a deep reactive ion etch (DRIE) is done by means of a Bosch cycle, alternating steps of passivating the wafer surface and etching deeply into the silicon substrate without undercutting the etched features. A third etch, an anisotropic RIE, is done to remove any silicon artifacts left behind by the Bosch. After the etch is complete, the wafer is placed in a solution of tetramethylammonium hydroxide (90%) and hydrogen peroxide (10%) to remove fluorocarbons left over from the etch processes, and the nickel mask is stripped. At this point, a pedestal 6µm high is formed. Figure 4-7 illustrates the end of this stage in the fabrication process.

![Figure 4-7: The pedestal for pending waveguides is etched into the ARROW layers and silicon via RIE and DRIE, respectively. (© 2021 MDPI [9])](image)

4.4 Silicon Dioxide Growth by PECVD

The next general step in the fabrication process is to grow a silica film on the wafer. This silica is the material from which the solid waveguides and the ARROW channel sidewalls are
formed. The silica is grown by a plasma-enhanced chemical vapor deposition (PECVD) process. This process is fast compared to silica growth by thermal oxidation. PECVD also allows for adjusting the film’s refractive index by adjusting the gas ratios (silane and nitrous oxide) in the process chamber, and the film’s internal structural stress can be adjusted by the chamber’s pressure and gas flow rates. PECVD is a modification of chemical vapor deposition (CVD). In CVD, the wafer is heated to temperatures as high as 400°C; this temperature is high enough for the reactant gases introduced to the chamber to react and deposit silicon dioxide molecules on the hot surface. PECVD strikes a plasma in the gas mixture, adding electromagnetic energy to the gases that lowers the thermal requirement to temperatures at 250°C.

The biosensor wafer is placed in the Oxford Instruments PlasmaLab90 Plus PECVD chamber at 250°C. After the chamber pressure achieves the setting and process gases are introduced, a plasma is struck to catalyze silica growth. The silane/N₂O ratio is adjusted such that the silica film has a refractive index of 1.51. The film is grown to 6µm, and the process ends. Figure 4-8 illustrates the biosensor with the silica layer deposited. The wafer is removed from the PECVD chamber for further fabrication.

Figure 4-8: A silica layer is deposited on the biosensor device via PECVD. (© 2021 MDPI [9])
4.5 Solid Ridge Waveguide Etching

With the silica layer put down, the solid ridge waveguides (single mode excitation, MMI excitation, and collection) are etched. In a similar process as the pedestal mask patterning, the wafer is coated with a positive photoresist, AZ3330, and by photolithography is exposed and developed to leave a pattern of the ridge waveguides. Nickel is deposited on the wafer, and the undesired metal is removed by liftoff in acetone. The Trion RIE/ICP etches the silica layer with an isotropic RIE until the waveguides attain the designed height. The nickel mask is then stripped, leaving the biosensor to appear as illustrated in Figure 4-9.

Figure 4-9: Ridge waveguides are etched via RIE into the silica layer previously deposited. (© 2021 MDPI [9])

4.6 Silica Cladding Growth

It has been found that the optical transmittivity of the solid ridge waveguides degrades over time the PECVD silica absorbs atmospheric water. To counter this degradation, the biosensor wafer is given a second silica layer after the ridge waveguides are etched. Made with a lower refractive index (1.46) than the ridge structures themselves (1.51), total internal reflection is preserved in the solid waveguides. This cladding has shown to effectively preserve waveguide transmittivity in high humidity situations.
After the wafer has been ridge-etched, and the nickel mask has been stripped, it is placed in a 300°C dehydration bake for 15 hours to bake out any water present in the waveguides. At the end of this dehydration bake, the wafer is immediately placed in the PECVD chamber with the hotplate already at 250°C. The cladding layer is grown in the same procedure as the first silica layer, but a different process is selected with different gas flow ratios to have an index of refraction of 1.46. This cladding layer’s refractive index is sufficiently low for the waveguiding structures beneath to guide optical modes in the visible wavelength spectrum.

4.7 ARROW Core Etch-Out

At this point, all the needed material for the biosensor structure is in place, and the sacrificial SU-8 core must be etched out. Using a photolithographic process, the biosensor devices on the wafer are all given a protective AZ4620 coating, and the photoresist over the end sections of the ARROW channel is exposed and developed. The ends of the channel are then exposed to the atmosphere, and the wafer is submerged in a hydrofluoric acid (HF) solution with a 5% concentration. The acid etches away the silica layers that cover the SU-8 ends, but the channel silica structure beneath the AZ4620 coating is preserved. Typically, several tens of micrometers of SU-8 are exposed on each end of the channel structure.

After the SU-8 ends are exposed to atmosphere, the wafer is placed in a solution that is 60% hydrogen peroxide (H₂O₂) and 40% sulfuric acid (H₂SO₄). The solution is heated to 130°C, and the wafer is placed into the solution. The etch-out typically requires a week’s duration, with the solution being remade once a day. After the core has been etched out, the wafer is soaked in water for six hours to clear out the acid solution. The wafer is then placed in a Nanostrip™ solution (90% sulfuric acid) for six hours to etch away any trace amount of SU-8 leftover, and
then the wafer is placed in water for six hours again to clear out the Nanostrip™. The final
device appears as illustrated in Figure 4-10. At this point, the wafer is inspected for device yield
and ready for device testing.

Figure 4-10: Completed biosensor with the silica cladding layer deposited and SU-8
sacrificial core etched out. (© 2021 MDPI [9])
CHAPTER 5. SURFACE ROUGHNESS EFFECTS ON WAVEGUIDE LOSS

Some of my original work was to develop and advance the etching process of the biosensor’s ridge waveguides. As a side effect of etching by RIE or DRIE, micro- and nanostructures made from the etched material can form in the field and close to the designed features. These residue structures can increase optical loss in waveguiding devices. Increased loss (decreased transmittivity) is an adverse effect in the biosensors studied here. Fluorescent signals detected by the avalanche photodiode will be less pronounced, and the biosensors operate best when these waveguide losses are kept small. To this end, I investigated three microstructure formations that form from different RIE parameter combinations. Waveguides etched under these RIE conditions were optically characterized and compared. I further divided each of these residue categories into those that were and were not given a supplemental wet etch treatment.

5.1 Ridge Waveguide Etch Process Overview

To briefly reiterate, the ridge waveguides in the biosensor are formed by reactive ion etching (RIE). After the silica (1.51 refractive index) is grown over the biosensor pedestal and SU-8 sacrificial core, the wafer is coated in a positive photoresist, in this case AZ3330. The photoresist is patterned by photolithographic exposure to near-UV light, and the exposed portions are cleared away in chemical development. The remaining photoresist post-development leaves the core and solid waveguides exposed to atmosphere. A 120-nm thick layer
of nickel is deposited over the whole wafer, and in an acetone bath, the nickel on the photoresist is lifted off; the remaining nickel deposited directly on silica forms the waveguide patterns.

The wafer is then placed into the Trion ICP/RIE chamber and then subjected to a plasma etch process. When the process parameters are in a suitable combination, the silica on the wafer is etched isotropically, in a downward vertical direction. The etching is primarily done by chemical reaction, but some of it can be done by kinetic bombardment of plasma ions onto the exposed silica. The silica material beneath the nickel mask is preserved from the downward etch, and there is minimal undercutting of the ridges. The nickel mask is then stripped away by a nickel etchant. Ideally, this whole process (illustrated in Figure 5-1) results in a clean etch with no residue remaining and allowing a smooth interface between the ridge and cladding layer (refractive index 1.46). However real etches leave residue microstructures which can be detrimental to optical transmittance.

Figure 5-1: Summary illustration of the ridge etch process. (a) The process begins with ARROW layers on a silicon substrate. (b) Silica with a 1.51 refractive index is grown over the ARROW layers. (c) A nickel mask is applied to the silica by photolithography. (d) The silica is etched by RIE. (e) The nickel mask is stripped. (f) A silica cladding with a 1.46 refractive index is grown over the ridge. (© 2020 IEEE/OSA [7])
5.2 Microstructure Residues Origins

After the cladding layer was grown over the biosensor wafer, I observed clusters of microstructures in the field and near waveguide structures; in actuality, the microstructures were beneath the cladding, but the cladding made it obvious. I then decided to conduct a quick study of how microstructure formations change when changing process parameters of the RIE, with hope of finding a lead to reducing the residue microstructure density. Three microstructure formations were found in the quick study, and the process parameters are listed in Table 5-1.

<table>
<thead>
<tr>
<th>Etch Parameters</th>
<th>O₂/CHF₃ (nanotubes)</th>
<th>O₂/CHF₃ (“grass”)</th>
<th>CF₄ (“stalks”)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber Pressure</td>
<td>19mT</td>
<td>19mT</td>
<td>12mT</td>
</tr>
<tr>
<td>Gas 1 (flow rate)</td>
<td>O₂ (9sccm)</td>
<td>O₂ (9sccm)</td>
<td>CF₄ (50sccm)</td>
</tr>
<tr>
<td>Gas 2 (flow rate)</td>
<td>CHF₃ (125sccm)</td>
<td>CHF₃ (125sccm)</td>
<td>-</td>
</tr>
<tr>
<td>ICP Power Setting</td>
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<td>88W</td>
<td>270W</td>
</tr>
<tr>
<td>RIE Power Setting</td>
<td>70W</td>
<td>35W</td>
<td>75W</td>
</tr>
</tbody>
</table>

The first microstructure type, a result of the then-standard RIE process parameters, were clusters of tube-like pillars. These nanotubes, so termed because they were less than a micron in diameter, are shown in Figure 5-2a. A gas mixture of O₂ and CHF₃ were the chemical reactants. The nanotubes are made from a self-additive process in which silica particles removed from the material bulk re-attach to the wafer, forming nucleation sites for more etched particles [27,28].

The second found microstructure type has a form that appears “grass like” in the etched field, shown in Figure 5-2b. The “grass” here is ubiquitous in the field and in proximity to the waveguide structure. The process in this case was the same as the process that produced the nanotubes, except both the inductively coupled plasma (ICP) and RIE generators were set to a halved power. This “Half Power” process changed the micro-structuring from an additive process of recombined materials to a subtractive process. When the metal mask is bombarded by...
the plasma ions, metal particles are sputtered off and land in the silica field, forming micro-masks. This change in residue formation is consistent with findings in other works [27,28].

The third microstructure formation that was studied had the appearance of “stalks” scattered in the field. These stalks were thin (~0.3µm) and sparse in area density by visual inspection of scanning electron microscope (SEM) images, shown in Figure 5-2c. They are taller than the silica grass formation. The process that formed these stalks had a high ICP power setting and used CF₄ gas as a reactant. At the base of each stalk, there appears a hole in the silica beneath. It seemed these stalks were grown from an additive process that involved a high-energy ion bombarding the silica, milling through the material, and the displaced material forming the vertical stalk structure. Prior to optical characterization, it was conjectured that the stalks would cause the least optical waveguide loss of the three microstructure formations as they were thin and sparse. This CF₄ etch process also had the fastest etch rate of approximately 300nm/min.

![Figure 5-2: Microstructure formations under study. (a) Nanotubes, (b) “grass”, and (c) “stalks”. (© 2020 IEEE/OSA [7])](image)

5.3 Wet Etch Supplement

A wet etch supplement was applied to etched waveguide samples as an effort to clear away the microstructures grown in the RIE without investing time into altering the RIE
processes. The etchant chosen was a hydrofluoric (HF) acid solution with a 5% concentration. When the samples were submerged in this solution for 3-5s, there was significant removal or reduction of the microstructures while having a minimal effect on the waveguide structures themselves. After the waveguide samples were removed from the Trion, but before the nickel mask was stripped, they were submerged in the 5% HF solution for 4s with light agitation; they were then immediately rinsed with water. The nickel mask was stripped, and the samples were inspected. Both the grass and stalk structures appeared to remove, with some leftover grass clumps sticking to the waveguide sidewalls. The nanotubes were reduced in size, but they were so thick that they would not be completely removed before waveguide deterioration in the HF became a concern. The SEM images for each are shown in Figure 5-3.

![SEM images of microstructure residue after being treated with 5% HF. (a) Nanotubes, (b) grass, and (c) stalks. (© 2020 IEEE/OSA [7])](image)

A waveguide consisting only of the guiding core and without cladding has a smaller mode field diameter than a waveguide core with equal dimensions but that has been cladded with oxide. As a mode propagates down the waveguide length, it will experience a rapidly changing effective waveguide index of refraction caused by intermittent location of the microstructure residue near the ridge core and within the cladding.
A change in effective index creates a point at which light scatters or reflects (or both) rather than transmitting down the wavelength; more scatter/reflection points will incrementally degrade the waveguide transmission. Figure 5-4a illustrates the placement of the cladding over the microstructures, and Figure 5-4b is an image taken by SEM of cladding oxide grown over the microstructures. Figure 5-4c, however, shows the cross section of a ridge waveguide that was given the HF treatment prior to cladding, and the interface between the high index core and low index cladding is visibly cleaner.

![Figure 5-4: (a) Cross section schematic of etched ridge waveguide with microstructures in the field and cladding grown over. (b) SEM image of ridge cross section with microstructures present under cladding. (c) SEM image of ridge cross section that has been treated with 5% HF prior to cladding and featuring a clean interface between ridge core and cladding. (© 2020 IEEE/OSA [7])](image)

### 5.4 Optical Characterization

#### 5.4.1 Optical Transmission Measurement

To characterize the transmission effects of the microstructure residue on waveguide transmittivity, six sets of ridge waveguide samples were fabricated: one sample set of each RIE process that were given a PECVD silica cladding without being given the 5% HF treatment, and
one sample set of each RIE process that was given the 5% HF treatment prior to being cladded. The waveguides were made from silica by PECVD that had an index of refraction of 1.51 by on top of the ARROW layers, and then etched according to their respective sample set RIE/HF processes.

Each sample set had a series of ridge waveguides with three different ridge widths: 3µm, 6µm, and 10µm. Each wafer sample was cleaved into chips containing multiple sets of these waveguides. These chips were placed on an optical bench setup with one end of the waveguides placed in the focal point of an objective lens (Figure 5-5); this lens transmitted the image of the waveguide facet to an optical power photodetector. A fiber waveguide was aligned with the other end of the ridge waveguide chip; this fiber was connected to a 635nm laser source (Thor Labs® Model S1FC635) and guided the laser light to the ridge waveguide facet.

The chip and fiber were then fine-aligned with the objective lens and photodetector, and the laser generator’s power was set to 1mW, illuminating the waveguide. A visual example is given in Figure 5-6. An optical power reading was recorded as a P(x) value. This was done for each waveguide on the chip. The chip was then removed and the laser-guiding fiber brought to the objective lens focal point, and a power reading was recorded from the fiber facet as a P₀ value. Each waveguide power value was divided by the fiber facet power value to record a transmission (T) value. Each waveguide length was likewise recorded as an x value. The chip was then cleaved to shorten the waveguide lengths, and the entire process was repeated for the new waveguide length. This cycle was repeated five times for each waveguide in a practice called the cutback method [7].
Figure 5-5: Mode image of a 10µm ridge waveguide that has been aligned in the objective lens and captured with a CCD camera.

Figure 5-6: Top-down image of a fiber aligned with a ridge waveguide on a sample chip, guiding light from the laser source to the chip facet.

With a series of x-length measurements and a corresponding set of T values, the transmission values were then scatter-plotted along the x-coordinates. Due to an inherent inconsistency in facet transmissions due to cleaving the chips, the highest T values were selected for analysis. An exponential curve was fitted for each waveguide width’s highest T value for each x-length, and a curve equation for each waveguide width was calculated. The exponential slope was taken to be the calculated waveguide loss, given as $\alpha$. 
Figure 5-7: An example of a semilogarithmic plot of waveguide transmissions with respect to waveguide length. The transmissions are plotted as red Xs on a logarithmic y-scale and a linear x-scale. An exponential curve is fitted to the plotted points, which appears as a straight line on the semi-log plot. The exponential slope is taken to be the waveguide loss.

5.4.2 Propagation of Uncertainty Calculation

The optical transmission measurement process and cutback method has several variables that can introduce error into the system. Because of this, it was necessary to ascertain the error in the calculated loss values before each test case could be compared. Propagation of uncertainty is the calculation of error of a desired quantity when that quantity itself (waveguide loss in this case) is a calculation and multiple input variables are subject to error. These errors can be due to imperfections in the test system, user error when making fine measurements, etc. It becomes necessary to determine how much each input error contributes to the overall error.

Propagation of uncertainty calculations sum the error contributions of each input variable and the error contributions of covariant variables. Statistics are taken for each input variable, namely figure means ($\mu$), standard deviations ($\sigma$), and variances ($\sigma^2$). For each waveguide width,
in each test sample category, a sample was measured ten times for transmission. The P(x), P₀, and waveguide length (x) values were compiled from each of these measurements.

