**Fabrication of Long Range Surface Plasmon Polariton Biosensors
Incorporating Optical Waveguides and Encapsulated Microfluidic
Channels via Wafer Bonding**

by

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Abstract

A microfabricated structure incorporating optical waveguides and closed microfluidic channels is an essential component in realizing a practical long range surface plasmon polariton (LRSP) biosensor. This has been achieved through advances in fabrication processes to realize reliable optical waveguides, and by development of a reliable wafer bonding process to create closed fluid channels. An existing process for fabrication of gold waveguides was modified by introducing ultra-shallow trenches to recess waveguides and present a planar surface for bonding. An improved fluidic channel etching process was characterized and successfully employed, yielding very smooth channel surfaces free from curtaining and grass issues. Optical performance of the complete bonded chips was demonstrated and verified using a cutback measurement method, producing an attenuation loss of 4.92 dB/mm. An alternative hot embossing process for formation of microfluidic channels on TOPAS substrate was investigated. Embossing die was produced on 4-inch silicon wafer using deep reactive ion etching (DRIE) to create 29 μm raised channels, and the die was subsequently used to transfer the channel structure to the TOPAS material.

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Chapter 1: Introduction

Biosensors have been envisioned to play vital roles in human lives due to their countless applications. They are widely used in the fields of medicine, diagnostics, environment, biotechnology, food safety, and homeland security. The fabrication of biosensors has become an essential and advanced topic of current research in the field of science and engineering. Biosensors can be categorized into different types depending upon signal transduction. Optical biosensors are the most frequently reported type of biosensors [1]. The fabrication of optical biosensors is presented in this thesis. The processes and steps to fabricate the integral parts of a biosensor such as waveguides, microfluidic channels, cladding, and bonding, which constitutes a full working optical biosensor are discussed.

1.1 Motivation and Contribution

Optical biosensors offer direct, real-time, and label-free detection of many biological and chemical substances. Some of their benefits include high specificity, small size, accuracy, cost-effectiveness, and simplicity to use [1]. Optical biosensors eliminate the need for biomolecular labeling resulting in reduced testing time as well as sample size. There has been exponential growth in the area of research and technological development of biosensors in last decade. Optical biosensors can be categorized based on their construction and structure such as surface plasmon resonance, evanescent wave fluorescence, and optical waveguide interferometry. Optical biosensors are dominated by the technique of surface plasmon resonance (SPR) [2]. The SPR sensors are used to analyze molecular interaction kinetics. Thus, the pharmaceutical industry is the major user and target for such devices. In general, these devices use the Kretschmann-Raether configuration. The SPR mode is excited with a transverse magnetic polarized beam using a prism with a gold deposited film which makes them bulky and expensive. Long-range surface plasmon polaritons (LRSP), which are transverse magnetic surface waves that propagate on a thin metal strip or slab cladded by dielectrics of the similar refractive index [3], can help overcome some of the

limitations of conventional SPR systems. The propagation length of SPP's is $\sim 90 \mu\text{m}$ which can be extended to millimeters using LRSPPs, resulting in a longer interaction length and improvement in sensitivity. The sensing depth is also increased to about $1 \mu\text{m}$, which makes it possible to sense large objects like cells [2]. The LRSPPs can be excited in a compact manner using butt or grating-coupling to an optical fiber, which makes them small and compact, making them an attractive choice for many fields of application [4]. The LRSPPs are highly sensitive to conditions near the metal surface, and the magnitude of LRSP sensitivity is on the order of $1 \times 10^5 \text{ nm RIU}^{-1}$ (nm per Refractive Index Unit) comparable with $1.4 \times 10^4 \text{ nm RIU}^{-1}$ of a conventional SPR sensor operating at 850 nm wavelength [5].

This thesis presents several modifications and enhancements to the fabrication process for an LRSPP biosensor, which consists of gold waveguides and microfluidic channels. The waveguides are embedded in a symmetric cladding of a dielectric CYTOP. The CYTOP is a trademark and a product of AGC Ltd. The LRSPP is fabricated on two separate substrates and then bonded to realize a complete device. A lower CYTOP cladding is fabricated by spin coating of CYTOP on a silicon wafer, and the gold waveguide structure is patterned on the lower CYTOP cladding. An upper CYTOP cladding is fabricated on borofloat glass substrate by spin coating of CYTOP then microfluidic channels are etched in the upper CYTOP cladding. Cracking issue in the cladding due to CYTOP solvent ingress was resolved by separating the upper and lower cladding on separate substrates. Furthermore, the gold waveguides deformation issue was resolved by introducing modifications to waveguide patterning process. Finally, lower and upper claddings containing gold waveguides and microfluidic channels are bonded to realize an integrated LRSPP biosensor. A wafer bonding process involving gold waveguides and microfluidic channels was developed and successfully implemented. The thermal and optical properties of all constituent materials such as silicon, CYTOP, gold, and glass were exploited to fabricate and bond fully functional LRSPP. The measured

mode power attenuation (MPA) of the fabricated LRSPPs in this work is discussed in results section which is lower but close to the theoretical value of 7.2 dB/mm [6].

An alternative fabrication process incorporating a hot embossing process to create the microfluidic channels in an amorphous polymer TOPAS was investigated. TOPAS is a trade name for Topas Advanced Polymers' cyclic olefin copolymer (COC) [7].

The LRSP device structure was designed by Professor Berini's group at the University of Ottawa. The entire micro-fabrication of the LRSP biosensing device was performed by the author.

The training and assistance to perform AFM and SEM analysis at the various stages of fabrication were provided by Howard Northfield at the University of Ottawa. The device testing was performed at the University of Ottawa by Alex Krupin and Zohreh Hirbodvash. DRIE of silicon wafers for the fabrication of embossing die was assisted and facilitated by Jeremy Upham at the University of Ottawa.

1.2 Overview of the Thesis

This thesis consists of six chapters. The motivation behind this work and contribution is described in Chapter 1. The background study of the area of optical biosensors based on SPR and LRSPPs is presented in Chapter 2. Some basic theoretical concepts related to the field of optical biosensors, previous work, and literature review is also included in Chapter 2. Chapter 3 describes the fabrication process of the LRSP. The contribution made through this thesis, along with modifications and enhancements made to existing processes is explained in Chapter 3 as well. The wafer bonding process was used to realize a sealed device with glass lids. The wafer bonding process and results are presented in Chapter 4. The solutions and fixes to the issues with previous work are presented in Chapter 3 and Chapter 4. The device testing and results are also presented Chapter 4. Details of hot embossing process to create microfluidic channels, an alternative to channel etching, are described in chapter 5. The outcome of the work presented in this

thesis is summarized in Chapter 6. Some challenges related to the current work as well as the suggestions for future work are given in Chapter 6 as well.

Chapter 2: Background and Literature Review

Modern research in biosensors began about sixty years ago with the development of enzyme electrodes by a scientist Leland C. Clark. Researchers and scientists from different fields of science such as physics, chemistry, VSLI, and engineering have jointly worked to develop reliable, robust, and economical biosensing devices since then. The functionality and sophistication of these devices have improved so much that their application extends to almost every major field of life [8]. The work presented in this thesis is an attempt to contribute towards the subject of biosensors by modifying and introducing fabrication processes for a LRSPP biosensor involving waveguides and microfluidic channels. This chapter presents the theory, literature review, and the background information as well as summary of contributions of this work to the field.

2.1 Biosensors

A biosensor is a multi-component analytical device consisting of a biological element and a sensing component. It responds to a complex biochemical reaction, such as antibody-antigen binding or an enzyme-substrate reaction, and produces a signal suitable for interfacing with a measurement system [9]. Thus, the function of the biosensing device is to provide methods of obtaining rapid results with an integrated device suitable for online monitoring or routine analysis of samples. A biological element can be an enzyme, antibody, living cell or tissue, whereas the signal output could be an electric potential, electric current, or a complex EM quantity. The biological element may be attached to the sensing element using the following schemes: (1) in a membrane entrapment scheme, a semipermeable membrane is used to keep the analyte and biological element separate, and a sensor is attached to the biological element, (2) in a physical adsorption scheme, the biological element is attached to the sensor using a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces, (3) in a matrix entrapment scheme, a porous encapsulation matrix is created around the biological element that helps in binding it to the sensor, (4) in covalent bonding, the sensor surface is treated with a reactive group to which the

biological element can bind [8]. The biosensors can perform this function using different methods to sense biochemical reaction and transducing it to an output. Therefore, the classifications of biosensors depend upon the methods of the signal transduction: Electrochemical, thermal, magnetic, optical, and piezoelectric biosensors [1].

The characteristics of a good biosensor may include: (1) the biological element must be highly specific and stable (2) the reaction should be as manageable as independent of stirring, pH, temperature (3) the output should be accurate, precise, reproducible, and provide linear response over a concentration range (4) the sensor should provide real-time response (5) the device should be economical, small, portable and useable by semi-skilled person [10].

The topic of this thesis is optical biosensors, but other major classes of the biosensors are reviewed before the optical biosensors are discussed in detail. The elements and select components of typical biosensors are shown in Figure 2.1. Samples containing an analyte of interest can take a variety of forms, including cell cultures, bio-fluids, food samples, or environmental samples. Transduction of the analyte concentration to an electrical signal is achieved through a combination of a specific bioreceptor immobilized on the surface of an electrical interface device. In the electrical energy domain signal conditioning circuits and signal processing techniques transform the transducer output to a form suitable for logging or display.

Figure 2.1 - Elements and Select Components of a Typical Biosensor [10].

2.1.1 Electrochemical Biosensors

Electrochemical biosensors are devices that combine inherent bioselectivity of the biological element with the sensitivity of electroanalytical methods. The biological element in the sensor is used to sense the analyte. After the biological element is recognized, a catalytic or binding event takes place which produces an electrical signal controlled by a transducer that is proportional to the concentration of the analyte. The output of the electrochemical transducer may be measured in form of current, potential, or impedance. However, the techniques measuring the current are most commonly used. Electrochemical biosensors have two major categories based on biological recognition process; (1) biocatalytic devices, (2) and affinity sensors [11]. Biocatalytic sensors use enzymes as biological recognition element. A thin layer of an enzyme is immobilized on the surface of electrodes to create electrochemical probes which are called enzyme electrodes [12]. The enzymes have high biocatalytic activity and specificity. Biocatalytic sensors have a simple design and are economical to use due to inexpensive instrumentation required [10]. Biocatalytic sensors may also use other biological elements such as cells, or tissue slices to recognize the target analyte and produce electroactive species or other measurable output. Biocatalyst sensors have

many commercial and personal applications, and blood glucose monitors devices are the most prominent example. Some analytes are not amenable to detection by enzyme electrodes because of the unavailability of selective enzymes for the analyte or the analyte is not commonly found in all living systems. In such cases, affinity biosensors become useful. Affinity biosensors can produce an electrical output signal by using selective and strong binding of biomolecules such as antibodies and membrane receptors with the target analyte. The complementary size and shape of the binding site to the analyte of interest determines the molecular recognition in Affinity biosensors [11]. An important type of affinity biosensors is called immunosensors. These are antibody-based biosensors where the detection of an analyte, is performed by its binding to an antibody [13]. The binding event is converted to electrical output by the electrochemical transducer. The immunosensors can perform both qualitative and quantitative analysis. These can be used for the detection of bacteria, viruses, drugs, and hormones.