In a waveguide, the optical power transmission, waveguide length, and waveguide loss (α) can be related by the following exponential equation.

\[ T = \frac{P_{mean}(x)}{P_{0,mean}} = Ae^{-\alpha x} \]  

(5-1)

The A factor is calculated in the transmission curve fitting and accounts for the optical power coupled into the waveguide and the facet transmissions. To calculate the waveguide loss error, the equation is first rewritten to isolate α.

\[ \alpha = \ln \left( \frac{P_{mean}(x)}{P_{0,mean}} \right) \]  

(5-2)

With this equation, the partial differentials for each factor are calculated. These partial differentials are then substituted into (5-3), the square root of which is the propagated error of the waveguide loss [29].

\[ \sigma_\alpha^2 = \left( \frac{\partial \alpha}{\partial x} \right)^2 \sigma_x^2 + \left( \frac{\partial \alpha}{\partial P_{mean}(x)} \right)^2 \sigma_{P(x)}^2 + 2 \left( \frac{\partial \alpha}{\partial x} \right) \left( \frac{\partial \alpha}{\partial P_{mean}(x)} \right) \sigma_x \sigma_{P(x)} + \left( \frac{\partial \alpha}{\partial P_{0,mean}} \right)^2 \sigma_{P_0}^2 + 2 \left( \frac{\partial \alpha}{\partial P_{mean}(x)} \right) \left( \frac{\partial \alpha}{\partial P_{0,mean}} \right) \sigma_x \sigma_{P_0} \]  

(5-3)

### 5.5 Comparison of Waveguide Losses

The effects of microstructure residue on optical loss can now be examined after testing multiple waveguides of each set width and in each test sample category (RIE process and HF treatment), calculating waveguide losses from curve fitting, and calculating loss errors. The loss error can be added to the waveguide transmission plot, shown in Figure 5-8. All of the losses
and loss errors are compiled within Table 5-2. Within each test sample category, waveguides exhibited increasing losses with decreasing waveguide widths, consistent with expectations.

Figure 5-8: Waveguide transmission plot with error accounted. The solid line is the transmission with the fitted loss slope, and the dashed lines are the transmissions with the error added and subtracted from the fitted slope. (© 2020 IEEE/OSA [7])

<table>
<thead>
<tr>
<th>Ridge Width</th>
<th>Optical Loss (cm⁻¹)</th>
<th>O₂/CHF₃ Without 5% HF</th>
<th>O₂/CHF₃ With 5% HF</th>
<th>O₂/CHF₃ Half Power Without 5% HF</th>
<th>O₂/CHF₃ Half Power With 5% HF</th>
<th>CF₄ Without 5% HF</th>
<th>CF₄ With 5% HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>3µm</td>
<td>Loss</td>
<td>1.585</td>
<td>1.411</td>
<td>1.900</td>
<td>2.925</td>
<td>1.465</td>
<td>0.705</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>0.0625</td>
<td>0.0166</td>
<td>0.0226</td>
<td>0.199</td>
<td>0.2958</td>
<td>0.062</td>
</tr>
<tr>
<td>6µm</td>
<td>Loss</td>
<td>1.448</td>
<td>1.020</td>
<td>0.886</td>
<td>1.696</td>
<td>0.807</td>
<td>0.619</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>0.0394</td>
<td>0.0594</td>
<td>0.0455</td>
<td>0.319</td>
<td>0.0571</td>
<td>0.118</td>
</tr>
<tr>
<td>10µm</td>
<td>Loss</td>
<td>0.722</td>
<td>0.685</td>
<td>0.735</td>
<td>0.913</td>
<td>0.443</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>0.0688</td>
<td>0.0284</td>
<td>0.0964</td>
<td>0.230</td>
<td>0.1069</td>
<td>0.0121</td>
</tr>
</tbody>
</table>

The waveguides etched by the different RIE processes but not treated with 5% HF had observable differences in optical loss. The O₂/CHF₃ “Half Power” process had the highest losses in each waveguide width category. This seems to be caused by the high density of scatter and/or
reflection points as light propagates down “Half Power”-etched waveguides and the attendant “grass” microstructures. The CF₄ process had the lowest loss in each waveguide width category. The CF₄ process’s “stalk” microstructures were much less dense and offered fewer scatter/reflection points. The original “Full Power” O₂/CHF₃ process offers the intermediary as the nanotubes are thicker than both “grass” and “stalks”, creating more severe scatter/reflection points but are much less frequent to a propagating wave than the “grass” microstructures; the loss values correspond to this intermediary microstructure circumstance.

For the waveguides that were treated with 5% HF prior to cladding, differences from each non-HF RIE process category are observed. For waveguides etched by both the original “Full Power” O₂/CHF₃ process and the CF₄ process, the HF treatment reduced optical loss. The reduction in loss was greater for the CF₄ process than for the “Full Power” O₂/CHF₃ process. As was shown earlier in Figure 5-3a, HF-treated nanotubes were reduced in size but did not completely clear away. However, as Figure 5-3c shows, the “stalks” did completely clear away, offering no contribution to the waveguide loss. The 10µm waveguides had the least reduction in waveguide loss when treated with HF, and this is due to the ridges being sufficiently wide to minimizing the effects of microstructure residue presence from the start. Regarding loss error, the HF-treated waveguides exhibited a reduced loss error except for the 6-µm wide ridges in each process case.

It is notable that the HF treatment actually increased the waveguide loss of the “Half Power” O₂/CHF₃ process. It is not plain why this should be, but conjecture is offered here. Noticeable in Figure 5-3b, the HF-treated “grass” microstructures still left some debris but now plastered onto the sidewalls of the ridge waveguide. It is conjectured that this created worse scatter/reflection points than what was already present in “Half Power” conditions. Additionally,
in every waveguide width, HF-treated “Half Power” waveguides also had an increase in loss error. Figure 5-9 shows a comparison of waveguide modes in 3µm ridges (etched with the O₂/CHF₃ “Half Power” process) that were not and were treated with HF. However, the standard O₂/CHF₃ and the CF₄ processes did not exhibit the particle clumping post-HF, and they both showed decreased waveguide loss post-HF.

Figure 5-9: Comparison of waveguide modes in (a) a 3-µm wide waveguide that was not treated with 5% HF and (b) a 3-µm wide waveguide that was treated with 5% HF. These ridges were etched by the O₂/CHF₃ “Half Power” process. (© 2020 IEEE/OSA [7])

The RIE process used It is also notable that the CF₄ process generally results in lower waveguide losses than the O₂/CHF₃ processes. Changing the RIE process used for etching waveguides had the overall most significant impact on the waveguide improvement. However, applying the 5% HF treatment does further boost the performance of a waveguide when maximal transmittance is intended. Based on these results, the CF₄ process with a treatment of 5% HF was chosen to become the new standard for ridge waveguide etching in the biosensor fabrication process outlined in Chapter 4. The waveguide loss is the least in this sample category compared to all others. The etch rate was also fastest of all three studied etch processes, considered an
improvement in the fabrication process. This etch process has since been integrated and used in the fabricated biosensor devices described subsequently in this work.
CHAPTER 6. EXCITATION REGION THEORY

My central work in this dissertation was the development of a working theory behind the optofluidic flow-through biosensor’s signal behavior and how the various design parameters affect a device’s signal. The core of this theory is the excitation region (ER) of the biosensor. This region is the point where an optical mode intersects with the ARROW channel, through which a bio-sample passes through the illuminated volume. This theory integrates several fields of study: optics, waveguiding, fluorescence, collection efficiency, microfluidics, and photon signal counting. This chapter will outline the theory that has been developed and will relate how each component field affects the excitation region. In subsequent chapters, the theory will inform a predictive model that can simulate device designs pre-silicon.

6.1 Fluorescence and Signal Rates

Fluorescence is the means by which a bio-target’s presence is signaled. The bio-sample is processed such that a chosen fluorophore marker will bind to a target prior to passing through the biosensor. The fluorophore, when illuminated by the right wavelength, or range of wavelengths, will fluoresce at a wavelength slightly longer than the exciting wavelength. The rate of incident photons, the fluorophore’s properties, and the number of fluorophores on a target will determine the rate at which fluorescence photons are emitted from a target.

Fluorescence is one of two categories of luminescence, the other category being phosphorescence. Luminescence occurs when a particle or bulk substance is electronically
excited and subsequently electronically relaxes, converting the exciting energy into a photon. Fluorescence, specifically, occurs when a particle’s electron is excited from a ground state to a higher energy band and quickly relaxes back to the ground band, emitting a photon as the excess energy. The two determining factors are the short time duration of being excited before relaxation and that the relaxation energetically brings the particle back down to ground. If the relaxation took a long time-duration and the particle/substance did not relax to the original ground state, the phenomena would be categorized as phosphorescence. The fluorescent energy transition process is illustrated in the Jablonski diagram shown in Figure 6-1.

Figure 6-1: Jablonski diagram of the fluorescence process. An exciting photon is absorbed, exciting an electron from a ground state to a higher energy band. The electron then relaxes back across the bandgap and returns to a ground state. This relaxation emits another photon of slightly lower energy than the exciting photon. (Image is in the public domain, https://commons.wikimedia.org/wiki/File:Jablonski_Diagram_of_Fluorescence_Only.png)
It must be understood the rate at which a fluorophore will emit photons when using fluorescence as a biosensor signal that can be measured in terms of individual photon counts in a unit time. This requires an understanding of the relation between an incident photon rate and a photon emission rate. The time in which a particle is electronically excited, remains excited, relaxes, and can be excited again is the particle’s luminescent lifetime, $\tau$. For the purposes of generating a fluorescence signal, it would be ideal that a fluorophore emit a photon every time it is put into an excited state; this would be called the fluorophore’s natural lifetime, $\tau_n$, and is described mathematically in (6-1). In reality, not every fluorophore excitation results in a radiative relaxation. The fluorophore’s lifetime thus can be understood as the inverse of the sum of the fluorescence emission rate, $\Gamma$, and the nonradiative relaxation rate, $k_{nr}$; this is expressed mathematically in (6-2). A fluorescent lifetime is typically in the order of $10^{-9}$s to $10^{-8}$s, whereas phosphorescence lifetimes range $10^{-3}$ to $10^{0}$s [30].

$$\tau_n = \frac{1}{\Gamma} \quad (6-1)$$

$$\tau = \frac{1}{\Gamma + k_{nr}} \quad (6-2)$$

If a fluorophore has a given lifetime, then there will be a required incident excitation rate to keep it fluorescing continuously. This means there is a number of incident photons of a given wavelength that will equate to one emitted photon. This ratio of emitted photons to incident photon is called the quantum yield, $Q$. Ideally, this ratio would be 1:1, one emitted photon is caused by a single exciting photon. However, due to the fact that there will be some non-radiative relaxations, the quantum yield will be less than 1. The quantum yield can therefore be understood mathematically as the ratio of the fluorescence rate, $\Gamma$, to the sum of the fluorescence and nonradiative rates, $k_{nr}$, given in (6-3).
\[ Q = \frac{r}{\Gamma + k_{nr}} \tag{6-3} \]

Solving (6-2) for \( k_{nr} \) and substituting into (6-3) shows us that the quantum yield is the product of the fluorescence rate and lifetime. Solving for the fluorescence rate, this equation is shown in (6-4); as this is the maximum rate fluorophore can emit a photon, the rate’s symbol here is subscripted with an ‘m’. This equation is useful in calculating the fluorescent lifetime in a commercial fluorophore product as both the quantum yield and lifetimes are common values provided by producers from their statistical findings. For example, the ThermoFisher Scientific® Alexa Fluor 647 (AF647) fluorophore has a quantum yield of 0.33 and a lifetime of 1 ns published on their website [31]. Using these values as an example, the fluorescence generation rate of AF647 is calculated to be \( 3.3 \times 10^8 \) photons/s.

\[ \Gamma_m = \frac{Q}{\tau} \tag{6-4} \]

\[ \Gamma_m \geq \Gamma_e = Q\Gamma_{inc} \tag{6-5} \]

A fluorophore’s maximum emission rate (\( \Gamma_m \)) is important to consider when comparing it with the incident photon rate. The maximum emission rate will always be greater than or equal to observed or calculated emission rate (\( \Gamma_e \)), which is the product of the rate of photons incident on the fluorophore (\( \Gamma_{inc} \)) and the quantum yield, expressed in (6-5).

Because there is a maximum emission rate for a given fluorophore, there is a point at which the exciting optical intensity will maximize the emission rate, but increasing the intensity beyond that point will generate no additional signal. This will inform the modeler the maximum useful optical power setting may be used in generating a fluorescence signal. Developing the theory therefore requires relating the exciting optical intensity (power per unit area) to a number
of photons per area per unit time so that we can compare the incident photon rate to the maximum emission rate. The first step in answering these questions is to derive and calculate the fluorophore’s absorption cross-section.

**Figure 6-2: Illustration of the relation between the incident photons (a) on a fluorophore, Cy5 in this example, and the fluorescence photons (b) emitted by the fluorophore.** Knowing the maximum emission rate, we can calculate if the incidence rate approaches that limit when we calculate the statistically-derived absorption cross-sectional area, represented by the dashed line (c). (This figured is a modified version of an image in the public domain, https://commons.wikimedia.org/wiki/File:Cy3_Cy5_dyes.gif)

### 6.2 Fluorophore Absorption Cross-Section

The fluorophore has a given absorption cross-section ($\sigma_a$) with an area value (cm$^2$ for our purposes here) that can correspond with the incident number of photons per area per time, as illustrated in Figure 6-2 above. This value derived from the Beer-Lambert Law which relates a medium’s optical attenuation to its material properties. For a given medium, illustrated in Figure 6-3, the optical intensity differential ($\partial I$) is related to the medium’s thickness differential ($\partial x$) by the equation given in (6-6). The differential of optical intensity with respect to the medium’s
thickness is the negative product of intensity (I), the absorption cross-section, and the material’s number of molecules per volume (n) measured in particles per cubic centimeter. Integrating this equation with the boundary conditions of x = 0 and I = I₀, we arrive at the Beer-Lambert Equation in (6-7) where d is the medium’s thickness [29].

\[
\frac{\partial I}{\partial x} = -l\sigma_a n \quad (6-6)
\]

\[
\ln \frac{I_0}{I} = \sigma a nd \quad (6-7)
\]

Figure 6-3: Illustration of the Beer-Lambert Law. As light with an intensity I₀ passes through a medium of thickness d, the intensity will reduce to I(x=d).

The Beer-Lambert Equation has another form which will be component in calculating a fluorophore’s absorption cross-section. This form of the equation is given in (6-8) and relates
the change in optical intensity to the material’s concentration (C) in moles/liter and its molar extinction coefficient (ε) measured in M⁻¹ cm⁻¹; the extinction coefficient is another value commonly provided by fluorophore manufacturers, thus a relation between this value and the absorption cross-section is needed [32].

The two forms of the Beer-Lambert Equation can be equated and solved for σ. The natural logarithm of I(x)/I₀ is 2.303 times greater than the ten-base logarithm of I(x)/I₀, thus the absorption cross-section can be related to the extinction coefficient, molar concentration, and particle number density in (6-9). Since the particle number density, n, is related to the molar concentration, c, and Avogadro’s number, Nₐ, by (6-10), the absorption cross-section can be related directly to the extinction coefficient in (6-11).

\[
\log_{10} \frac{I_0}{I} = \varepsilon C d \tag{6-8}
\]

\[
\sigma_a n d = 2.303 \varepsilon C d \tag{6-9}
\]

\[
n = \frac{N_A C}{10^3} \tag{6-10}
\]

\[
\sigma_a = 3.824 \times 10^{-21} \varepsilon \tag{6-11}
\]

Continuing with AF647 as an example, ThermoFischer Scientific published an extinction coefficient of 270,000M⁻¹cm⁻¹ [33]. This value allows for the absorption cross-section to be calculated to be 1.032×10⁻¹⁵ cm² (1.032×10⁻¹⁹ m²). With this area value, the rate of incident photons can be calculated as it relates to a given optical intensity value, and therefore it can be known how much this incident photon rate will approach the fluorophore’s maximum emission rate.
6.3 Optical Intensity, Incident Photon Rate, and Maximum Fluorescence Photon Emission Rate

Photon rates can be converted from measurable or calculable optical intensity values. Optical intensity is defined here as the amount of optical power in a given area (A). Power (P) itself is a rate of energy change in a unit time (t) measured in seconds, and optical energy can be converted into a number of photons of a given wavelength (λ). The photon flux (Φ), measured in photons per area per time, relates all of these factors and is given in (6-12), where h is Planck’s constant and c is the speed of light.

\[ \Phi = \frac{P\lambda}{Ahc} \]  

(6-12)

Multiplying the incident photon flux of an exciting light by the absorption cross-section of a fluorophore will give the incident photon rate (Γ\text{inc}) as shown in (6-13) above. This equation gives us four input variables for which the modeler should be able to account: input power setting, optical wavelength, the relevant illuminated area, and the fluorophore absorption cross-section. The power and wavelength values are fairly straightforward, but some consideration should be given to the relating the illuminated area (A) and absorption cross-section (\sigma_a).

\[ \Gamma_{\text{inc}} = \Phi_{\text{inc}}\sigma_a = \frac{P\lambda}{Ahc}\sigma_a \]  

(6-13)

The abovementioned relation between area values is depends on the intensity profile of the illuminated area. If the illuminated area has a uniform intensity profile, then the calculation is simple and can be done simply by dividing the known total power value by the total illuminated area. If the intensity profile is not uniform, then some area resolution within the intensity profile is required. This area resolution is best chosen to be the smallest rectangular area that can encapsulate a fluorescing particle; assuming the particle approximates a sphere or
circle, the resolution area’s dimensions are chosen to match the particle’s diameter as illustrated in Figure 6-4. This resolution is important in calculating the amount of fluorescence that is generated described later in this chapter and creating the simulation mesh described in the next chapter.

![Figure 6-4](image)

**Figure 6-4:** An illustration of how optical intensity profiles, shown as large red circles, relate to the fluorescing particle’s cross-section, shown as small black circles. (a) If an intensity profile is uniform, the rate photons incident on the particle is constant regardless of the particle’s position within the illuminated region. (b) If an intensity profile is nonuniform, the rate of photons incident on the particle will vary with the particle’s position. Area resolutions are constructed to be the smallest rectangular area that encapsulates particle cross-section.

The question regarding how incident photon rates compare to the maximum fluorescence photon emission rate can now be answered. The key to this question is the point at which increasing optical intensity provides no additional fluorescence signal. Using AF647 again ($\Gamma_m = 3.3 \times 10^8$ photons/s, $\sigma_a = 1.032 \times 10^{-19} \text{ m}^2$) and a uniform optical intensity profile, we can calculate an example evaluation. As an initial calculation, if we assume an optical power setting of 1mW, an excitation wavelength of 633nm, and an illuminated area of $20 \mu\text{m}^2$ ($2 \times 10^{-11} \text{ m}^2$), we
can calculate from (6-13) that the incident photon rate on an AF647 fluorophore is 
\[ \Gamma_{\text{inc}} = 1.644 \times 10^7 \text{ photons/s}. \]
When we calculate the AF647 fluorescence photon emission rate as given in (6-5), we can see that the emission rate is \(5.426 \times 10^6\) photons/s, nearly two entire orders of magnitude below the maximum emission rate. Alternatively, using (6-5) and (6-13), we can calculate the maximum power setting that will maximize the fluorescence emission rate, keeping other variables the same, and arrive at 60.8mW to achieve the maximum fluorescence rate.

While a uniform intensity profile provides an instructive example, and there are some situations that call for modeling a uniform optical intensity, it is far more common in practice for the modeler to use nonuniform intensity profiles. Most illuminated regions can be approximated with a Gaussian distribution. This requires a resolution area to be calculated within the intensity profile to approximate the fluorescing particle’s cross-section (\(A = 1.314 \times 10^{-19}\) m\(^2\) for the case of AF647). Performing the same operation for finding the maximizing power in an intensity profile resolution area that was done above, we calculate this power value to be \(4 \times 10^{-10}\)W within just that resolution area. However, calculating the incident photon rate of every location in the illuminated area would be tedious without the assistance of computing, and this example is simply for comparing the cases of uniform and nonuniform optical intensity profiles. In the next chapter, a succinct description of the calculation done in a simulation shall be given.

6.4 Exciting Optical Intensity Profile and Accumulating Incident Energy

The material in this chapter prior to this point extensively describes the modeler’s concern with signal-generating fluorescence. From this point on in this chapter, the theory’s subcomponents will be related to each other and how they influence the generation of the fluorescence signal shall be described. For the purposes of orientation, Figure 6-5 illustrates that
the particle’s path down the ARROW channel and through the excitation region follows the z-direction, while the channel width follows the x-direction and the height follows y.

Figure 6-5: Illustration of the excitation region orientation to follow for the remainder of this work. The width of the ARROW channel is oriented with the x-axis, and the height with the y-axis. The fluorescing particles will travel down channel along the positive z-axis and across the optical intensity profile. (© 2021 MDPI [9])

The first component in constructing the excitation region is the exciting optical intensity profile. This was discussed in the previous section regarding the relation between incident excitation photons and emitted fluorescence photons. Here, the general shape and power distribution of the illuminated region will be considered.

At the time of writing, there are two illumination formats: planar (a.k.a. side) and top. The planar illumination format is done by coupling a laser to the side facet of a solid ridge waveguide. This solid waveguide then guides the mode to the ARROW channel and forms a Gaussian-like profile. Depending on the ridge cross-sectional geometry, the mode profile may be large and have lower optical intensity, or it may be small and well confined with a high optical intensity. More will be described in the following chapter on model construction, but the modeler can create a mode profile using software such as Lumerical® Mode Solutions™, export
this profile, and distribute the simulated laser power setting across the Gaussian-like mode profile. This distribution will have the peak power at the mode intensity peak, and the remaining power distributed accordingly.

In a top-illumination format, a laser is focused onto the ARROW channel from above. Devices used in this format have an optically blocking and possible reflective layer deposited over the chip; one or several windows are etched or milled through this blocking layer such that light can illuminate the ARROW beneath. The laser spot is usually sufficiently large compared to the window dimensions that the intensity profile incident on the window is approximately uniform. This makes the optical excitation energy constant regardless of a passing fluorescing particle’s position in the ARROW.

As the fluorescing particle passes through the illuminated region, it will fluoresce according to the intensity of light incident on it. The amount of power incident on a passing particle is summed. This summed power value will be converted into an energy value by multiplication with the time the particle spends in the excitation region. The time value must first be calculated as a function of the particle’s x-y position within the ARROW channel.

6.5 Flow Velocity and Time Profiles in the ARROW Channel

Calculating the duration that a particle spends in the excitation region is simple, dividing the excitation region depth by the velocity at which the target particle flows in the sample stream. The depth of the excitation region is simply defined as the width of the optical mode, which is in the z-direction. What does require attention is the calculation of the particle’s velocity. First, I assumed that the particle’s velocity is the same as the fluid flow velocity at its
location in the ARROW cross section; the particles are less dense than the surrounding fluid matter, so slippage within the fluid streams is not anticipated.

The next consideration is the fluid flow velocity of a rectangular duct. Fluid flow in this optofluidic flow-through biosensor platform has a laminar regime. Laminar flow is a condition characterized by no internal fluid turbulence. The fluid particles flow in layered sheets without mixing. However, the velocities of these sheets are not necessarily equal. Within a duct, the fluid sheets adjacent to the sidewalls of the duct have zero velocity while the sheets in the center of the duct flow at the highest velocities. From an entry point into a duct that is invariant down its length, the fluid flow velocity profile develops until it attains a steady state conditions called being “fully developed”. The length of the duct over which the fluid develops to this steady state is called the hydrodynamic entrance length or entry length, $L_H$. After a fluid flow regime fully develops, the velocity profile attains a parabola-like distribution, as illustrated in Figure 6-6.

![Figure 6-6: Illustration of a parabolic flow velocity profile developing in a laminar flow regime across the distance of a fluid duct. The arrows represent fluid flow velocity, and they gradually increase in magnitude closer to the center as the fluid develops. The fluid eventually becomes fully developed at which point the velocity profile attains a steady state; the distance for this profile to fully develop is the entrance length, $L_H$.](image)
As the particle’s position in the ARROW cross-section has some determination over its flow velocity, it becomes necessary to calculate a two-dimensional velocity profile. The first step is to confirm whether the fluid flow is fully developed at the point the exciting light intersects with the ARROW channel, so the entrance length is to be calculated and compared with the exciting light’s position along the ARROW length. The entrance length is calculated by the equation (6-16), where $Re$ is the Reynold’s number and $D_H$ is the hydraulic diameter of the rectangular duct [34,35].

The Reynold’s number is a unit-less ratio of a fluid’s flow velocity and its dynamic viscosity; fluids with a Reynold’s number less than 2300 are within the laminar flow condition. The fluid’s density in kg/m$^3$ is symbolized by $\rho$, the mean velocity in m/s by $u_m$, the dynamic viscosity in Pa·s by $\mu$, and the duct’s hydraulic diameter in meters by $D_H$. The hydraulic diameter is so termed because the theory of hydrodynamic development began with circular ducts for simplicity, but it can be calculated for noncircular ducts with the equation given in (6-15) where $A$ is the duct cross-sectional area and $P$ is the duct’s inside perimeter [35, 36].

To quickly check the biosensor’s entry length, we can set the ARROW cross-section to be $12\times6$ µm$^2$ ($7.2\times10^{-11}$ m$^2$) and a typical mean fluid velocity of 0.02 m/s. Water is the fluid commonly used to suspend bio-samples in this biosensor platform ($\rho = 997$ kg/m$^3$, $\mu = 8.9\times10^{-4}$ Pa·s). With these parameters, we calculate the Reynold’s number to be 0.163, well below the critical number of 2300 where laminar transitions into turbulent flow, and the entry length comes out to be $6.511\times10^{-8}$ m (0.065 µm). As the excitation region is either 55 µm or 245 µm from the inlet to the ARROW, depending on chip orientation during testing, the model can incorporate a hydrodynamically fully developed flow velocity profile.
The flow velocity profile can be calculated now that it is determined to be hydrodynamically fully developed. The cross-section of the ARROW channel can be described in Figure 6-7, with the horizontal dimension (x-axis) given as 2a, the vertical dimension (y-axis) given as 2b, the fluid flowing along the z-axis, and the aspect ratio of the channel dimensions given as \( \alpha = 2b/2a \).

The equation for fluid momentum in this duct is expressed in (6-17), where \( g_c \) is the proportionality constant in Newton's second law of motion (\( g_c \) is 1 and dimensionless in SI units), \( p \) is the value for pressure in the flow direction, and \( \nabla^2 \) is the Laplacian operator for two dimensions [37,38]. As the pressure drop is constant in the x- and y-directions, the operator is set to \( c_1 \). As the ARROW cross-section is set on the x-y plane, (6-17) can be rewritten as (6-18). The boundary conditions are set to no-slip, such that the velocity at the sidewalls have zero velocity, i.e. \( u = 0 \).

\[
\nabla^2 u = \frac{g_c}{\mu} \frac{dp}{dx} = c_1
\]

\[
\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} = c_1
\]
Figure 6-7: Cross-section and orientation of the ARROW channel.

Solving (6-18) for velocity with this boundary condition, the velocity profile can be described mathematically in (6-19), with the mean velocity written in (6-20). These equations are computationally complex in this form, but they can be approximated by (6-21) if the aspect ratio is less than or equal to 0.5, which is typical of this biosensor platform [37]. The exponents \( m \) and \( n \) are expanded in (6-22) and (6-23), respectively.

\[
\begin{align*}
\mathbf{u} &= -\frac{16c_1a^2}{\pi^3} \sum_{n=1,3,\ldots}^{\infty} \frac{1}{n^3} (-1)^{\frac{n-1}{2}} \left[ 1 - \frac{\cosh\left(\frac{n\pi y}{2a}\right)}{\cosh\left(\frac{n\pi b}{2a}\right)} \right] \cos\left(\frac{n\pi x}{2a}\right) \\
\mathbf{u}_{\text{mean}} &= -\frac{c_1a^2}{3} \left[ 1 - \frac{192}{\pi^5} \left(\frac{a}{b}\right) \sum_{n=1,3,\ldots}^{\infty} \frac{1}{n^5} \tanh\left(\frac{n\pi b}{2a}\right) \right] \\
\frac{\mathbf{u}}{\mathbf{u}_{\text{max}}} &= \left[ 1 - \left(\frac{y}{b}\right)^n \right] \left[ 1 - \left(\frac{x}{a}\right)^m \right] \\
\mathbf{m} &= 1.7 + 0.5\alpha^{-1.4} \\
\mathbf{n} &= \begin{cases} 
2, & \alpha \leq \frac{1}{3} \\
2 + 0.3 \left(\alpha - \frac{1}{3}\right), & \alpha \geq \frac{1}{3}
\end{cases}
\end{align*}
\]
Integrating (6-21) over the dimensions of the ARROW channel gives two equations that relate the velocity profile and maximum velocity, (6-24) and (6-25), respectively, to a given mean velocity [37]. The modeler can decide upon a mean velocity around which the flow velocity profile will be constructed, and the equations given in (6-22) through (6-25) will construct the profile. A flow velocity profile with a parabolic distribution will be calculated, as Figure 6-8 illustrates with a mean velocity of 2 cm/s, and a 12 µm × 6 µm ARROW channel; this results in a maximum velocity of 4.28 cm/s in the center of the channel.

\[
\frac{u}{u_{\text{mean}}} = \left( \frac{m+1}{m} \right) \left( \frac{n+1}{n} \right) \left[ 1 - \left( \frac{y}{b} \right)^n \right] \left[ 1 - \left( \frac{x}{a} \right)^m \right]
\]

(6-24)

\[
\frac{u_{\text{max}}}{u_{\text{mean}}} = \left( \frac{m+1}{m} \right) \left( \frac{n+1}{n} \right)
\]

(6-25)

Figure 6-8: Illustration of a calculated fully developed flow velocity profile.

With the velocity profile now theorized and calculated, the modeler can quickly calculate the profile of the ARROW channel regarding the amount of time a fluorescing particle passes
through the excitation region. The depth of the excitation region is defined by the width of the optical mode or otherwise illuminated region within the excitation region. This depth is then divided by the velocity profile to create a time profile with respect to the particle’s x-y position within the channel cross section.

The no-slip boundary condition which states there is zero velocity at the duct’s walls implies there is an infinite time value at the locations on the walls. This presents a case in which the model must consider distance from the duct walls at which a particle will flow in a finite time. This exclusion zone will be defined in greater detail in the next chapter, which outlines the virtual construction of the model and the simulation of sample tests. For the purpose of constructing a model theory, the outermost molecules in the fluid adhere to the duct walls and the remaining fluid flows over this layer of molecules.

![Time Profile of a Fully Developed Fluid Flow Cross Section](image)

**Figure 6-9:** Illustration of the amount of time a fluorescing particle passes through the excitation region as a function of its x-y position within the ARROW.
In this time profile, the longest time values are found in the corners of the ARROW followed by the positions immediately adjacent to the channel perimeter, if the stream sheets adjacent to the sidewalls are excluded. The general flatness of the internal section of this profile is determined by the ER depth and ER velocity profile. Continuing with the example calculated and illustrated in Figure 6-8, a time profile calculated from a 2 µm excitation region depth is shown in Figure 6-9. This Figure, as well as subsequent Figures showing profiles that incorporate fluid flow, has the outermost layer of molecules removed from consideration such that infinite time be not an illustrated factor.

6.6 Fluorescence Generation and Collection

Now that the optical intensity and ARROW flow-time profiles are defined, they can be used to calculate the amount of fluorescence energy a fluorescing particle will emit while passing through the excitation region. In a laminar flow condition, the particle will not change streamlines, i.e. its x-y position will not change, in the time it takes to cross the excitation region depth. Therefore, the power incident on the particle can be summed as it tracks across the particle’s z-path in the optical mode; this is done by summing the power values within the individual area resolution cells on the particle’s path.

This power value is then multiplied by the time value associated with the particle’s x-y position to create an incident energy value; multiplying this incident energy value by the chosen fluorophore’s quantum yield will give a fluorescence energy value. Expanding this across the ARROW cross-section creates a profile of fluorescence energy generation; this is illustrated in Figure 6-10, continuing with the previously stated parameters and with a side-illuminated optical
mode set at 1mW. The regions of this profile that correspond to optical high intensity, long time periods for the particle’s passing, or both.

![Fluorescence Energy Generation](image)

**Figure 6-10: Illustration of the amount of fluorescence energy generated by x-y position.**

Not all fluorescence energy that is generated will be collected by the collection ridge waveguide and guided to the biosensor detection system. There are two considerations to calculating the fraction of fluorescence that can be collected as a signal: the amount of fluorescence that is coupled into a mode that is guided by the ARROW, and the transmittance of the ARROW itself. It has been previously examined that the center of the ARROW cross-section is the most coupling efficient point, with the efficiency dropping as the fluorescing particle’s position moves closer to the walls of channel [39]. Previous studies have also examined the waveguide loss of the ARROW [23,40,41].