The use of graphene and graphene-based nanocomposites for the fabrication of electrochemical biosensors have shown promising results with improved analytical performance, as reported in [14]. The fabrication of high selectivity and reagent-less electrochemical biosensors has become possible due to the direct electron transfer between redox enzymes such as glucose oxidase and the underlying electrode surface. The graphene functionalized electrodes show excellent analytical performance with negligible interference from biological elements metabolites [15]. Thus, graphene-/graphene-based nanocomposite modified electrodes (G/GNE) are being advocated for proteins, DNA, biomarkers, heavy metals, biomarkers, organic, and inorganic analytes of clinical, bioanalytical, and environmental significance [14]. Currently, electrochemical biosensors are being employed for the determination of neurotransmitters. The neurotransmitters are biomolecules that control the behavioral and physiological functions in the nervous system. The study and analysis of neurotransmitters in biological samples is critical in pharmaceutical and clinical aspects. Electrochemical biosensors fabricated using conductive polymers

(CPs) have the potential for such determinations of neurotransmitters being robust, selective, sensitive, and real-time measurements [16].

2.1.2 Magnetic Biosensors

Magnetic biosensors are developed to measure biomolecules and cells with high sensitivity that can be used to prevent diseases and gain valuable insight from biological systems. A magnetic biosensor is an integrated device in which a magnetic transducer converts magnetic field changes into a variation of voltage, current, and frequency. These biosensors are fabricated exploiting different types of magnetic effects such as Halls, magnetoelastic, inductive, magnetoresistance, and magnetoimpedance [17]. Most of the biological samples do not show significant magnetic susceptibility; therefore, magnetic nanoparticles have been utilized for biosensing, magnetic separation, and thermal ablation therapy. Diagnostic magnetic resonance (DMR) is currently employed to detect DNA/RNA, proteins, enzymes, drugs, pathogens, and tumor cells. DMR uses magnetic nanoparticles as proximity sensors that modulate the spin-spin relaxation time of neighboring water molecules which can be quantified using clinical MRI scanners or tabletop nuclear magnetic resonance (NMR) relaxometers. Most recently miniaturized biosensing method using magnetic nanoparticles are inexpensive to produce, biocompatible, physically and chemically stable and environment-friendly [18]. A chip-based NMR (micro NMR) detector systems is able to perform highly sensitive measurements of microliter sample volumes. The micro NMR consist of microcoils for NMR measurements, microfluidic channels for sample handling and mixing, miniaturized NMR electronics, and small portable magnet to generate polarized magnetic fields [19]. A recent study reported in [17] described the use of magnetic biosensors to study the effect of nanoparticle entering the human body. A nanoparticle below the size of 50 nm penetrates the tissue barriers and can be toxic for parenchymal and stromal cells. Thus, the MNPs have enormous potential in magnetic bio-detection in medicine and environmental control. The study in [17] also reports the fabrication of stable ferrofluids (FF) using electrostatic or steric stabilization of iron oxide MNPs. Controlled quantities of FF were used in

vitro experiments with human mesenchymal stem cells to understand the interaction between living cells and MNPs to realize advanced magnetic biosensors [20] [21].

2.1.3 Optical Biosensors

An optical biosensor is an integrated analytical device containing a biological sensing element integrated with an optical transducer system. The concentration of the measured substance (analyte) is converted to a proportional signal by the optical biosensor. There are many biological substances, including enzymes, nucleic acids, antibodies, whole-cell, receptors, and tissues which can be used by optical biosensors [1]. The optical biosensor may use direct detection (label-free) or optically labeled probes. The detected signal may result from changes in absorbance, fluorescence/phosphorescence, chemiluminescence, reflectance, refractive index, or light scattering. Optical biosensors are constructed to use many different optical techniques for detection. However, biosensors based on surface plasmon resonance, interferometry, fluorescence spectroscopy and spectroscopy of guided modes in optical waveguide structures such as a grating coupler and resonant mirror (RM) are common. The adaptability of the optical biosensors can be improved by coupling fiber optic with any optical technique mentioned above [22]. An illustration of an optical biosensor is shown in Figure 2.2. As in the previous figure, the analyte is introduced and binds to a biorecognition element immobilized on the transducer surface. There are several possible optical transducer elements that use the evanescent field in the proximity of its surface to detect the interaction of the binding event. The output of the optical transducer is converted to an electrical signal for logging or display.

Figure 2.2 - Illustration of an Optical Biosensor [1]
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Fluorescence is one of the most commonly used optical techniques for biosensing due to its selectivity and sensitivity. Fluorescence phenomena are associated with an energetic transition from a high energy state to a lower energy level resulting in photon emission. Another physically observable property of luminescence is polarization. It is due to the unique symmetries and orientations of wave functions and electric moment involved in electronic transitions [23]. In a fluorescence-based optical biosensor, the optical signal produced by a laser propagates in an optical fiber to a section of the fiber where antibodies are immobilized [24]. The detection system received the optical radiation produced by antigen fluorescence. These sensors are used in biochemical analyses of chemical compounds as well as in vivo (living organism or cells in human and plants) and in vitro (organism or cells outside biological context) analyses of cells and tissues. The fluorescence-based optical biosensors are also used in DNA sequencing due to their ability to reach a high readout speed [25]. The fluorescence-based optical biosensors can be divided into three types. The first type is direct sensing in which a molecule is detected before and after the change or reaction takes place. The second type is indirect sensing, where addition of a dye optically transduces the presence of a specific target molecule. The added dye can emit light when excited by a certain wavelength. The presence of a specific target molecule affects the fluorescence intensity, which is measured and analyzed. The green fluorescence protein (GFP) is a commonly used fluorescence tag that