For the modeler’s purposes, both of these considerations can be calculated at once by way of finite-difference time domain (FDTD) simulated propagation [42]-[44]. This method
allows the modeler to define the boundary conditions, geometry, and indices of refraction in which the simulated electromagnetic wave propagates. This volume geometry is broken up into finite mesh cells. The wave’s propagation is calculated by a numerical analysis in which the partial differentials of the time dependent Maxwell equations, listed in (6-26) through (6-29), are discretized to produce finite-difference equations. Within the mesh cells, the finite difference equations are solved for both electric and magnetic field components, the mesh cells become boundary conditions for the adjacent set of mesh cells, and the process repeats until all the cells in the simulated region are solved.

$$\frac{\partial B}{\partial t} + \nabla \times E = 0 \quad (6-26)$$

$$\frac{\partial D}{\partial t} - \nabla \times H = J \quad (6-27)$$

$$B = \mu H \quad (6-28)$$

$$D = \epsilon E \quad (6-29)$$

Using software that performs the FDTD method, such as Lumerical® FDTD Solutions™ (illustrated in Figure 6-11), the modeler builds the ARROW channel geometry to match the distance between the excitation region and the facet where the collection ridge waveguide collects the fluorescence. The modeler then sweeps a dipole source across the ARROW cross-section, performs the FDTD calculations at each location point, and measures the fraction of coupled and transmitted optical power for each dipole location. The modeler then creates a collection efficiency profile by compiling all the transmission values according to their associated dipole x-y position. Figure 6-12 illustrates an example profile for an ARROW cross
section, showing that the collection efficiency has a peak that runs through the vertical center of
the channel and reduces close to the channel boundaries.

Figure 6-11: Example of ARROW geometry construction for FDTD calculation, built within Lumerical® FDTD Solutions™.

Figure 6-12: Collection efficiency profile done by FDTD sweep across the ARROW cross-section.
The fluorescence energy generation is multiplied by the collection efficiency to produce a collected fluorescence energy profile. This in effect is the profile that describes the excitation region, thus it will also be called the “excitation region profile”. A fluorescing particle that passes through this profile will emit fluorescence energy (that will also be collected) that corresponds to the particle’s x-y position. This energy is convertible to a number of photons, given the fluorescence wavelength. This excitation region profile is illustrated in Figure 6-13, continuing with the example parameters outlined in Figures 6-8 through 6-10. While this example ER profile is useful for illustrating the model theory, the profile shape varies depending on the optical mode power, intensity, and number of mode lobes. This aspect will be explored later in Chapter 8 when multiple design variations are compared.

The next chapter will follow a similar outline to this chapter, but it will focus on the model’s construction for simulation purposes.

![Figure 6-13: Excitation Region profile of collectible fluorescence energy according to x-y position within the ARROW channel.](image)
CHAPTER 7. MODEL CONSTRUCTION AND SIMULATION

The previous chapter described the theory behind the formation of the excitation region (ER) within the ARROW channel. The excitation region is the core of the model, and the model’s construction will be outlined in this chapter.

7.1 Fluorescing Particle Selection and Model Mesh Configuration

The first consideration in the model’s construction is setting the mesh size, and the number of mesh cells, in the various components described in the previous chapter. It was previously stated that the resolution area of the optical mode profile was to be set to the smallest rectangular area that encapsulates the fluorophore absorption cross-section. This resolution area becomes the mesh size for all other model factor profiles to be constructed. Every resolution area in the mode profile will later be attributed a power value as a fraction of the total mode power.

If a single fluorophore molecule were the target particle, the particle’s small size would require a fine mesh to accurately approximate the amount of optical power incident on it. If nucleic acid chains hundreds of base pairs long were the target, the resolution area should be calculated to be tens of nanometers long on one side. The number of fluorophores on the chain would also have to be factored in as a multiplier.

Because there is a wide variety of potential fluorophores that can be simulated, it becomes prudent to keep to a constant particle for the remainder of this work. As will be
discussed in subsequent chapters, ThermoFisher Scientific® FluoSpheres™ Carboxylate-Modified Microspheres 625/645 Crimson (0.2µm diameter) are often used in testing fabricated biosensor chips for device sensitivity without processing a biosample [45]. The physical test results described later in this work used these “beads”. This bead has also been evaluated to create an equivalent with published AF647 fluorescence data as this bead has the same excitation/emission wavelength spectra [8].

This bead will inform the mesh size and fluorophore parameters for the remainder of this work. The mesh size has been set to be 0.2×0.2µm² in all subsequent model components in this work. This mesh size is much larger than would be needed for smaller biological targets, but it is useful to illustrate the various model components and to compare the simulation to later-described physical tests that use the 200nm beads.

7.2 Optical Intensity Profile

7.2.1 Side-Illuminated Modes

Using the Lumerical® MODE Solutions™ software, cross-sectional geometries of the excitation ridge waveguides are constructed. The geometry indices of refraction are set, the mesh size entered, and the simulation region is defined. The mode profiles are then generated by eigenmode formulation, which is a finite differences eigenvalues operation [46]-[48]. This produces multiple possible mode profiles contained in the simulation region and waveguide geometry. Examples of the simulation cross-section and intensity mode profiles are given in Figure 7-1.
The mode profiles to be used in model construction are exported. The intensity profile matrices are converted into a MATLAB data file (or a .txt file that can be read into MATLAB). The real-number component of this intensity profile is extracted and set to absolute values, shown in Figure 7-2a. This intensity matrix (\( \mathbf{I} \)), with \( i \times k \) rows and columns, is then normalized to the sum of intensity values in this matrix and multiplied by the input laser power setting (\( P \)), mathematically described in (7-1); this distributes the input laser power across all mesh cells in the profile. If the excitation ridge waveguide transmission is known, it can be factored into this calculation; otherwise this \( T_{\text{WGex}} \) factor is set to 1. The sum-normalized and power-distributed optical mode profile is illustrated in Figure 7-2b.

\[
P_{i,k} = P_{\text{laser}} T_{\text{WGex}} \frac{I_{i,k}}{\sum_{i,k} I}
\]  

(7-1)
Figure 7-2: (a) Optical mode intensity profile exported to MATLAB with a peak value of 1. (b) Profile of the laser power distributed over the intensity profile.

7.2.2 Top-Illuminated Windows

The model construction for top-illuminated devices is somewhat less involved than the side-illuminated modes. It can be done without software outside of MATLAB, though that option still remains if the modeler decides to utilize it. But within MATLAB, the modeler defines the window dimensions by setting the number of mesh cells in each direction. If a window that transmits the exciting light is 4µm wide in the z-direction and covers the 12µm ARROW channel width, the matrix defining the window is set to 20 rows and 60 columns (keeping the 200nm mesh dimensions).

As it is assumed the optical intensity incident on the window is uniform, the window matrix is initially set to contain only values of 1. Then, following the operation shown in (7-1), the intensity is normalized according to the sum of intensity values, and the optical power is distributed across this matrix. The power profile is then defined to be uniform.
7.2.3 Summed-Power Vector

As the fluorescing particle tracks across the mode profile in the z-direction, the model sums up the accumulated power values in each mesh cell. Because of the laminar flow condition in biosensor devices, the particle’s z-path does not change its y-position across the mode width. Also, as the fluid flow velocity is fully developed at this point, the velocity and time values will not vary across the ER depth. Following these points, the summed power value can be calculated entirely from a single row of the optical mode matrix, expressed mathematically in (7-2), to be later multiplied by a selected time value. Summing all the rows of the optical mode matrix results in a summed-power vector displayed in Figure 7-3, using the side-illumination mode profile example. This summed power vector is then saved for subsequent multiplication with a time matrix.

\[
P_{\text{sum},i} = \sum_k P_{i,k} = \begin{bmatrix} P_{\text{sum},1} \\ P_{\text{sum},2} \\ \vdots \\ P_{\text{sum},i}^{\text{th}} \end{bmatrix}
\] (7-2)

Figure 7-3: Summed power values across the mode profile width, i.e. the ER’s depth.
### 7.3 Fluid Velocity Profile and Flow Time Profile

The fluid velocity profile is calculated according to the process outlined in Chapter 6. To generate the velocity profile, a fully developed flow velocity function is imported into MATLAB and used to calculate the velocity in every defined mesh cell [49]. Using this function, the x-y ARROW dimensions and number of mesh units are set, and a user-inputted mean flow velocity is used to calculate the velocity in every mesh cell. The resulting velocity profile is given in Figure 7-4 for a 200nm mesh size, 2 cm/s mean velocity, and a 12µm × 6µm channel.

**Figure 7-4: Flow velocity profile with a 2 cm/s mean velocity.**

With the flow velocity matrix constructed, it will be divided by the depth of the excitation region, $d_{ER}$. This produces the time matrix which will be used to calculate the amount of excitation energy that is incident on the passing fluorescing particle. At this point, an exclusion zone must be defined to account for the no-slip boundary condition described in Chapter 6. The model here defines the exclusion zone as the outermost rows and columns in the various
ARROW profile matrices (time, fluorescence generation, and excitation region). This is corroborated by findings that the 200nm beads do not appear to flow less than 200nm away from the duct walls [50]. The model at this time defines the exclusion zone to be the outermost row and column of the ARROW profiles with a resolution area sized to the simulated particle. The time matrix with the defined exclusion zone on the duct walls is illustrated in Figure 7-5.

\[ t_{i,j} = \frac{d_{ER}}{v_{i,j}} \]  

(7-3)

Figure 7-5: Time profile of an excitation region with a 2µm depth.

7.4 Incident Energy Profile

With the summed-power vector \( P_{\text{sum}} \) and time matrix \( t \), the incident energy matrix can be calculated. A new matrix (Figure 7-6a) is created with \( i \times i \) rows and columns. The values of the vector \( P_{\text{sum}} \) are set along the primary diagonal of this matrix, with all other values set to zero. This matrix is then matrix multiplied with \( t \) to create the incident energy matrix, \( E_{\text{inc}} \), which is
i \times j \) rows and columns. Multiplying this incident energy matrix by the fluorophore quantum yield, \( Q \), will produce the fluorescence energy generation matrix, shown in Figure 7-6b.

\[
diag(P_{sum,l}) = \begin{bmatrix}
P_{sum,1} & \cdots & 0 \\
\vdots & \ddots & \vdots \\
0 & \cdots & P_{sum,lth}
\end{bmatrix}
\]  

\[
E_{inc;i,j} = diag(P_{sum,l}) \ast t_{i,j} 
\]

\[
E_{fluor;i,j} = Q \cdot E_{inc;i,j} 
\]

Figure 7-6: (a) Profile of excitation energy incident on a passing particle as a function of the particle’s x-y position. (b) Profile of the amount of energy a fluorescing particle will emit.

7.5 Collection Efficiency Profile

As stated in the previous chapter, the collection efficiency profile is done by sweeping a dipole source across the ARROW cross section and performing finite-differential time domain (FDTD) calculations at every sweep point. The FDTD calculations described in this work were done using the Lumerical® FDTD Solutions™ software suite [51]. The dipole is set emit light at 670nm wavelength to match that of the beads’ emissions. A monitor measuring power
transmission is set at a distance of 245µm from the excitation region, as this is a typical distance to the collection ridge waveguide facet in fabricated devices.

The modeler selects sweep points to be located at every mesh cell location in the ARROW cross section. This sweep is also done three times at different dipole orientations, along the x-y-z axes, to account for the three polarization extremes. The transmissions from each dipole polarization are averaged to simulate the emissions of an isotropic point source. As these calculations have intensive computing resource requirements and are done in many iterations, it is prudent to make use of a supercomputer that can handle high-resource jobs in large batches. After all the FDTD calculations are returned, the three transmission profiles of varying dipole polarizations are averaged, and an effective collection efficiency matrix (CE) is produced, shown in Figure 7-7.

![Fluorescence Collection Efficiency Profile](image)

**Figure 7-7: Effective collection efficiency profile.**
7.6 Excitation Region Profile

With the fluorescence generation and collection efficiency matrices created, the fluorescence energy collection profile (a.k.a. “excitation region profile”) can be created. The excitation region profile $E_{ER}$ is calculated by finding the Hadamard product of $E_{fluor}$ and $CE$, as expressed in (7-7). The Hadamard product is an elementwise multiplication process in which the value found in a given $i,j$ index of one matrix is multiplied by the value in the equivalent $i,j$ index of another matrix [52]. The resulting excitation region profile is shown in Figure 7-8.

$$E_{ER;i,j} = E_{fluor;i,j} \odot CE_{i,j} = \begin{bmatrix}
E_{fluor;1,1}CE_{1,1} & \cdots & E_{fluor;1,jth}CE_{1,jth} \\
\vdots & \ddots & \vdots \\
E_{fluor;i,1}CE_{i,1} & \cdots & E_{fluor;i,jth}CE_{i,jth}
\end{bmatrix} \quad (7-7)$$

![Excitation Region Profile](image)

Figure 7-8: Excitation region profile to be sampled for biosensor simulation.
7.7 Excitation Region Sampling and Signal Simulation

With the excitation region model constructed, it can be sampled for signal simulation. In MATLAB, a set number of random samplings are taken from $E_{ER}$ with a uniform probability distribution and placed in a vector array ($E_{sample}$) for further calculation; this is to simulate a number of particles (equal to the number of sampled ER values) passing through the excitation region. Published observations of biosensor detection indicate that flowing fluorescing particles do not flow within 0.2-0.5\(\mu m\) of the ARROW sidewalls [50]. To match this observation, the outer 0.2\(\mu m\) of the ER profile are excluded from random sampling.

The collected fluorescence energy values in $E_{ER:sample}$ are converted to a number of photons according to the equation (7-8). If the various waveguide and facet transmission fractions are known or definable, they can be factored in; otherwise they are set to 1 to examine the ER solely. Also, if a detector efficiency ($\eta_{det}$) is known or definable, it can be factored into the photon count calculation as well. The resulting photon counts are kept in a vector array.

$$\text{Photon count}_m = E_{ER:sample}T_{WG}T_{facet}\eta_{det}\left(\frac{A}{hc}\right)$$ (7-8)

The biosensor signal is measured as an intensity unit, photon counts per 0.1\(ms\). The remaining signal calculation step is to divide the number of photons by the time ($t_{sample}$) the corresponding simulated particles spend in the excitation region, shown in (7-9). This time is converted from being measured in seconds into intervals of 0.1\(ms\). This signal vector then available for signal analysis.

$$\text{Signal}_m = \frac{\text{Photon count}_m}{t_{sample:m}} \ast 10^{-4}$$ (7-9)
Running the simulation gives a distribution of signal magnitudes that can be analyzed. The shape of this distribution informs the sensitivity of the biosensor design being simulated. Instructive signal statistics include the signal mean, standard deviation, and coefficient of variance. The coefficient of variance (CV) in this work is defined as the ratio of the signal standard deviation to the signal mean. An effective biosensor has a distribution with a high mean signal value as well as a small signal variance; biosensor designs typically have these qualities to varying degrees.

An example signal distribution is shown in Figure 7-9, where 1000 particles were simulated using the example ER profile given in Figure 7-8; the transmission factors in (7-9) were set to 1 in this example, while the detector efficiency was set to 65%. Table 7-1 lists all the model parameters that factored into the generation of the excitation region profile. There is a high number of low-signal events, and the number of events gradually decreases with increasing signal magnitude; this corresponds with the distribution of energy values in Figure 7-8. The mean signal in this example is 3861 counts/0.1ms, and the standard deviation is calculated to be 4375 counts/0.1ms; the CV is 1.133, which is not characterized as representing a small variance.

<table>
<thead>
<tr>
<th>Table 7-1: List of Model Parameters used in Chapter Continuing Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Mode Profile Height/Width</td>
</tr>
<tr>
<td>Optical Mode y/z Mesh Cells</td>
</tr>
<tr>
<td>Optical Mode Width (ER Depth)</td>
</tr>
<tr>
<td>Optical Mode Power</td>
</tr>
<tr>
<td>ARROW Height/Width</td>
</tr>
<tr>
<td>ARROW y/x Mesh Cells</td>
</tr>
<tr>
<td>Mean Flow Velocity</td>
</tr>
</tbody>
</table>

While the signal distribution will vary depending on the random sampling of the ER profile, simulating increasing numbers of particles (number of samplings) will exhibit greater consistency in simulated results. The primary factor determining the signal distribution is the
excitation region itself. In the following chapters, the impact of biosensor design parameters on the excitation region will be investigated.

Figure 7-9: Example signal distribution of a simulated biosensor design.
The purpose of the model and simulation described in this work is to create a tool that can predict the sensitivity of a biosensor design before investing time and resources into the design’s fabrication. To validate the model’s accuracy, three biosensor design variations with the side-illumination format were fabricated and tested for signal performance. These designs were also modeled and simulated, and the simulated signals and the signals from the fabricated devices were compared. Additionally, this work gave the opportunity to test new designs, the excitation regions of which could be conjectured but were unknown otherwise. This chapter will outline this validation work.

8.1 Three Biosensor Designs

8.1.1 Standard ARROW and Excitation Ridge

The first of the compared designs was the Standard device design. The ARROW core was set to be 6µm high and 12µm wide. The excitation ridge waveguides were made from 1.51 refractive index silica 6µm thick; the ridge was etched down 3.15µm and were 5µm wide. The fabrication process closely followed the process outlined in Chapter 4. Figure 8-1 illustrates this design’s cross-section.
The excitation ridge waveguide in this design is sufficiently large to support multiple likely mode profiles. Two excitation modes are typically observed, one with a single lobe and another with two intensity lobes. This presents a dynamic in which a single design offers possible ER tradeoffs. Modes with a single lobe concentrate the optical power and intersect with the collection efficiency peak in the ARROW, but this is also where fluorescing analytes spend the least time fluorescing. Double-lobe modes divide the optical power over two areas and do not intersect with the collection efficiency peak, but they place the power in portions of the ARROW where flow speeds are lower and fluorescing times are greater.

8.1.2 “Three-Micron” ARROW and Excitation Ridge

The next biosensor device that was studied was a new, previously untested design. This design features an ARROW channel that is 12µm wide and only 3µm high. The excitation ridge waveguide was made from a 1.51-index silica film that was 3µm thick and etched down 2.25µm
and had a width of 2µm. This design was dubbed the “Three-Micron” design and is illustrated in Figure 8-2.

![Figure 8-2: Excitation ridge waveguide cross-section of the Three-Micron biosensor design. (© 2021 IEEE/OSA [8])](image)

The intent behind this design was to restrict the optical mode to a single Gaussian-like lobe that would illuminate the entire height of the ARROW channel such that the possibility of a particle passing through a negligibly-intense part of the ER is mitigated. The intensity peak would intersect with the collection efficiency peak. Before this design was modeled and fabricated, it was anticipated that a fully-illuminated ER would raise the mean signal, compared to the Standard design.

### 8.1.3 “Sandwiched” Excitation Ridge

The third design that was modeled for comparison was a second new and previously untested design. The ARROW was designed to be 12µm wide and 5µm high. The excitation waveguide was made by first depositing a layer of 1.46-index silica, 1µm thick, over the biosensor pedestal and channel sacrificial core. Then, a 3µm-thick layer of 1.51-index silica was
deposited over the prior low-index silica layer. The 1.51-index silica was etched through the whole 3µm thickness, leaving a 2µm-wide rectangular waveguide sitting atop the low-index silica. A second layer of low-index silica was grown over the biosensor device. The resulting waveguide cross-section was a high index rectangular core “sandwiched” between layers of low-index silica; this design is thus dubbed “Sandwich”. The cross-section of this design is illustrated in Figure 8-3.

Figure 8-3: Excitation waveguide cross-section of the Sandwich biosensor design. (© 2021 IEEE/OSA [8])

The design intention for this device was to guide a tightly confined mode profile to the peak of the collection efficiency profile in the excitation region. Prior to simulation and fabrication, it was speculated that this design would have a very high maximum signal peak detected, but there would also be a very large variance in signal magnitudes overall. There would be portions of the ER that were very dimly illuminated, resulting in a high number of low-magnitude signal peaks.
8.2 Testing and Simulating the Three Designs

These biosensor designs were fabricated and tested by an associated research group at the University of California – Santa Cruz. The testers flowed a sample of ThermoFisher Scientific® FluoSpheres™ 625/645 Crimson beads as the fluorescing analytes [45]. A helium-neon laser with a 633nm wavelength was used as an excitation source, and the Perkin Elmer® SPCM-AQR-14-FC was used as a detecting avalanche photo diode (APD) with a 65% detection efficiency [53].

Experiment parameters were measured for input laser power, excitation waveguide transmission, and average flow velocity in each device test. Signal results were also reported for the number of detected fluorescent beads, sample test time, and the number and magnitude of signal peaks. The recorded signal peaks also allowed for calculations to be made regarding maximum signal, mean and median signals, standard deviation, and coefficient of variance (CV). Additionally, 90% Confidence ranges were calculable for mean and standard deviation statistics.

The three devices were modeled and simulated based on the reported experiment parameters (input laser power, flow velocity, waveguide transmission, etc.). These parameters are outlined in Table 8-1. Crucially, at this point it was determined to simulate the biosensor tests based on the number of AlexaFluor™ 647 equivalents to a single ThermoFisher FluoSphere bead of 200nm diameter. The FluoSpheres’ fluorescence parameters (lifetime, quantum yield, etc.) at the time of writing are not published, nor is it reported exactly which fluorophore populates the bead. For the purposes of validating the model against the physical experiments, the single-lobe mode Standard biosensor excitation region was used to scale up a multiplication factor on the simulated signal counts until the simulated mean signal matched the tested mean signal. The found scaling factor was 610, which corresponds to an equivalent number of
illuminated and fluorescing AF647 fluorophores on a 20nm-diameter bead; this factor was used
in all other simulations.

The simulation results were compared to the physical experiment results. Similarity
between the signal statistics and distributions were used to validate the veracity of the model
theory. All statistical figures are compiled in Table 8-2, after the three designs’ experiments and
simulations are described.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td>Laser Power (mW)</td>
<td>3.9</td>
</tr>
<tr>
<td>Waveguide Transmission (Fraction)</td>
<td>0.15</td>
</tr>
<tr>
<td>Average Flow Velocity (cm/s)</td>
<td>0.90</td>
</tr>
<tr>
<td>Sample Test Time (s)</td>
<td>40</td>
</tr>
<tr>
<td>Sample Test Volume (nL)</td>
<td>56</td>
</tr>
<tr>
<td>Number of Detected Beads</td>
<td>512</td>
</tr>
</tbody>
</table>

8.2.1 Standard Design

The Standard design was modeled and simulated with two potential excitation regions.

One ER was modeled to be illuminated by a single-lobe waveguide mode, and the other was
illuminated by a double-lobe mode. As was stated above, the single-lobe mode was used to scale
up the fluorophore-equivalence factor used in the other substitutions; the mean signal results will
be identical in this case. Figure 8-4 shows the waveguide modes used to illuminate the
excitation region as well as the resulting ER profiles.
The results from the physical experiment detected 512 beads at a mean signal magnitude of 241 counts/0.1ms. The mean signal for the single-lobe mode simulation was matched with the physical results, and the mean signal for the double-lobe mode simulation was 249 counts/0.1ms.
The Standard experiment maximum signal was 1769 counts/0.1ms with the single-lobe and double-lobe simulations being 55% and 26% smaller, respectively. The experiment median signal was measured at 161 counts/0.1ms, and the single-lobe and double-lobe simulations calculating a 7% decrease and a 19% increase, respectively. The physical experiment had a recorded CV of 0.67; the single-lobe simulation had a lower CV at 0.62, but the double-lobe had a higher CV at 0.77. The histogram distributions given in Figure 8-5 show a 94% intersection between the single-lobe simulation and the physical experiment data and a 95% intersection between the double-lobe simulation and physical experiment data [54].

![Figure 8-5: Signal distributions of the simulated single- and double-lobe mode Standard design, as well as the signal distribution from physical experiments. Gaussian fit lines are added to each distribution. (© 2021 IEEE/OSA [8])](image-url)
Comparing the signal distributions, given in Figure 8-5, the narrower Gaussian curves on the single-lobe simulation and physical experiment data indicate the lower variance as compared with the double-lobe simulation. However, it can be said that the signal statistics and distribution data appear to be similar between simulation and experiment.

8.2.2 Three-Micron Design

Continuing the comparison between model and experiment, the Three-Micron design (Figure 8-6) experiment reported 443 detected beads at a mean signal of 1144 counts/0.1ms. The simulated mean signal was calculated to be 1251 (9% greater than the experiment results). The simulation also calculated a maximum signal of 4113 counts/0.1ms which was 23% greater than the experimental maximum signal of 3339 counts/0.1ms. The simulation also returned a CV of 0.68, and the experiment had a calculated CV of 0.55. The signal histograms in Figure 8-7 have a 93% intersection between simulation and experiment.

Figure 8-6: Excitation waveguide mode and excitation region profile of a Three-Micron design biosensor device. (ER profile © 2021 IEEE/OSA [8])
**8.2.3 Sandwich Design**

The remaining design to be examined was the Sandwich biosensor design. Its mode profile and ER profile are given in Figure 8-8. The physical experiment detected 1770 particles. The simulation calculated a mean signal of 826 counts/0.1ms, and the experiment resulted in a mean signal of 802 counts/0.1ms (3% difference). The maximum signal predicted by the simulation was 2366 counts/0.1ms, and the physically tested max signal was 3252 counts/0.1ms (27% difference). The Sandwich CV was 0.56 for the simulation and 0.51 for the experiment. The signal distribution histograms (Figure 8-9) have a 92% intersection between simulation and experiment.

![Figure 8-7: Signal distributions of the simulated and physically tested Three-Micron devices. Gaussian fit lines are added to each distribution. (© 2021 IEEE/OSA [8])](image-url)
Figure 8-8: Excitation waveguide mode and excitation region profile of a Sandwich design biosensor device. (ER profile © 2021 IEEE/OSA [8])

Figure 8-9: Signal distributions of the simulated and physically tested Sandwich devices. Gaussian fit lines are added to each distribution. (© 2021 IEEE/OSA [8])
Table 8-2: Signal Statistics of Simulations and Physical Test Results

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Signal (cts/0.1ms)</td>
<td>796</td>
<td>1317</td>
<td>1769</td>
<td>4113</td>
<td>3339</td>
</tr>
<tr>
<td>Mean Signal (cts/0.1ms)</td>
<td>241</td>
<td>249</td>
<td>241</td>
<td>1251</td>
<td>1144</td>
</tr>
<tr>
<td>Mean Signal – 95% Confidence Range (cts/0.1ms)</td>
<td>228-254</td>
<td>232-266</td>
<td>227-255</td>
<td>1171-1331</td>
<td>1086-1202</td>
</tr>
<tr>
<td>Median Signal (cts/0.1ms)</td>
<td>231</td>
<td>203</td>
<td>209</td>
<td>1226</td>
<td>1070</td>
</tr>
<tr>
<td>Standard Deviation (cts/0.1ms)</td>
<td>150</td>
<td>192</td>
<td>161</td>
<td>855</td>
<td>626</td>
</tr>
<tr>
<td>Standard Deviation – 95% Confidence Range (cts/0.1ms)</td>
<td>142-160</td>
<td>182-205</td>
<td>152-172</td>
<td>802-915</td>
<td>588-670</td>
</tr>
<tr>
<td>CV</td>
<td>0.62</td>
<td>0.77</td>
<td>0.67</td>
<td>0.68</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*Simulation used to scale up to the fluorophore-per-bead factor. This scalar was then kept constant across all other simulations.

8.3 Discussion of Experiment/Simulation Comparisons

The various simulations appear to be in good agreement with their respective experimental results. There are some differences in each statistic comparison, but the greatest differences between corresponding simulation/experimental values are the maximum and median signal values; the primary values for determining device sensitivity (mean signal and CV) are approximate.

The greatest visual difference in signal distributions is that between the simulation and experimental signal of the Sandwich design (Figure 8-9). The simulation distribution does not fit
into as tight of a peak as the experimental signal distribution, and there appear to be a few local peaks in the simulated distribution. There were no observed phenomena in the experiment that explains the tight experimental peak as opposed to the wider simulated distribution. Possible conjecture is that there may have been an unintended hydrodynamic focusing phenomenon in the Sandwich device being tested, whereas the simulations randomly sample locations in the ER with a uniform probability. However, the signal statistics are similar between simulation and experiment, and all of the simulation-to-experiment signal distributions feature a histogram intersection of greater than 90%. Differences notwithstanding, all of these comparisons lead me to claim the model theory is an accurate description of the behavior of optofluidic flow-through biosensors.

With the claim of a validated model, comparisons between the sensitivities of the three designs can be commented. The Three-Micron had the greatest mean signal of the three designs. However, it had the slowest sample flow rate of 0.53nL/s and target detection rate of 8 beads/s. The Standard design had the weakest mean signal of the three with a sample flow rate of 1.4nL/s and a target detection rate of 14 beads/s. The Sandwich design, however, had an in-between mean signal magnitude in simulation and experiment; the experiment also exhibited a sample flow rate of 1.36nL/s and target detection rate of 32 beads/s.

Both the Three-Micron and Sandwich designs feature some improvements over the Standard design. In testing scenarios in which test times are not critical but a strong signal intensity is needed, the Three-Micron design offers the best option of the three designs studied here. The Sandwich design offers perhaps the most robust improvement over the Standard design. The mean signal is improved by nearly 4×, the CV is comparable, but primarily it offers
the fastest sample processing and detection rates of all three designs. Scenarios that require short
test return times would call for the Sandwich device to be utilized.

With the biosensor model theory validated and new device designs studied, the model can
be used to project the sensitivities of an array of different illumination formats and fluid flow
regimes.
CHAPTER 9. PROJECTING FUTURE DESIGNS

With a validated model, it becomes prudent to anticipate biosensor designs that have not been fabricated and tested. Some designs can be sophisticated compared to the designs described in the previous chapter, implementing different illumination formats and fluid flow regimes. This increased sophistication comes with an increased complexity in the fabrication process. The model described in this work allows the designer to test out design ideas without investing time and resources into fabricating a device with undefined sensitivity. Some of these ideas regard illumination direction and hydrodynamic focusing, and they have been to some degree experimented [25,26,50,55-57]. Utilizing the biosensor model enables the designer to evaluate each of these ideas in a variety of combinations.

The illumination format used by the various designs and examples prior within this work have predominantly used the side (planar) illumination method of guiding a laser mode to the ARROW with a ridge waveguide built into the biosensor chip. Top illumination rather suspends a laser source above the biosensor chip, pointing down onto the ARROW. This method is intended to have a quick-alignment that does not require the fine alignment needed when butt-coupling a fiber waveguide to the facet of an on-chip ridge waveguide.

There are a few options to set up a top illumination biosensing format. The laser can be focused onto a spot on the ARROW; this will allow for great excitation intensity, but it also would drastically increase noise levels and make signal detection difficult. The chip can also be coated with a blocking material, such as aluminum, with windows milled through above the
ARROW channel. This would allow enough laser light to excite fluorescence but cut back on noise levels; multiple windows would provide a multiplexing function for the chip [57]. Another option is to diffract the laser into several lines of divided optical power and align the line pattern over the ARROW channel to create multiple illumination points, again for multiplexing purposes. A comparison of side and top illumination methods is shown in Figure 9-1.

Figure 9-1: (a) Side illumination by guiding an optical mode to the ARROW, planar with the biosensor chip. (b) Top illumination by focusing a laser onto a window that admits light into the ARROW. (© 2021 MDPI [9])

Hydrodynamic focusing is a method utilized to constrain the sample stream to a confined cross-section within the ARROW channel. The motivation here is to have this focused stream intersect the portion of the excitation region that is most optically intense and/or most collection efficient. A sample fluid can be hydrodynamically focused either two-dimensionally or three-dimensionally. Two-dimensional hydrodynamic focusing (2DHF) is done by compressing the sample stream horizontally with a buffer fluid inlet on either side of the channel [25,52,58]. Three-dimensional hydrodynamic focusing (3DHF) has buffer inlets above, below, and on the sides of the main channel to focus the sample stream both horizontally and vertically [25,59]. A comparison of 2DHF and 3DHF methods is given in Figure 9-2.
Some of my work was to adapt the model to simulate an array of biosensor designs that vary according to illumination/flow regime combination. Each simulated design was placed in a category of side or top illumination and either a laminar parabolic, 2DHF, or 3DHF flow regime. The side and top illumination intensity profiles were based on those of fabricated and tested devices [57], and the HDF stream cross-sections were based off reported cross-sections [25,52]. There were therefore six design combinations based off of published parameters. At the end of this comparison, I further optimized the design combination with the strongest signal to find an even more sensitive design.

9.1 Validating the Top Illumination Format

Before the new design combinations could be compared, the top illumination format had to be validated; the previous chapter validated the side illumination format. Two devices underwent bead tests, one with the side illumination format and the other top-illuminated. These devices were tested with a multiplexing excitation region; the side-illuminated device utilized its multi-mode interferometer (MMI) to create seven optical spots on the ARROW, while the top-
illuminated device featured seven windows, each $14\mu m \times 4\mu m$, illuminated by a suspended laser overhead. The laser was focused by an objective lens to an elliptical spot with an $85\mu m$ long diameter and a $22\mu m$ short diameter.

Because the laser spot is optically less intense than a guided mode, even if the mode is split into seven spots, the laser power settings for each illumination format were different. The side-illuminated test had its laser power set to 5.3mW; 151µW were measured in each MMI spot. The top-illuminated test had a laser power set to 19mW; 553µW was incident on each window [57]. Because the spot was much larger in area than the windows, it is assumed that the intensity gradient across each window was near-uniform. Because different power settings were used in the two physical test categories, validating the model requires that the physical and simulated signals be measured in counts/(mW·0.1ms).

In each device category, 136 beads (ThermoFisher Scientific© FluoSpheres™ 625/645 Crimson 0.2µm) were detected and their signal distributions were recorded. The physically tested mean signals were 161 counts/0.1ms for the side-illuminated device [57]. Normalizing for incident power ($151\mu W/spot \times 7 \text{ spots} = 1.057\text{mW}$), the mean signal becomes 152 counts/(mW·0.1ms). The top illumination physical tests resulted in a mean signal of 195 counts/0.1ms. Normalizing for power again ($553\mu W/\text{window} \times 7 \text{ windows} = 3.871\text{mW}$), the mean signal becomes 50 counts/(mW·0.1ms). The ratio of power-normalized mean signals was 3:1:side:top; this ratio was kept in mind during the simulations. The waveguide and facet transmissions in the physical experiments were not measured and are unaccounted for.