helps the researchers to study dynamics of molecular events of living cells as well as locations and structures. However, this type of fluorescence biosensors is subject to some challenges and difficulties. The binding interactions between an activated signaling molecule and its target could be challenging to detect because of difficulty in seeing the localized interaction over background fluorescence. The third type of fluorescence biosensing is mentioned as fluorescence energy transfer (FRET). In this technique, a unique fluorescence signal is generated. As mentioned above, when the fluorophore (fluorescence compound or dye) is excited by a specific wavelength of light, it emits light at a different wavelength. However, when two fluorophores are paired in such a way that the emission wavelength of one overlap with the excitation wavelength of the other, the excitation of one of them will stimulate fluorescence of the complementary pairing fluorophore. This occurs especially when the fluorophores reside within about a few angstroms from each other. FRET is a very useful technique because the unique fluorescence signal generated under these conditions can be used to visualize and quantify the position and concentration of the interacting fluorophores [26]. The fluorescence spectroscopy can be used to repeatedly excite and detect single molecules to produce a bright signal at the single-cell level. This helps to study the dynamic nanoscale behavior of molecule in complex local environment [27]. Despite of the fact that fluorescence biosensing method is very sensitive and powerful, but it requires labeling the molecules as mentioned in GFP and FRET techniques. Thus, it requires additional preparation steps and is deemed laborious. However, there are optical biosensing methods which do not require labeling and refractive index (RI) detection is one of them. The optical label-free sensing is performed through following major optical structures: (1) surface plasmon resonance, (2) interferometer-based biosensors, (3) resonant mirrors, (4) emerging technologies such as optical ring resonators, photonic crystals, and optical fiber-based biosensors [28]. In label-free RI detection target molecule are not labeled or altered but those are detected in their natural form. RI based detection methods measure the change in refractive index induced by molecular interactions. The RI change is proportional to the sample concentration or surface

density instead of sample mass. Thus, the detection signal does not depend on sample volume. In most of the label-free optical biosensors, such as RI biosensors, the sensing light is concentrated close to the surface of the sensor where an evanescent field exponential decay into the bulk solution with a decay length ranging from a few tens to a few hundred nanometers. Therefore, the sensor can detect the RI changes induced by the analyte binding within the decay length [29]. The decay length characteristic can be exploited to minimize the size of device in the range of micrometers to nanometres. The sensitivity and detection limit (DL) are the two important parameters of the evanescent based sensors. In general, the sensitivity is determined by the light-matter interaction. For evanescent based sensors, the sensitivity is the fraction of the light in solution or the light intensity at the surface of the sensor. The DL is inferred by accounting for the noise in the transduction signal.

Figure 2.3 - Evanescent Field Sensing by an Adsorbed Layer [30].
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Fiber-optic biosensors are an important member of the optical biosensor family. An optical fiber is a cylindrical waveguide constructed using silica where light is transmitted along its axis. It uses the phenomenon of total internal reflection. A fiber is comprised of a core and a cladding. The core is made from dielectric material surrounded by a cladding, which is also a dielectric material with a lower refractive index. In this arrangement, most of the light is internally reflected, but there is a small component of light

which propagates into the cladding. This component of light is called the evanescent field as shown in Figure 2.3. In most of the standard fiber structures, the evanescent field decays to near zero at the surface of fiber if the cladding is much thicker than the core. However, the cladding diameter can be reduced by tapering it down to get a diameter less than the core diameter. In such a case, the light is guided by the cladding and not by the core. Thus, the evanescent field interacts with the surrounding environment and its magnitude enhances in the tapered region of cladding [26]. This phenomenon, combined with other optical sensing techniques, can be used for biosensing purposes. The fiber optic biosensors can be classified into two categories: intrinsic and extrinsic sensors. In an intrinsic sensor, the interaction with analyte takes place within a component of optical fiber. In extrinsic sensor, the optical fibers are utilized to couple the light only. The fiber optical biosensors can be combined with several optical techniques such as refractive index, absorbance, fluorescence, chemiluminescence measurements, and other spectroscopic approaches [31].

With advancement in technology and fabrication processes, the dimensions of optical fibers are reduced to a submicron size, which can be used to sense individual cells. Fiber Bragg gratings (FBG's) are the most prevalent fiber-based optical sensors. FBG's are periodic structures which are written using UV radiation. FBG's are created imprinting directly into the core region of optical fiber. These structures are comprised of periodically varying refractive index on several millimeters on the fiber core. The periodicity of the FBG's works as wavelength sensitive reflectors. Thus, light propagating down the fiber is partially reflected at each of the refractive index variations. These reflections interfere destructively at most of the wavelengths resulting in the light propagating down the fiber if it was of uniform refractive index. However, at some wavelengths, called Bragg wavelength, from each successive period create constructive interference being in phase and light is reflected backward in the fiber. Therefore, the resulting structure acts as a band rejection filter, reflecting a narrow band of light at the Bragg wavelength. The shifts in the

Bragg wavelength are monitored; the device acts as a refractive index sensor, which is used for biochemical sensing [29] [32]. The evanescent field can be exposed from fiber Bragg grating using different strategies: (1) creating a surface grating on the side of the fiber by bending and polishing the fiber to expose the core and then grating is physically patterned on the surface , or (2) etching the optical fiber by a chemical mean down to its core [33] [34] [35]. Recently, Long-period grating (LPG) has gained attention for biochemical sensing. Due to development in fabrication technology, the LPGs with longer periodicity ranging from 100 μm to 1 mm can be manufactured. These are three to four orders of magnitude larger than conventional FBGs. Core modes couples into the cladding modes of the fiber when using an LPG [36].

The FBGs technology has many advantages such as self-referencing capacity, minimum size distributed sensing, and multiplexing [26]. Schematics of fiber Bragg grating sensor, fiber Fabry-Perot interferometer sensor, and an etched-eroded fiber are shown in Figure 2.4 [34]. Figure 2.4 (a) shows an etch-eroded fiber Fabry-Perot interferometer (FFPI). The narrow resonance spectral features that can be achieved results in higher sensitivity than a fiber Bragg grating, and it can detect variation in the order of 10^{-5} RIU. Figure 2.4 (b) shows an etched-eroded single fiber Bragg grating used to measure the indices of liquids for biosensing applications [34].