Validating the model to the physical tests was done by adjusting the optical power setting in the models until the mean signals of the side and top illumination simulations matched the measured means in the physical tests. The various waveguide and facet transmissions were
ignored in the model due to their unknown values in the physical tests and to minimize the number of variables involved; ignoring these transmission values also allows us to isolate the excitation region behavior for analysis. The lack of these transmission values would result in larger signals than were measured in the physical experiments. The power setting that matched the experimental signal means was 26.4µW for the side format and 80µW for the top format; these values correspond to the amount of power incident on the ARROW, summed from each of the seven spots or windows.

Power-normalizing the mean signals of the simulations, the side format had a mean signal of 6098 counts/(mW·0.1ms), and the top format had a mean signal of 2438 counts/(mW·0.1ms). The side:top ratio for the power-normalized signal means was 2.50. This ratio is close to the one calculated for the physical experiments, and the difference is likely due to the unaccounted waveguide and facet transmissions in the physical experiments.

Figure 9-3: Signal distributions used for validating the side and top illumination formats. (a) Simulation results. (b) Physical test results. (© 2021 MDPI [9])

Histogram intersections were calculated for the experiment/simulation signal histograms in the side and top format categories [54]. The intersections were 85% for the side illumination
histograms and 92% for the top illumination histograms. Because of the similarity in the power-normalized mean signal ratios and the histogram intersections, the differences between the side and top illumination formats can be considered validated. The signal distributions used in the validation process are shown in Figure 3-9.

9.2 Design Combinations

Six designs were initially considered and compared. The two illumination categories each had a laminar-parabolic flow profile, a 2DHF flow profile, and a 3DHF flow profile. Devices with laminar-parabolic flow profiles have been examined extensively, most commonly with side illumination; top illumination experiments have only recently been studied. Side illumination 2DHF and 3DHF devices have also been studied. However, a comprehensive array of designs of illumination format and flow regime combinations have yet to be fabricated and tested. The remainder of this chapter will show the modeled results of this array.

9.2.1 Preliminary Information

For the sake of simplicity in comparison, a single excitation spot/window was used in each simulation, and all simulations used a 12µm × 6µm ARROW channel. The top illumination windows were modeled to have the same dimensions as those used in the validation section above. The 2DHF and 3DHF flow profiles were modeled to match the focused stream cross-sections found in previously fabricated devices and published findings [25,52]. The 2DHF cross-section was defined to be 4µm wide and to run the height of the ARROW channel; because particles have previously been found to not occur within 0.2µm of the ARROW channel boundaries, the 2DHF cross-section is 22.4µm². The 3DHF cross-section was defined to be 3µm wide and 4µm high. Smaller cross-sections will take longer times to pass a set number of
particles through the excitation region; Table 9-1 lists the cross-sections for each flow regime and the time to pass 1000 particles. I will describe my own optimization of these cross-sections later in this chapter.

**Table 9-1: Excitation Region Cross-Sections and Estimated Test Times**

<table>
<thead>
<tr>
<th>Flow Regime</th>
<th>Parabolic</th>
<th>2DHF</th>
<th>3DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-Section (µm²)</td>
<td>65.0</td>
<td>22.4</td>
<td>12.0</td>
</tr>
<tr>
<td>Estimated Test Time (s)</td>
<td>73</td>
<td>168</td>
<td>237</td>
</tr>
</tbody>
</table>

Aside from the two optical illumination profile categories and the three flow regime categories, the model and simulation parameters were kept constant as much as possible. These parameters are listed in Table 9-2.

**Table 9-2: General Model Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Simulated Particles</td>
<td>1000</td>
</tr>
<tr>
<td>Input Power</td>
<td>1 mW</td>
</tr>
<tr>
<td>Channel Height</td>
<td>6 µm</td>
</tr>
<tr>
<td>Channel Width</td>
<td>12 µm</td>
</tr>
<tr>
<td>Mean Flow Velocity</td>
<td>2 cm/s</td>
</tr>
<tr>
<td>Bead Diameter</td>
<td>0.2 µm</td>
</tr>
<tr>
<td>Excitation Wavelength</td>
<td>633 nm</td>
</tr>
<tr>
<td>Emission Wavelength</td>
<td>645 nm</td>
</tr>
</tbody>
</table>

### 9.2.2 Side-Illuminated Designs

Because of its Gaussian-like optical intensity profile, side-illuminated/parabolic flow design has a high degree of variance in its excitation region profile (Figure 9-4). Sampling from this excitation region results in a large number of low signal counts and a decreasing number of medium and high signal counts. Simulating 1000 particles in this design resulted in a mean signal of 5040 counts/0.1ms and a coefficient of variance (CV) of 1.071.
The side/2DHF design had an ER with similar variance to the side/parabolic design. This is because the sampleable cross-section was restricted horizontally and not vertically. The mean signal was greater than the side/parabolic design at 9499 counts/0.1ms. The CV was 1.042. The signal distribution again had a high number of low signal counts, but the medium-to-high signal counts had a somewhat uniform distribution and extended further along the signal magnitude scale. The excitation region and signal distribution are shown in Figure 9-5.

Figure 9-5: (a) Excitation region profile of the side/2DHF design. (b) Signal distribution of the side/2DHF design. (© 2021 MDPI [9])
The side/3DHF design had the least variance of sampleable energy values in its ER profile. The mean signal was 19,901 counts/0.1ms. Because vertical focusing was integrated into this ER profile, the CV was 0.672. The distribution was fairly uniform. Figure 9-6 shows the side/3DHF ER and signal distribution.

![Figure 9-6](image-url)

Figure 9-6: (a) Excitation region profile of the side/3DHF design. (b) Signal distribution of the side/3DHF design. (© 2021 MDPI [9])

9.2.3 Top-Illuminated Designs

Because the top-illuminated designs have a uniform optical intensity distribution in the ER, the variance in sampleable energy values is not due to optical excitation variance. The variance in the parabolic flow category therefore is attributed to the excitation time and collection efficiency factors in the ER. As the sample streams are hydrodynamically focused, the time and collection efficiency variances decrease, and the ER energy in turn decreases.

The top/parabolic design had a mean signal of 2575 counts/0.1ms. This design’s CV was calculated to be 1.190, similar to the corresponding side/parabolic design’s CV. The signal distribution of this design has its peak slightly removed from the zero-end of the magnitude scale. The ER profile and signal distribution are shown in Figure 9-7.
The top/2DHF design had a mean signal of 4243 counts/0.1ms. The CV was 0.748. The signal distribution approaches a normal distribution as compared with the top/parabolic design, and the peak of the distribution appears to be in the middle of the signal range. The ER profile and signal distribution are shown in Figure 9-8.
The top/3DHF design had a mean signal of 5300 counts/0.1ms. The CV was 0.332, the smallest of all pre-optimized design combinations. The signal distribution was the most left-skewed of the pre-optimized signal distributions. The top/3DHF design’s ER profile and signal distribution are shown in Figure 9-9. The signal statistics of all pre-optimized design categories are listed in Table 9-3.

![Figure 9-9: (a) Excitation region profile of the top/3DHF design. (b) Signal distribution of the top/3DHF design. (© 2021 MDPI [9])](image)

**Table 9-3: Signal Statistics of Pre-Optimized Biosensor Design Combinations**

<table>
<thead>
<tr>
<th>Flow Regime \ Side Illumination</th>
<th>Parabolic Mean Signal (cts/0.1ms)</th>
<th>2DHF CV</th>
<th>3DHF CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side Illumination</td>
<td>Mean Signal (cts/0.1ms)</td>
<td>5040</td>
<td>9499</td>
</tr>
<tr>
<td>Top Illumination</td>
<td>Mean Signal (cts/0.1ms)</td>
<td>2575</td>
<td>4243</td>
</tr>
<tr>
<td>CV</td>
<td>1.071</td>
<td>1.042</td>
<td>0.672</td>
</tr>
<tr>
<td>CV</td>
<td>1.190</td>
<td>0.748</td>
<td>0.332</td>
</tr>
</tbody>
</table>

**9.2.4 Discussion of Pre-Optimized Design Simulations**

The side-illuminated devices had higher mean signals than the top-illuminated, but the tops trended smaller CVs than the sides. Within each illumination format, the designs that
featured 3DHF flow regimes exhibited the highest mean signals and smallest CVs. While they had the best results in each illumination format, the 3DHF designs also require the longest time to pass 1000 particles through the ER, $3 \times$ longer than the laminar-parabolic flow regimes. A clinician in a practical test setting would have to consider the tradeoffs: strong signals or fast test times.

Side illumination offered higher mean signals than the top illumination format, but they require aligning and butt-coupling a fiber-guided laser source to the ridge waveguide facet; this will add time to the test procedure. Further, the above simulations do not account for waveguide facet transmission, and this depends on the quality of the chip’s cleave. The top illumination format offers a simpler and faster alignment procedure as the exciting laser can be focused onto the chip’s channel, and the lack of exciting waveguide/facet transmission factors remove that layer of complexity from the system. However, this added simplicity comes at the cost of optically intense ERs. Each of these tradeoffs can be considered for application in a clinical setting.

9.3 Optimizing the Side/3DHF Design

To re-iterate, the array of design combinations described above was to explore untested device designs and compare them with tested designs. The hydrodynamic focusing cross-sections within the ARROW were based off of published works. Using the model, I chose the above-described side/3DHF design and optimized the simulated HDF cross-section. The cross-section height and x-position in the channel were swept, looking for a maximized mean signal and a minimized CV.
9.3.1 Sweep of 3DHF Stream Heights

The first parameter sweep was to simulate the side/3DHF sample stream cross-section heights while keeping them centered within the ARROW. The sweep was done in 1-µm increments, beginning with the 4µm height described above and continuing until a cross-section 1µm high is simulated. Shortening the height in effect increases the degree of hydrodynamic focusing in the vertical direction.

As the simulated HDF cross-section is reduced in height, the mean signal progressively increases and the CV progressively decreases. The 1µm height has the highest mean signal at 42,932 counts/0.1ms and the lowest CV at 9%. As the sweep increases the HDF focus, the signal distribution approaches a normal distribution. To pass 1000 particles through this shortest HDF cross section will require an estimated 797 seconds of time. Figure 9-10 shows the ER profiles and signal distributions of this parameter sweep.

9.3.2 Sweep of 3DHF Stream x-Position

The other parameter sweep was to adjust the x-position of the HDF stream cross-section. Measuring at the cross-section center, the position swept from 6µm and out to 10µm. This kept the edge of the HDF stream at least 0.5µm away from the ARROW sidewall. The height of the cross-section was kept at a constant 4µm. The mean signals resulting from this sweep stayed roughly equivalent until they decreased at 9µm and 10µm; the lowest mean signal was 10,653 counts/0.1ms at the 10µm x-position. The CVs stayed roughly equivalent across the sweep. The ER profiles and signal distributions for this parameter sweep are shown in Figure 9-11. The signal statistics for this optimization exercise are listed in Table 9-4.
Figure 9-10: The ER profiles and signal distributions of the side/3DHF optimization sweep of HDF cross-section heights. (a) 4µm, (b) 3µm, (c) 2µm, and (d) 1µm. (© 2021 MDPI [9])
Figure 9-11: The ER profiles and signal distributions of the side/3DHF optimization sweep of HDF stream x-position. (a) 6µm, (b) 7µm, (c) 8µm, (d) 9µm, and (e) 10µm. (© 2021 MDPI [9])
Table 9-4: Signal Statistics of Side/3DHF Optimization Parameter Sweeps

<table>
<thead>
<tr>
<th>HDF Cross-Section Height</th>
<th>4µm</th>
<th>3µm</th>
<th>2µm</th>
<th>1µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Signal (cts/0.1ms)</td>
<td>19,901</td>
<td>27,121</td>
<td>36,488</td>
<td>42,932</td>
</tr>
<tr>
<td>CV</td>
<td>0.672</td>
<td>0.481</td>
<td>0.249</td>
<td>0.091</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HDF Cross-Section x-Position</th>
<th>6µm</th>
<th>7µm</th>
<th>8µm</th>
<th>9µm</th>
<th>10µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Signal (cts/0.1ms)</td>
<td>19,901</td>
<td>19,643</td>
<td>20,295</td>
<td>17,730</td>
<td>10,653</td>
</tr>
<tr>
<td>CV</td>
<td>0.672</td>
<td>0.680</td>
<td>0.684</td>
<td>0.681</td>
<td>0.720</td>
</tr>
</tbody>
</table>

9.3.3 Discussion of Optimization

Based on the parameter sweeps done to optimize the side/3DHF design combination, keeping the HDF stream centered in the ARROW channel and decreasing the cross-section height, i.e. increasing the degree of focus, improves the design sensitivity most. However, as the focusing increased until the HDF stream is 1µm high, the test time increases by over 3× from the initial 4µm height and 11× over the parabolic flow regime.

The optimization of the HDF cross-section height, however, was manually done and assumes the hydrodynamic focusing design parameters were already defined and optimized to produce each cross-section. In reality, the various 3DHF design parameters must be calculated to produce each cross-section.

9.4 Conclusion of Design Combination Projections

The work exhibited in this chapter showcases the potential of the model theory and simulation to project biosensor performance. Even when waveguide and facet transmissions are unknown, it is possible to compare devices with differing design parameters and evaluate worthwhile fabrication.
CHAPTER 10. FUTURE WORK AND CONCLUSION

The original work presented in this dissertation was the genesis and development of a model theory that explains the behavior of optofluidic flow-through biosensors. Understanding this model enables the engineer to construct a model and use it to simulate biosensor designs. This model has exhibited a utility to inform the engineer’s biosensor design process.

While the theory has been established and the utility validated, there a number of things I recommend to develop the model into a robust designer’s tool. This chapter will outline the model/simulation improvements that should be included, as well as what future developments in the biosensor technology naturally follow the current state of the art.

10.1 Recommended Improvements to the Model

To become a robust designer’s tool, the model and simulation must incorporate a statistical error function in calculating the excitation region profile. I have in mind a Monte Carlo algorithm that accounts for statistical error in design parameters. For example, multiple device dies on a single wafer are fabricated at once. When the wafer is cleaved into multiple device chips, the waveguide facets will not all have the same optical transmission fraction values. Creating a probability distribution of facet transmissions and incorporating it into the device simulation will give a realistic expectation for what that transmission value will be. This is one factor to consider in the biosensor simulation. Creating a probability distribution for all
design parameters will be an involved process, but this will make an overall more accurate simulation of a physical device.

Corner tests are another useful function to add to the model. Because of the many structures on the biosensor device, this will also be an involved process. Using a ridge waveguide as an example, the modeler can take the expected minima and maxima extremes of waveguide height and width and calculate the waveguide’s loss.

Another feature future modelers will want to incorporate is a function that predicts noise levels for a given optical excitation power setting. The noise predictor would serve to show noise floor that the signal of a given design would have to rise above to be useful. Furthermore, it could be used in conjunction with the Monte Carlo statistical algorithm to predict the range of possible noise levels as a function of statistical variation in the fabrication process.

I consider an extensive finite-differential time domain simulation to be the most obvious method to calculate noise levels. This is a simple approach in concept as the biosensor can be virtually constructed, an excitation source defined, and monitors placed at the ends of the collection waveguides will be able to collect stray optical power that corresponds with noise levels; the noise levels can be normalized with the power setting of the optical source. While it is conceptually simple, the execution will be quite involved. The virtual construction of the biosensor will be a complicated effort. Even after the device is virtually constructed and the simulation region defined, the resource requirements to perform this FDTD calculation will be extreme. It may be prudent to use an eigenmode expansion (EME) [60]-[62] method as an alternative, but the three-dimensional topography of the biosensor device may have to be approximated rather than explicitly defined.
General user-interface improvements to improve the modeler’s user experience would also be beneficial.

10.2 Future Work within the Biosensor Development

Continued refinement of the illumination formats will be an ongoing process. Practical concerns such as alignment time will be critical in making the biosensor a technical and economic quality tool. Side-illuminated devices that can quickly be aligned will have the benefit of both high-sensitivity and fast test times. Top illumination formats that can increase the excitation power while minimizing noise levels will merit further development as the spot alignment will be much simpler than a fine-aligned butt-coupled fiber to a planar ridge waveguide. A side-illuminated device with the avalanche photodetector suspended above the ARROW channel could bypass the need for a collection waveguide and a collection efficiency profile in the model and simulation, but the I conjecture the signal-to-noise ratio of this setup will be quite low without some way to filter out excitation wavelengths.

In development are biosensors that utilize a nanopore inlet that connects the sample reservoir to the ARROW channel directly [63]-[67]. This development will help with single-particle detection, as one bioparticle at a time would pass through the aperture.

10.3 Conclusion

The optofluidic flow-through biosensor offers fast and accurate detection of target bioparticles. The sophisticated design requires a great deal of ongoing improvement. The model theory and construction outlined in this work will aid in this development, saving time and resources until a given design is estimated to be valuable enough for fabrication.
REFERENCES


APPENDIX A. MATLAB CODE FOR SIMULATION

In this Appendix, I list example MATLAB code for the model simulation. The code can be altered to match variations in the modeler’s investigation. Prior to running the MATLAB simulation program, optical intensity modes to be simulated are generated in Lumerical® MODE Solutions™ and exported to MATLAB. This is for the side illumination format; the top-illumination format does not require the Lumerical software.

A.1 Generating the Optical Mode Intensity Profile

1. In Lumerical® MODE Solutions™, construct the excitation waveguide cross-section geometry (Figure A-1a) from the “Structures” menu and set the needed material parameters, refractive index most important of all.
2. Define the differential eigenmode simulation region (Figure A-2) from the “Simulation” menu. The geometry is set similar as in Step 1. The number of mesh cells in the x- and y-directions is defined based on the size of the fluorescent particle to be simulated and the simulation region x-y dimensions.
3. Run the simulation, and the Eigensolver Analysis window opens. The “Mesh Structure” process allows the user to confirm the geometry meshes correctly. Enter the number of modes to simulate; greater numbers of modes will require more computing resources, but large mesh sizes (such as the one used in these example figures) will mitigate resource requirements. Specify the refractive index or range of indices to “Calculate Modes” will run the number of eigenmode results specified, and each mode can be selected from the Mode List for analysis and export. See Figure A-3 for a visual example.

![Eigenmode Analysis window](image)

**Figure A-3: Eigenmode Analysis window.**

4. Export the intensity profile matrix to MATLAB. Make sure “E Intensity” is the selected modal field component; the modeler may find utility for other field options, but “E
Intensity” is needed for the MATLAB program. Make sure cartesian coordinates are selected. Select “Data Export” from the Options drop-down menu, and select “matlab”. “Modes selected” is a necessary selection, but other options may be exported as the modeler sees fit. See Figure A-4.

Figure A-4: Tool for exporting the intensity profile to MATLAB.
A.2 Excitation Region Generator for Side Illumination

The code for the Side Illumination Excitation Region generator is copied below.

```matlab
clear all
close all

% run this file to generate Side/Planar excitation regions

powerTotal = 0.001; % Watts

unitArea = 4e-14; % m^2 area of a mesh cell; you have to calculate this by
% hand if you change the mesh

% all "BEAD" mentions reference that the mesh size corresponds to 200nm x
% 200nm to encapsulate a 200nm-diameter bead

% this is where you load the different mode profiles

load('MABRW5DoublePoyntingBEAD.mat');
load('MABRW5DoublePoyntingBEADtake3.mat');
load('MABRW5SinglePoyntingBEAD.mat');
load('MABRW5SinglePoyntingBEADtake2.mat');
load('MABRW5SinglePoyntingBEADtake3.mat');
load('SideIlluminationBEADpoynting.mat');
load('Standard6umMMIPoyntingBEAD.mat');

% set the mode profiles to an absolute value; uncomment the mode profile
% that you want to simulate; try them all so you know which profile
% looks like what

% absreal = abs(MABRW5DoublePoyntingBEAD');
% absreal = abs(MABRW5DoublePoyntingBEADtake3');
% absreal = abs(MABRW5SinglePoyntingBEAD');
% absreal = abs(MABRW5SinglePoyntingBEADtake2');
% absreal = abs(MABRW5SinglePoyntingBEADtake3');
absreal = abs(SideIlluminationBEADpoynting');
% absreal = abs(Standard6umMMIPoyntingBEAD');

% the 'absreal' below helps to manually adjust the size of the mode loaded
% from Lumerical: higher exponents result in tighter modes; try running with
% it commented out and with it uncommented
absreal = absreal.^5.4;

figure(1);
mesh(absreal)
set(gca,'FontSize', 13)
% xticks([0 100 200 300 401])
% xticks([0 20])
% xticklabels({'0','3','6','9','12'})
xticklabels({'0','4'})
```
xlabel({'Optical Mode','z-Position (\mum)'})
% yticks([0 34 67 101 134 168])
yticks([0 30])
% yticklabels({'0','1','2','3','4','5'})
yticklabels({'0','6'})
ylabel({'Optical Mode','y-Position (\mum)'})
% zlim([0 0.1])
zlabel('Fraction of Peak Intensity')
title({'Optical Intensity Profile','Normalized to Peak'})

absrealmx = max(max(absreal));
absrealsum = sum(sum(absreal));

% calculates 'effective' mode profile by including only the e^-2 diameter
eff = absreal;
eff(eff < absrealmx.*exp(-2)) = 0;
effsum = sum(sum(eff));

nonZ = eff(eff ~= 0);
umnonZ = nnz(eff);
nonZsum = sum(sum(nonZ));

modeArea = unitArea*numnonZ;
absrealPower = absreal.*unitArea;

absrealPowerSum = sum(sum(absrealPower));
Pmult = powerTotal/absrealPowerSum;
absrealAdj = Pmult.*absreal;

figure(2);
mesh(eff)

norm = (absreal./absrealsum).*powerTotal;

normsum = sum(sum(norm));
normMax = max(max(norm));
normMean = mean(mean(norm));

normPoynt = norm./unitArea;

figure(3);
% contourf(norm)
mesh(norm)
set(gca,'FontSize', 13)
% xticks([0 100 200 300 401])
xticks([0 20])
xlabel({'Optical Mode','z-Position (\mum)'})
yticks([0 30])
ylabel({'Optical Mode','y-Position (\mum)'})

intensity_sum = sum(norm,2);
newx = 1:length(intensity_sum);
figure(4);
plot(intensity_sum,newx)
set(gca,'FontSize', 13)
xlabel('Summed Power (W)')
ylabel('Channel y-Position (\mum)')

velocity
u = integrated_rect_flow_veloc(0.02, 6, 12, 29, 59);
maxu = max(max(u));
figure(5);
set(gca,'FontSize', 13)
mesh(u)
xlabel('Channel x-Position (\mum)')
ylabel('Channel y-Position (\mum)')

create time profile
time = excitation region depth / velocity
excite_time = 2e-6./u;
excite_time(excite_time == Inf) = 0;
figure(6);
mesh(excite_time(2:29,2:59))
% mesh(excite_time(2:299,2:599))
set(gca, 'FontSize', 13)
% xticks([0 100 200 300 401])
% xticks([0 599])
xticks([0 58])
% xticklabels({'0','3','6','9','12'})
xticklabels({'0','12'})
xlabel('Channel x-Position (\mum)')
% yticks([0 34 67 101 134 168])
% yticks([0 299])
yticks([0 28])
% yticklabels({'0','1','2','3','4','5'})
yticklabels({'0','6'})
ylabel('Channel y-Position (\mum)')
% zlim([0 0.1])
zlabel('Time in Excitation Region (s)')
title({'Time Profile of a', 'Fully Developed Fluid Flow Cross Section'})

% incident power
raw_fluorprod = diag(intensity_sum)*excite_time;

figure(7);
mesh(0.33*raw_fluorprod(:,2:59))
set(gca, 'FontSize', 13)
% xticks([0 100 200 300 401])
% xticks([0 599])
xticks([0 58])
% xticklabels({'0','3','6','9','12'})
xticklabels({'0','12'})
xlabel('Channel x-Position (\mum)')
% yticks([0 34 67 101 134 168])
% yticks([0 299])
yticks([0 30])
% yticklabels({'0','1','2','3','4','5'})
yticklabels({'0','6'})
ylabel('Channel y-Position (\mum)')
% zlim([0 6e-8])
zlabel('Energy (J)')
title({'Fluorescence Energy Generation Profile'})

% Coupling Efficiency Component
load('CEbeadXYZtake2.mat')
CE = CEbeadXYZtake2;

figure(8);
mesh(CE)
set(gca, 'FontSize', 13)
xticks([0 60])
xticklabels({'0','12'})
xlabel('Channel x-Position (\mum)')
FluorProd_wCE = CE .* raw_fluorprod;
FluorProdSim = FluorProd_wCE(2:29, 2:59); % edges get clipped from simulation here
% FluorProdSim = FluorProd_wCE(:, 29:30);

usim = u(2:29, 2:59); % Parabolic
usim mean = mean(mean(usim)); % Parabolic
timesim = excite_time(2:29, 2:59); % Parabolic
timesimMean = mean(mean(timesim));

figure(9);
mesh(FluorProdSim)
% mesh(FluorProd_wCE)
set(gca,'FontSize', 13)
xticks([1 15 30 45 58])
% xticks([1 15 30 45 60])
xlabel('Channel x-Position (\mum)')
yticks([1 5 10 15 20 25 30])
% yticks([1 5 10 15 20 25 30])
ylabel('Channel y-Position (\mum)')
zlabel({'Collected Fluorescence','Energy (J)'}

title('Excitation Region Profile')
% zlim([0 3e-11])

A.3 Excitation Region Generator for Top Illumination

This generator’s code is similar to the side illumination format, but the optical intensity profile is generated without a matrix import from MODE.

```matlab
clear all
close all

% run this file to generate Top excitation regions

powerTotal = 0.001; % Watts

unitArea = 4e-14; % m^2 area of a mesh cell; you have to calculate this by hand if you change the mesh

% all "BEAD" mentions reference that the mesh size corresponds to 200nm x 200nm to encapsulate a 200nm-diameter bead
% unlike the side-illuminated ERs, if it is assumed the optical intensity is uniformly distributed then the optical excitation profile can be constructed here
uniform = ones(20,60);
absreal = abs(uniform./numel(uniform));
absrealsum = sum(sum(absreal));

figure(1);
mesh(absreal)

absrealmax = max(max(absreal));

eff = absreal;
    eff(eff < absrealmax.*exp(-2)) = 0;

nonZ = eff(eff ~= 0);
umnonZ = nnz(eff);
nonZsum = sum(sum(nonZ));

modeArea = unitArea*numnonZ;

figure(2);
mesh(eff)

% mesh(absrealAdj)

% distributes 1mW power across the excitation window
norm = (eff./absrealsum).*powerTotal;

normA = norm; % peak line, if multiple windows have different intensities
% normB = 0.99.*norm;
```
% number of excitation windows/spots
normSum = normA;
% normSum = 7*normA; % if I wanted to say there were 7 excitation windows

figure(3);
mesh(normSum)

% sum the accumulated power as the bead tracks through ER
intensity_sum = sum(normSum,1);

figure(4);
plot(intensity_sum)

% velocity
% u = integrated_rect_flow_veloc(mean veloc, y-microns, x-microns, no. y-mesh cells, no. x-mesh cells);
% here, enter one less mesh cell number than you want to result; e.g., if you
% want 30 y-cells, then enter 29
u = integrated_rect_flow_veloc(0.02, 6, 12, 29, 59); % Parabolic
maxu = max(max(u));

% create time profile
% time = excitation region depth / velocity
excite_time = 4e-6./u; % 4um window width
excite_time(excite_time == Inf) = 0;
% ^ the outermost edges will go to infinite time (zero velocity); the outer
% edges get clipped in simulation anyway, so for simplicity set Inf -> 0

figure(5);
mesh(excite_time(2:29, 2:59))
% zlim([0 0.01])

% incident energy profile creation
bufferstep = ones(30,60).*max(max(intensity_sum));
raw_fluorprod = bufferstep.*excite_time;

figure(6);
mesh(raw_fluorprod)

% Coupling Efficiency Component
load('CEbeadXYZtake2.mat');
CE = CEbeadXYZtake2;

figure(8);
mesh(CE)
% xlim([0 410])
% ylim([0 410])
% zlim([0 1.2])
FluorProd_wCE = CE .* raw_fluorprod;
FluorProdSim = FluorProd_wCE(2:29, 2:59);
usim = u(2:29, 2:59);  \% Parabolic
usim_mean = mean(mean(usim));  \% Parabolic
timesim = excite_time(2:29, 2:59);

figure(9);
mesh(FluorProdSim)
set(gca,'FontSize', 13)
xticks([1 15 30 45 58])
xticklabels({'0.2','3','6','9','11.8'})
xlabel('Channel x-Position (\mum)')
yticks([1 5 10 15 20 25 28])
yticklabels({'0.2','1','2','3','4','5','5.8'})
ylabel('Channel y-Position (\mum)')
zlabel('Collected Energy (J)')
zlim([0 1e-10])

A.4 Code for Signal Simulation

While the excitation region from one of the above illumination formats is saved, run this
code to simulate the signal distribution.

close all

h = 6.626E-34;
c = 299792458;

% quantum yield values, ie statistically 33% of incident photons on AF647
% result in an emitted fluorescence photon
% Q = 0.27; % QY Cy5
Q = 0.33; % quantum yield AF647
% Q = 0.36; % QY AF680

% wavelength of incident laser
wavelength = 633E-9; % m

% absorption cross sections of various fluorophores and the beads
absorptionAreaAF647 = 1.03248E-19; % m^2; fluorophore absorption cross
sectional area; AF647

% absorptionArea = 6.9906e-20; % m^2; AF680
beadArea = 3.14159265e-14; % m^2; bead
% absorptionArea = 9.56E-20; % m^2; Cy5
% number of particles you want to simulate
numberParticles = 1000;

nelements = numel(FluorProdSim);
randices = randsample(1:nelements, numberParticles, true);
R = FluorProdSim(randices);
urand = usim(randices);
timerand = timesim(randices);

% histogram of incident power
figure(1);
H = histogram(R) % R+1
axis([0 11 0 200])
xlabel('Incident Excitation Energy')
ylabel('Detected Photon Counts')

% scatter plot of incident power and flow velocity
figure(2);
scatter(urand,R,'b')
axis([0 0.04 0 0.5])
xlabel('Velocity (m/s)')
ylabel('Fluorescent Production (A.U.)')
title('Standard Design')

F = 610; % number of AF647 equivalents on a bead

timeconvert = mean(timerand)./1e-4;

photonCap = inf;
photonCap = 137500*F*0.65;

% convert fluorescence energy to a number of photons
% the '0.65' refers to the APD efficiency, and if you know and want to
% include waveguide transmissions then you can add them to the line
numPhotons = Q*F*R*(wavelength/(h*c))*(absorptionAreaAF647/unitArea)*0.65;

numPhotonMean = mean(numPhotons);
numPhotonSum = sum(numPhotons);

% signal is number of counts/0.1ms
Signal = numPhotons./timeconvert;

figure(4);
H2 = histogram(nonzeros(Signal))
set(gca, 'FontSize', 14)
title('Signal Distribution')
xlabel('Counts/0.1ms')
ylabel('No. Events')
% xlim([0 2e4])
% ylim([0 70])

% uncomment these if you want to account for known noise values...but that
% needs experimental data, and the model doesn't predict that yet
% MIN = Signal > 18; % standard noise floor
% Signal = Signal(MIN);

maxPhotonSig = max(Signal) % photons/ms

n = sum(Signal~=0);
n(n==0) = NaN;
meanPhotonSig = mean(Signal);

medianPhotonSig = median(Signal)

urand_mean = mean(urand)

StDev = std(Signal)

CV = std(Signal)/meanPhotonSig

meanPhotonSig = mean(Signal)
APPENDIX B. FDTD SWEEP PROCEDURE FOR COLLECTION EFFICIENCY

The process of creating the collection efficiency profile described in Chapters 6 and 7 requires many FDTD calculations. The ARROW geometry is constructed and meshed virtually, and a dipole acts as the fluorescent particle light source. The dipole is swept across the ARROW channel, and an FDTD calculation is performed at every location point. The resulting transmission values are recorded by a monitor at a set distance down the ARROW, and the multiple transmission values are collated with each dipole location point. This is done for the three extremes of the dipole’s x-y-z polarization, and the three polarization profiles are averaged to create an effective collection efficiency profile.

The size of the x-y intervals depends on the size of the fluorescent particle being simulated. Smaller particles require more dipole locations to create an accurate profile matrix and therefore more FDTD calculations. Each calculation has intensive computing resource requirements, especially when simulating an ARROW hundreds of microns in length. Saving each dipole position as a file to be run and submitting the batch of file jobs to a supercomputing service is the method used in this dissertation. This Appendix will outline the process used.
B.1 Create FDTD Parameter Sweep

1. Construct the ARROW geometry using Lumerical® FDTD Solutions™, as shown in Figure B-1. Define the various material refractive indices. This is a similar task as described in Appendix A.1.