Figure 2.4 – Fiber Bragg Grating Sensor and Fiber Fabry-Perot Interferometer Sensor [34].
(a) Schematic of a Single FBG and FFPI Sensors (b) SEM Image of an Etched-eroded Fiber
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Photonic crystals (PC) biosensors are another type of label-free optical sensor. Like FBGs, PCs are also periodic dielectric structures with periodicity. The periodicity of PCs is on the order of a wavelength which forms the periodic bandgap. The structure of the PC does not allow the incident light to propagate whose wavelength lies within the photonic bandgap resulting in a wide bandgap appears on the transmission or reflection spectrum. The PC's periodic structure can be locally disturbed to form a photonic defect within the bandgap. Thus, the light resonant with the defect mode can propagate in the PC. Consequently, the defect mode appears as a relatively sharp peak within the bandgap on the transmission or reflection spectrum. The spectral location of the defect mode is very sensitive to the variations in the locality around the defect; it can be exploited to sense transduction signal upon RI changes as a result of the binding of molecules to the defect [29]. The defect mode wavelength can be adjusted across the photonic bandgap by performing fine-tuning of spectral parameters. Also, the PCs can confine the light very strongly due to

the photonic bandgap. Due to these characteristics, PCs have become very attractive for their use in sensing application. The PCs can be configured to detect RI changes in waveguide configuration [37] and microcavity laser configuration [38]. A SEM image of typical PC based biosensor is shown in Figure 2.5 [39]. The periodicity of PC as shown in the figure, is on the order of a wavelength.

Figure 2.5 – A SEM Image of a Photonic Crystal Microcavity Based Biosensor [39].

Waveguides are an important structure which are commonly used in optical biosensors. The waveguides can be integrated with many optical biosensors as sensing elements. For example, waveguides are used as sensing element in interferometer-based biosensors, whereas in resonant mirrors the leaky mode at the waveguide-substrate boundary is used as a sensing element. There other waveguide structures employed in optical biosensors such as surface plasmon resonance (SPR), metal clad waveguides (MCWG) reverse symmetry waveguides, and symmetrical metal cladding waveguides as shown in Figure 2.6. The figure shows both metal and dielectric waveguide structures. The immobilized antibodies shown indicate the sensing surface. Conventional waveguides take advantage of the total internal reflection that occurs at both waveguide-solution and waveguide-substrate boundaries [29]. The light is confined in the core of the waveguide; however, there a component of the guided light that extends toward the interface

between waveguide and surrounding material. The extended component of the guided light is mentioned as an evanescent field, which may extend to hundred of nanometers. In a waveguide transduction process, a receptor is deposited on the surface of the waveguide. As soon as an analyte is mobilized on the same surface, a biomolecular interaction between a receptor molecule and its complementary analyte produces a change in the RI at the sensor surface that induces a change in the optical properties of the guided light through the evanescent field (EF) [30]. Thus, the transduction occurs due to evanescent field sensing (EFS) in optical waveguide biosensors.

The waveguide structures can support different transverse modes for light propagation. An SPR, as shown in Figure 2.6 (a), can operate at only transverse magnetic (TM) mode. However, resonant mirrors (RM), as shown in Figure 2.6 (b), supports both transverse magnetic (TM) and transverse electric (TE) modes, which have different resonant angles [40]. The metal clad waveguides (MCWG), shown in Figure 2.6 (c), are comprised of the metal layer. In the case of MCWG, the metal layer acts as a spacer between low RI waveguide and high RI substrate. Thus, light is guided in a low RI layer. Therefore, the sensitivity is increased through increasing matter-light interaction by pushing extra light into the solution layer. The reverse symmetry waveguides, depicted in Figure 2.6 (d), follow the design of MCWG to push more light into the solution for increased light interaction with biomolecules such as cells [29] [41] [42].

Figure 2.6 - Illustration of Waveguide Structures depicting an antibody functionalized surface [29].

(a) Surface Plasmon Resonance (b) Resonant Mirror (c) Metal Clad Waveguides

(d) Reverse Symmetry Waveguide (e) Symmetrical Metal Cladding Waveguide

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The SPR biosensors are based on the phenomenon of surface plasmon resonance (SPR). It is the interaction of light with thin metallic film usually silver or gold. The metal film is coated on a transparent medium. The SPR occurs due to the generation of charge density oscillations called surface plasmon polaritons (SPPs) at the interface of two media with the dielectric constant of opposite signs such as metal and dielectric, as mentioned above in this case.

The biosensing device fabricated and presented in this thesis is based on SPR. The details of the work along with necessary background information is described in the following sections.

2.1.3.1 Surface Plasmon Resonance Biosensors

The phenomenon of anomalous diffraction on diffraction gratings because of the excitation of surface plasmon waves was described first time at the start of the twentieth century by Woods [43]. Optical excitation of surface plasmons using the method of attenuated total reflection was demonstrated by

Kretschmann and Otto in the late sixties [44]. However, the first demonstration of surface plasmon resonance (SPR) biosensor was presented in 1983 by Liedberg et al. for biosensing application [45]. Since then SPR biosensors have become most intensively studied biosensors. The SPRs are powerful biosensing devices to study the interaction between the target analytes and bio recognition molecules. The SPR biosensors are very popular due to their label-free biosensing applications [29]. The SPR is a charge density oscillation which may exist at the interface of a metal and a dielectric. The charge density wave is associated with an electromagnetic (EM) wave. The field vector of EM wave is maximum at the interface and decays evanescently into both media. This surface plasma wave or surface plasmon polariton (SPP) is a TM polarized wave [46]. To characterize SPP, the dispersion and spatial profiles are considered. The dispersion relation of SPP propagating at the interface of two media is given by the following wave vector [46];

$$K_{sp} = K_0 \sqrt{\frac{\epsilon_d \epsilon_m}{\epsilon_d + \epsilon_m}} \quad (1)$$

Where K_{sp} is frequency dependent SPP wave vector, K_0 is the free space wave vector, ϵ_m and ϵ_d are frequency dependent permittivity of metal and dielectric respectively. The equation shows that the condition is satisfied for metals and dielectric since ϵ_m and ϵ_d have opposite signs. The SPP propagates along the interface of two media with an evanescent EM field confined at the metal-dielectric boundary, and it gradually attenuates due to absorption in the metal [46]. The distribution of the magnetic field intensity of SPP for two different wavelengths at the interface of gold and dielectric in the direction perpendicular to the interface is shown in Figure 2.7 [47]. The dielectric refractive index is 1.32, which is very close to the refractive index of CYTOP, which has a refractive index of 1.34. CYTOP is a proprietary amorphous fluoropolymer used as cladding (dielectric) material in the fabrication of biosensing devices in work reported in this thesis.

An SPR optical biosensor consists of an optical system, a biofunctionalized transducing medium and an electronic system supporting optoelectronic components of the sensor and allowing data processing. The biomolecular recognition element on the surface of metal recognizes and captures analyte present in a sample producing a local increase in the RI at the metal surface. The RI increase gives rise to an increase in the propagation constant of SPP propagating along the metal surface, which is accurately measured by the optical method [47]. The changing conditions can be tracked and captured by measuring the resonant angle, resonant wavelength, resonant intensity change, or the phase or polarization of the light wave modulated by SPPs in a biosensor [29]. The optical system used to excite the SPP is also used for interrogation of SPR concurrently. Thus, increasing the interaction length, the sensitivity of SPR sensor does not increase. As follows from equation (1), the SPP cannot be excited directly by an incident optical wave at the planar metal-dielectric interface because the propagation constant is always higher than that of optical wave propagation in the dielectric. Therefore, the momentum of the incident optical wave needs to be enhanced so that it matches the momentum of the SPP. The enhancement in the momentum is achieved through a technique called attenuated total reflection (ATR). Figure 2.8 illustrates several common approaches to couple optical signals to surface plasmons. Figure 2.8 a) illustrates the use of a prism very close to or in contact with a metal film, in order to match the wave vector of the photon with that of the SPP. Figure 2.8 b) shows coupling from a waveguide in contact with a metal film and Figure 2.8 c) illustrates the use of a grating to match wave vectors.

Figure 2.7 - Distribution of Magnetic Field Intensity of SPP [47].
Calculated for two different wavelengths at gold and dielectric interface
(dielectric refractive index 1.32) in the direction perpendicular to the interface
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One of the key requirements for a modern-day biosensor is integration with other components and systems. This can be achieved through miniaturization and integration of the biosensors. Thus, planar waveguide structures can be used to fabricate SPR biosensors. Planar optical waveguides are already being employed, which are fabricated through thin-film technologies. These waveguides are multilayer structures of varying optical refractive index that can support a propagating light mode. Thus, using semiconductor fabrication techniques, multiple sensing components can be integrated into a single device [48]. In optical waveguide-based SPR sensors, the light wave is guided by the waveguide, and a SPP is excited at the metal-dielectric interface similar to all other SPR structures. The guided modes are in phase in these structures [44]. As mentioned above the sensitivity of the waveguide based SPR sensors is lower than other structures that use ATR such as prism coupler.

Figure 2.8 - Excitation of Surface Plasmon Polaritons [47].

- (a) Using a light beam Through Prism Coupling, (b) Using a guided mode of the optical waveguide, (c) Using light diffraction on a diffraction grating**
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Waveguide based SPR biosensors are appropriate for large scale and planar integration. The resonance coupling of light to SPP includes a significant phase shift to the incident light. This phase change can be measured using heterodyne or interferometry methods. The integrated SPR biosensor utilizing this method consists of a waveguide channel that splits into two arms, a sensing arm and a reference arm using a Y-splitter. The phase-dependent interference between the two guided modes is produced by combing both arms, as shown in Figure 2.9 [48].

Figure 2.9 - Illustration of a Waveguide Integrated SPR Biosensor [48].
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The major application areas of SPR biosensors are the detection of biological analytes and analysis of biomolecular interactions where SPR provides advantages of label-free real-time analytical technology. The main performance characteristics of the SPR biosensors are sensitivity, linearity, resolution, accuracy, reproducibility, dynamic range, and limit of detection. Sensitivity is the ratio of the change in sensor output to the change in quantity measured such as analyte concentration. Consequently, it depends on the sensitivity of the sensor output to refractive index and efficiency of conversion from the biochemical reaction into the change in the refractive index. The resolution of SPR sensor is defined as the minimum change in the bulk refractive index, which produces a detectable change in the output of the sensor. The accuracy is defined as the degree to which a biosensor output can reflect the true measured value from the sample. Repeatability is the ability of biosensor to produce the same output signal under the same measurement setup over a short interval of time. The lowest detection limit refers to the lowest concentration of target which can be measured by the sensor which is limited by noise caused by intensity fluctuations of the light source, noise associated with photon statistics and conversion of light intensity into electric signal [49] [50]. However, single-interface SPP is also characterized by high attenuation, limiting the scope of its applications. For a metal waveguide bounded by an ideal dielectric, the attenuation is caused by free electron scattering in the metal and at short operating wavelengths, by

interband transitions. In addition, roughness along the interface can cause additional attenuation [3]. The SPR sensor performance can be enhanced by improving the above-mentioned performance characteristics or by reducing the attenuation. The sensitivity is one of the most important parameters which can certainly improve the performance of SPR sensor. Using silver as a metal for SPR sensors can help to increase sensitivity. However, silver is subject to oxidation. The sensitivity of the SPR biosensor can be improved by employing a propagating mode called long-range surface plasmon resonance (LRSP) by reducing or controlling attenuation. The utilization of LRSP mode yields biosensors with improved sensitivity as compared to conventional SPR biosensors, as discussed in the following sections.