![Figure B-1: Construction of the ARROW geometry. Define each component’s dimensions and refractive indices.](image)

2. Place the dipole from the “Sources” menu. Define its position, polarization (using the theta and phi angle values), and the fluorescence wavelength. Place the monitor from the “Monitors” menu, and define its dimensions and position at the desired distance down the ARROW from the dipole. See Figure B-2.
3. Define the FDTD simulation region by selecting it from the “Simulation” menu, and setting its position and dimensions. Set the boundaries of the FDTD region a minimum of a half-wavelength from the dipole, monitor, and optically relevant dielectric interfaces. Define the desired mesh accuracy on the sliding scale from 1-8, 8 being most accurate. More accuracy requires more computing resources, but this accuracy is needed for smaller structures; a structure scaled in hundreds of microns can be simulated with a 1 or 2 accuracy setting. The standard FDTD boundary conditions are sufficient for this model, but the modeler may consider these parameters at his or her own interest. See Figure B-3.
4. A single simulation job is now constructed. Using this, a parameter sweep may be constructed that will override the dipole’s position. From the “Optimizations and Sweeps” window, create a new parameter sweep. Edit the sweep to override the dipole y-position. Add a parameter to be swept and provide a label (e.g. “y pos”) and select the dipole’s parameter “y” as the parameter to be swept. Make sure the type of sweep is defined as “Length (microns)”, and define the y-position range. In Figure B-4, the range is listed as 2.499 to -2.499 as the ARROW core is centered at y = 0; the 0.001 micron difference is to keep the dipole inside the ARROW. The y-range can be altered to fit the design in question. Set the number of y-points to sweep from the range extrema, 25 points in this example. Add a result to collect, give it a label (“250um trans” in this
example), and define it to be the “T” transmission value of the monitor. Click “OK” to save this sweep.

![Edit Parameter Sweep dialog box](image.png)

**Figure B-4: Create sweep of the dipole’s y-position.**

5. Right-click the sweep just created, and select “Insert parameter sweep”. This will nest the sweep just created into another sweep. This new function will sweep the dipole’s x-position. Edit the new mesh. Give it a label (“BEADmesh” in this example), and select the dipole’s “x” parameter to be swept. Again, make sure the type of sweep is defined to be “Length (microns)”, and define the sweep range to be the ARROW core width, -5.999 to 5.999 in this example. Set the number of x-points to simulate, 30 in this example. Add a result to measure; label this result, and select the result defined in the nested sweep (same as in Point 4 above). See Figure B-5.
6. Right click the sweep, and click “Save to files”. This will create a new folder where the FDTD .fsp file is saved. The dipole will then be swept across the ARROW core at every defined parameter point, and save a .fsp file for each point. Submit this folder to a supercomputing service for FDTD calculation. The submission process for the BYU Supercomputer is described in the next sub-section.

7. When the batch is completed, replace the saved .fsp files with their completed versions. In FDTD Solutions, right click the parameter sweep and select “Load from files”. This will concatenate all the files and produce a transmission matrix that corresponds to a collection efficiency matrix for that dipole polarization. Right click on the parameter sweep, click “Visualize”, and select the concatenated results. In the visualization window, make sure the matrix is set to an absolute value scalar operation. Click the “Export to…” drop-down menu and select “Text”. This will export the matrix to a .txt
file which can be imported into MATLAB. Repeat this process for all polarizations, and average the resulting transmission matrices to create an effective collection efficiency profile. See Figure B-6.

![Visualization window of the concatenated FDTD calculation sweep.](image)

**Figure B-6: Visualization window of the concatenated FDTD calculation sweep.**

### B.2 Submitting a Batch of FDTD Jobs to the BYU Supercomputer

Brigham Young University offers supercomputing services for its student researchers. In this subsection, I will describe the process I use to submit my FDTD jobs for mass calculation through this service.

1. Acquire an account with the Office of Research Computing at rc.byu.edu.
2. Place a folder containing the job batch in the “compute” directory.
3. In the home directory, place the .sh files “run-fdtd.sh” and “submit.sh” using the SLURM language.

   a. run-fdtd.sh contents:

   ```bash
   #!/bin/bash
   #SBATCH --time=1:10:00
   #SBATCH --ntasks-per-node=28
   #SBATCH --nodes=65
   #SBATCH --mem=8G
   #SBATCH --switches=1
   #SBATCH -C 'rhel7
   #SBATCH --mail-user=[USER EMAIL ADDRESS]
   #SBATCH --mail-type=END
   #SBATCH --mail-type=FAIL
   #SBATCH --output=/fslhome/[USER NETID]/compute/slurm_output/slurm-%A_%a.out
   # Error out on unset variable, just in case
   set -e -u
   # Get rid of possibly interfering modules and load FDTD
   module purge
   module load lumerical/fdtd
   # Which file are we running with?
   FSP_FILE=$(find $INFILE_DIRECTORY -name "*.fsp" | sed -n ${SLURM_ARRAY_TASK_ID}p)
   # Run fdtd-engine on one this task's fsp
   # have mpiexec get number of cores from Slurm
   mpiexec -n $SLURM_NTASKS fdtd-engine-mpich2nem $FSP_FILE
   ```

   b. Resource requests:

   i. Time: Walltime allocated for a single job to run; if not enough time is allocated, the job will not finish and the next job will be run. However, requesting more time will also de-prioritize the job’s queue position.

   ii. Ntasks-per-node: Number of cores attached to a node. Keep this at 28.

   iii. Nodes: Request the number of nodes. Requesting more nodes will allocate more processing power for the batch.
iv. Mem: Request memory for each node. Obtain estimated memory requirements for a job from FDTD Solutions software, and then divide the needed memory by the number of nodes requested. Request a little more than that number to provide some buffer in case the estimate is an underestimate.

v. The remaining parameters may be kept as is.

vi. The more resources requested, the further back in the supercomputer’s prioritization queue the batch will be placed.

c. submit.sh contents:

```bash
#!/bin/bash

# Export the infile directory (allows the job array to find it)
export INFILE_DIRECTORY=$HOME/compute/CEyArray

# How many fsp's do we have?
TASK_COUNT=$(find $INFILE_DIRECTORY -name "*.fsp" | wc -l)

# Submit an array of TASK_COUNT jobs
sbatch --array=1-$TASK_COUNT run-fdtd.sh
#sbatch --array=1-$TASK_COUNT run-fdtd.sh
```

d. Use run-fdtd.sh to request resources, and use ./submit.sh to run the batch.

e. Because of the large number of jobs in each batch, it may take several weeks or months for the batch to complete. This is due in part to the supercomputer’s queue algorithm which may pause running the batch so that it may allocate its resources to another user’s submission for a time.
APPENDIX C. STANDARD BIOSENSOR FABRICATION PROCESS

The outline given in Chapter 4 was an overview of the fabrication process, but each step described actually had multiple sub-steps. This appendix will give a detailed process for biosensor fabrication. This following is the standard fabrication process, but it may be altered to adapt to different design parameters.

C.1 ARROW layer deposition

The fabrication begins with a silicon wafer with commercially-sputtered dielectric layers. The company Nova Electronic Materials was the supplier used for these wafers. The ARROW layers were alternating layers of silicon dioxide (265 nm thickness, 1.47 refractive index) and tantalum pentoxide (102 nm thickness, 2.107 refractive index); the first layer deposited was silicon dioxide, and a layer of tantalum pentoxide would be the last and topmost layer deposited.

C.2 Pre-Core

- Ebeam: 100nm Cr @ 4 A/sec
- AZ3330: 5000 rpm @ 5000 rpm/s, 60 sec
- Soft bake: 90°C, 60 sec
- Exposure:
  - Use *Arrow Splitter Precore* mask
  - North aligner (no filter)
  - **Intensity: 7.45 mW/cm^2** *(recorded from aligner)*
  - **Time: 10 sec** *(adjust based on intensity to get desired dose)*
- **Dose:** 75.5 mJ/cm²
  - Develop: ~60 sec in 300 MIF
  - Hard bake: 110°C, 2 min
  - Descum: 150 W, 60 sec
  - Cr Etch: Leave in Cr Etch for ~5 min or until all removed
  - Remove AZ3330 with Acetone and IPA on Spinner
  - Descum: 150 W, 60 sec
  - Dehybake ~20 min (or more)

- **C.3 Core**
  - SU-8 10: 500 rpm @ 100 rpm/s, 6s → ~4200 rpm @ 1200 rpm/s 60s → 6000 rpm @ 6000 rpm/s 2s
    - *The middle rpm speed can vary anywhere from 3500-4400rpm in order to get the proper height*
  - SB: 65°C, 8 min -> 95°C, 8 min -> 65°C, 7 min
  - Exposure
    - *MMI Core* pattern mask (depending on wafer design)
    - South aligner (filter)
    - Intensity: 8.80 mW/cm² (recorded from aligner)
    - Time: 55 sec (adjust based on intensity to get desired dose)
    - **Dose:** 484 mJ/cm²
  - PEB: 65°C, 6 min -> 95°C, 6 min -> 65°C, 7 min
  - Develop: SU8 Developer ~60 sec
  - **Measure core height:** 6.0-6.5um is goal
    - *The 1st and 2nd hard bakes will decrease the height by ~0.5um*

***If height is too low (below 6.0um) strip off SU8 in Nanostrip at 90C for 30min and redo core step***
  - 1st Hard bake: 65C -> 200C, 12min -> 65C, 20 min
  - **Measure Core Height:** 5.5-6.0 is goal
  - Descum: 50W, 60s
- 2nd Hard bake: 65°C -> 250°C, 8min -> 65°C, 23 min
- Descum: 50W, 60s
- **Measured Core Height**: 5.5-6.0um is goal -> higher leads to more throughput

***If height is too low (below 5.5um) strip off SU8 in Nanostrip at 90C for 30min and redo core step***

### C.4 Pedestal Protection

- HMDS (use same spin rate as AZ4620)
- AZ4620: 2800 rpm @ 1100 rpm/s, 30 sec → 6000 rpm @ 6000 rpm/s, 2 sec
- Softbake: 70°C, 60 sec; 90°C, 60 sec; 110°C, 20 sec
  - Note for this step don't ramp up, just take the wafer off of the hot plate and put it on the other plate that is already set at the desired temperature
- Exposure: Create the pedestal pattern
  - Use the appropriate pedestal mask (see note at top)
  - North aligner (no filter)
  - Intensity: **7.45 mW/cm^2**
  - Time: **55 sec**
  - **Dose: 409.75 mJ/cm^2**
- Flood Exposure: Allows core to stick out above photoresist so Nickel can cover it
  - No mask for flood exposure
  - North aligner (no filter)
  - Intensity: **7.45 mW/cm^2**
  - Time: **9 sec**
  - **Dose: 67.05 mJ/cm^2**
- Develop: 400K:H2O 1:3, ~3min
- **Measure height over core**: Greater than 1.8um, ideal is 2.0-2.4um
  - If it is too short, develop for longer or possibly flood expose longer

***If core sticks out too much is too high (above 2.4um) remove AZ with A&IPA and redo pedestal mask step***
• Descum 50W, 30s
• HCL Dip: (HCL:H20, 1:2) for a few seconds (less than 5 sec)
• E-beam: 110nm Ni
• Lift off with acetone
  o Place wafer gently in a beaker full of Acetone
  o Leave wafer without shaking until enough Ni is lifted off
• Descum 50W, 30s

***Check pattern under microscope. If pattern doesn't look right (Ex. too much Ni lifted off) strip off Ni in Ni Etch (acid bench) for ~5 min (or until Ni is all removed) and redo pedestal mask step***

C.5 Pedestal Etch

• Trion "SAPoxide" recipe -> target 1.3um deep
  o 1.3um target is to etch through all of the ARROW layers
  o Wafer will turn a black color during this step
  o Time depends on rate -> etch for 300 sec and then measure the rate (nm/sec) to get remaining time
  o It is not a huge issue if you etch too deep on this step
  o Time: 1300sec usually works

• Measure Pedestal Height: ~1.3um

• Trion "Bosch" recipe -> target 5.4um
  o The Bosch recipe consists of a 16 step process with all of the odd steps, except step 1, being an 11 sec etch -> 7 etch steps total
  o Calculate rate by completing an entire Bosch cycle and then measuring the depth (based off of your previouid height)
  o Count the number of full cycles and extra etch steps needed to reach target
  o Ususally 3 full cycles, but keep going until you reach the target height

• Measure Pedestal Height: ~5.4um

• Trion "Si_isotropic" -> target 6.0um
  o This recipe etches at a fast rate
Etch rate is roughly 0.5um/60 sec
Pedestal has greater tolerance as long as you are above 6um total height it should be fine
Time: 60 sec usually works

- **Measure Pedestal Height: ~6.0um (or greater)**
- TC1 (H2O2:R6 1:10) soak: 10 min
- Ni Etch: Leave in Ni Etch for ~5 min or until all removed
- TC1 (H2O2:R6 1:10) soak: 10 min
- Descum: 50 W, 30 sec
- **Measure Pedestal Height: ~6.0 (or greater)**

***Place wafer in Dehybake until ready for the next step (or for at least 20 min)***

C.6 Oxide Layers

- Target: 6um (6000nm) of high index oxide (1.51)
- "0 Stress 1.51" recipe
  - Record Elipsometry Numbers:
    - **P1:** ~95
    - **A1:** ~45
    - **P2:** ~185
    - **A2:** ~135
- **Test Growth Time:** ~1 min 20 sec
- **Measured Thickness:** ~95 nm
- **Calculated Rate:** ~80 nm/min
- **Run Time:** ~1 hr 20 min
- **Index:** ~1.51
C.7 Ridge Ni Mask

- AZ3330: 1000 rpm @ 1200 rpm/s, 60 sec → 6000 rpm @ 6000 rpm/s, 1 sec - (no HMDS)
- SB: 90°C, 7 min
- Exposure: Get the pattern
  - Use appropriate ridge pattern mask (see note above)
  - North aligner (no filter)
  - Set exposure distance to 30um
  - **Intensity: 7.45 mW/cm^2** *(recorded from aligner)*
  - **Time: 35sec** *(adjust based on intensity to get desired dose)*
  - **Dose: 260.75 mJ/cm^2**
- Develop: 300K, ~1 min
- Exposure:
  - Ridge pattern mask (depends on wafer design)
  - North aligner (no filter)
  - **Intensity: 7.45 mW/cm^2** *(recorded from aligner)*
  - **Time: 6 sec** *(adjust based on intensity to get desired dose)*
  - **Dose: 260.75 mJ/cm^2**
- Develop: 300 MIF, 45 sec
- Descum: 100 W, 60 sec

***Check Feature size, If the size is undesired or alignment is off remove photoresist with A&IPA start over before deposing Ni***

- HCL Dip: (HCL:H2O, 1:2) for a few seconds (less than 5 seconds)
- E-beam: Ni 110nm @ ~4 A/sec
- Liftoff with acetone in Sonicator
  - Place beaker in sonicator
  - Turn on the sonicator
  - Quickly, but gently, place wafer in beaker
  - Watch until enough Ni has been removed
  - Remove wafer
• Make sure Ni is removed in undesired locations
  o If pattern looks wrong then remove Ni in Ni Etchant for 5min (or until all Ni is removed) and try again
• Descum 100W, 60s

C.8 Overnight Bake
• Place Wafer in Cleanoven 1
• Start at 70°C and ramp it up to 300°C at 4°C/min
• Hold at 300°C for 14 hours and ramp back down at same rate
• Once bake is done put immediately into PECVD for cladding growth or into the dehybake until ready.

C.9 Cladding Growth
• Target: 6um (6000nm) of low index oxide (1.44)
• "Steve Low Stress Low Index" recipe
  o Record Elipsometry Numbers:
    o P1: ~95
    o A1: ~45
    o P2: ~185
    o A2: ~135
• Test Growth Time: ~1 min 20 sec
• Measured Thickness: ~95 nm
• Calculated Rate: ~80 nm/min
• Run Time: ~1 hr 20 min
• Index: ~1.44
• Place immediately in Dehybake after
C.10 Core Expose

- *With the Core Expose step it is important to keep the wafer as dehydrated as possible*
  - If it is not dehydrated you may see bubbles appear in the photoresist the end of this process
  - If bubbles are found you may need to do the long overnight bake in order to dehydrate it

- AZ4620: 1500rpm, 60s
- Soft bake: 80°C 15min
- Let rest (not on metal surface) for ~15min @ RT
- Exposure
  - Core Expose pattern mask (depends on wafer design)
  - North aligner (no filter)
  - **Intensity: 7.45 mW/cm^2**
  - **Time: 60sec**
  - **Dose: 447 mJ/cm^2**
- Dev 400K:H2O 1:3 (~3min)
- Hard bake 100°C 2 hours

**Check to make sure there are no bubbles in photoresist. If bubbles are present restart.**
- Descum: 150W, 60s
- BHF Etch ~700sec (check to make sure oxide is gone)

C.11 SU8 Core Etching

- Place in Piranha @ 130°C, change acid daily until cores have fully etched. Piranha = 60ml:40ml H2O2:Sulfuric Acid
- Check each device after a couple days to see if any cores are broken
- **Before putting back in acid fill cores with water at the acid bench to prevent breaking**
- When etched place in, H2O -> Nanostrip -> H2O @ RT (at least 6hrs each soak)
- Before shipping check each device for defects and take notes