2.1.3.2 Long Range Surface Plasmon Polaritons

Long-range surface plasmon polaritons (LRSP) are optical surface waves that propagate along a thin symmetric stripe or slab over a considerable length, which can extend to centimeters. LRSP was first predicted in [51] about four decades ago followed by the work reported in [52] describing the observation of first ever excitation of LRSP mode on a thin metal film surrounded by index matching dielectric. LRSP is a practical way to reduce the SPP attenuation by using a thin metal film stripe surrounded by a dielectric operating the structure in the long-range mode. The long-range refers to the LRSP attenuation which is at least a factor 2 to 3 lower than that of the single-interface SPP. The range extension mitigates the limitation of the single-interface SPP, but the confinement is reduced as a result of this extension. However, the range extension can offset the decreased confinement, possibly enabling improved and competitive applications. The symmetric metal slab is the structure that can couple the LRSPs. Symmetry requires that dielectrics that are bonded on both sides of the metal have the same permittivity. In the symmetrical metal slab, the bound single-interface SPPs supported by individual metal-dielectric interfaces at large thickness " t ", couples as " t " is reduced. This results in forming two TM-polarized bound supermodes. These modes are referred as coupled modes as well. The supermodes are denoted as a_b for asymmetrical bound and s_b for symmetrical bound since their major transverse electric field component

changes either symmetrically or asymmetrically across the structure as shown in Figure 2.10. Thus, metal slab bounded by semi-infinite dielectrics can support two bound SPP modes, a_b and s_b . The metal slab is symmetrical if $\epsilon_{r,1} = \epsilon_{r,3}$, and it is asymmetrical otherwise [3].

Figure 2.10 – A Metal Slab of Thickness t Bounded by Semi-infinite Dielectrics [3].

In Figure 2.10, $\epsilon_{r,2}$ is relative permittivity of the metal slab, $\epsilon_{r,1}$ and $\epsilon_{r,3}$ are relative permittivity of dielectrics bounding the metal the slab. In a symmetric structure with lossless cladding, as thickness “ t ” is decreased, the effective index and attenuation of the s_b mode decreases. However, the mode fields are increasingly ejected from the metal film and penetrate deeper into the dielectric. The LRSP is the s_b mode of the thin metal film whereas the a_b mode is lossy and it is subject to increased attenuation [3]. The symmetric waveguides structures consisting of a thin lossy metal film of finite width embedded in an infinite homogenous dielectric supporting the purely bonded electromagnetic modes of propagation can be characterized at optical wavelengths [53]. The work in [53] [54] describes that the supported modes can be categorized into four families, depending on the symmetry of their fields. However, there are higher-order modes in addition to the fundamental modes. The fundamental modes are aa_b^0 (asymmetric-asymmetric bond), as_b^0 (asymmetric-symmetric bond), sa_b^0 (symmetric-asymmetric bond), and ss_b^0 (symmetric-symmetric bond). The letters “a” and “s” refers to asymmetry and symmetry of the major

electric field component along the y and x confinement directions respectively. The letters “b” and “0” show a bound or leaky mode, respectively. The properties of the thin metal film with finite width and infinite width are similar. However, the finite width thin metal films additionally provide two-dimensional field confinement. The finite width thin metal films support propagation of both asymmetrical and symmetrical modes, but due to inherent two-dimensional confinement, these modes show field variations in both the vertical and lateral directions. The ss_b^0 mode due to its unique characteristics makes the finite-width metal film waveguide is an attractive option for short distance applications such as polarizing devices. This type of waveguide offers 2-D confinement in the transverse plane, which is useful for signal transmission and routing as well as passive components, thus, making it suitable for integrated optics technology [53]. Figure 2.11 shows the dispersion characteristics of a thin film metal waveguide with a width of 1 μm whereas thickness is varied. As the film thickness is decreased, the ss_b^0 mode evolves towards TEM wave supported by the background. This evolution is similar to that shown by the s_b mode in metal film slab waveguides. It is observed that its losses and phase constant incline asymptotically towards those of TEM waves. Thus, by decreasing the film thickness, losses are reduced. It is also reported in [53] that decreasing the film width can reduce the losses well below those of the s_b mode supported by the corresponding metal film slab waveguide. The ss_b^0 mode can be selected as the only long ranging mode supported in a structure by a careful selection of the film thickness and width. Thus, the dimensions of the metal strip used in the fabrication of biosensor reported in this work supports the fundamental long range ss_b^0 mode termed as LRSP.

Figure 2.11 - The Dispersion Characteristics with Thickness of First Eight Modes Supported by a Metal Film Waveguide of Width 1 μm [53].

(a) Normalized phase constant (b) Normalized attenuation constant

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An LRSP wave can be confined to propagate in a waveguide structure comprising of a thin metal film of finite width embedded in an optically infinite homogenous dielectric, as shown in Figure 2.12 [55]. Attenuation and excitation measurements of the LRSP mode, supported by waveguide structures consisting of thin Au film and an index-matched curing gel (Nye Lubricants Inc., OCK-433) to form the polymeric upper cladding, are reported in [55]. The waveguide structure shown in Figure 2.12 is used to couple LRSPs at the operating free space wavelength $\lambda_0 = 1550 \text{ nm}$. It is important that dimensions of the waveguide structure are carefully selected in such a way that only ss_b^0 mode is coupled, whereas other modes are cut off.

Figure 2.12 - Illustration of Waveguide Structure for LRSP Coupling [55].
(a) 8 μm wide metal film, (b) 4 μm wide metal film, (c) Two of 2 μm wide metal films,
(d) Three 2 μm wide metal films separation 2 μm
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Figure 2.13 - Theoretical and Experimental Attenuations of Au Waveguides [55].
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Figure 2.13 shows a comparison between theoretical and experimental attenuation of different waveguide dimensions. It is observed that experimental attention values well match with the theoretical ones. In addition, the propagation length reported in [55] is 138 times longer than that of single-interface SPP. These findings make the reported structures an excellent choice for integrated optical applications such as LRSPS that can be used for biosensing, as discussed in the next section.

2.1.3.3 Long Range Surface Plasmon Polariton (LRSPS) Biosensors

The preliminary proposed LRSPS biosensors were an adapted form of conventional SPR prism-based biosensors. The low index polymer films were inserted between the prism and metal slab, which were used for bulk sensing and bacteria [4] [56]. However, the LRSPSs can also be implemented using waveguide structures consisting of a gold stripe embedded in a low index polymer such as CYTOP with an etched microfluidic channel. To keep the sensor size minimum, the LRSPS can be excited in a compact fashion using a butt or grating coupler. This structure also allows the LRSPS to be confined in the waveguide in the plane transverse to the propagation direction. Thus, it is possible to fabricate various integrated circuits such as straight waveguides, Y-Junctions, S-bend, Mach-Zehnder Interferometer (MZIs) and Bragg grating. In addition, gold is a preferred sensing surface because thiol-based recognition may be easily used [4] [57].

The LRSPS biosensors fabricated in this research work were waveguide biosensors consisting of gold stripe cladded in CYTOP, with etched fluidic channels. Thus, the fabricated LRSPS has symmetric dielectric cladding around gold waveguide to take advantage of the ss_b^0 mode coupling for enhanced sensitivity. The refractive index of the CYTOP used for cladding is 1.3348, at 1300 nm wavelength, which is very close to the water-based sample. The CYTOP is proprietary amorphous fluoropolymer of AGC Ltd. It shows high transmission in the visible light spectrum and electrical insulation. It repels oil and water and has resistance to many chemicals such as acids, alkaline, and organic solvents used in semiconductor

processes. In the previous work of [58] an excitation of LRSP mode along thin gold stripes on CYTOP covered with an index-matched aqueous buffer is reported. In this work, the attenuation measurements were performed at the operating wavelength of 1310 nm. The waveguides were excited using a broadside fiber coupling method with tapered fibers. The mode power attenuation (MPA) was measured very close to the theoretical value for the structure, which is 7.2 dB/mm. The reported work gives the rationale of the LRSP waveguide sensing application, using the structure mentioned above to support the excitation and propagation of the ss_b^0 mode for LRSP biosensing [49]. The work of [59] included LRSP biosensors based on gold waveguides with integrated fluidic channels. The LRSP were excited by butt-coupling a PM single-mode fiber to the input of sensor chips. Using the cutback method, LRSP attenuation of 5.0 dB/mm was measured for fully cladded waveguides. The chips were also tested for biosensing, and a 0.1 dB response was measured due to the binding of BSA protein on the functionalized gold surface. A cross-sectional view of the fabricated LRSP biosensing device during this work is illustrated in Figure 2.14.

Figure 2.14 - A Cross-sectional Illustration of Fabricated LRSP Biosensor.

The biosensing device reported in this research work consists of gold waveguides and symmetric dielectric cladding. The waveguides are 35 nm thick and 5 μm wide surrounded by about 9 μm thick symmetrical CYTOP cladding. The biosensing devices are supported by a silicon substrate, optically isolated by 9 μm thick cladding. The microfluidic channels were etched using RIE plasma etching. The reference waveguides are fully embedded in CYTOP to isolate them from the sample. The thickness of the gold waveguide is critical to the device performance, as gold waveguides that are too thick will cause rapid signal attenuation. The presence of a thin layer of bio-sample on the surface of a waveguide causes the attenuation constant and velocity to change. As a result, a shift in the propagation length of LRSP mode occurs. The attenuation can be detected in a straight waveguide by monitoring the output power and change in velocity can be detected in an optical interferometer such as MZI by monitoring the shift in the phase of the output beam [49]. During a biosensing operation, the gold waveguide in the fluidic channel, which is excited by an LRSP signal, is surrounded by a diluted aqueous solution containing a target analyte. The amplitude and phase of the LRSP mode are used to determine the presence of the target material in the sample.

The fabrication process steps of the LRSP biosensor are described in detail in the next chapter. This also includes previous work on the topic, related issues, improvements, and implementation of the new process.

Chapter 3: Fabrication of LRSPP Biosensors

In this chapter, the fabrication process of the sensor is discussed in detail. The LRSPP waveguides and micro-fluidic channels are fabricated on CYTOP cladding. The process presented in this chapter includes wafer preparation, lower CYTOP cladding, waveguide patterning, upper CYTOP cladding, etching of fluidic channels, and wafer bonding. The fabrication procedure is based on process steps and parameters presented in [60] [61] [62] [63] [64] [49]. However, some of these process parameters are modified where needed to obtain required results. These modifications are also discussed in this chapter. The fabrication process is made robust yet simple by introducing innovative processing, characterization of critical process steps and parameters, and implementation of the parameters through strict process controls. The process innovation is introduced by fabricating upper and lower cladding independently on silicon and glass wafers. Thus, the biosensor devices are fabricated on two separate substrates, a Si substrate which carries Au waveguides fabricated on lower CYTOP cladding and a glass substrate which contains microfluidics channels etched into upper CYTOP cladding. These individually fabricated components of biosensor devices are integrated by CYTOP-CYTOP bonding, which is performed in a wafer bonder. The resultant devices have glass lid eliminating the need for annealing as reported in [65]. The waveguide fabrication process is adapted by introducing an ultra-shallow trench to house the waveguide to ensure a planar bonding surface. The integration of these processing steps has yielded a fully functional biosensing device without deformation and cracking of waveguides and CYTOP. The processing steps for the fabrication of LRSPP biosensor are illustrated in Figure 3.1.

Figure 3.1 - Illustration of Process Flow for Fabrication of LRSPP Biosensor.
W-1 to W-9 show waveguides on lower clad, C-1 to C-10 show fluidic channels on upper clad.

The figures (W-1) to (W-9) show the processing of waveguide structure on CYTOP cladding whereas the figures (C-1) to (C-10) depict the processing of fabrication of glass substrate containing microfluidic channels etched into CYTOP cladding. A cleaned and salinized starting silicon wafer to be used as a structural base for devices is shown in figure W-1. The lower CYTOP cladding is formed by spin coating of 3 layers of A-grade CYTOP on silicon substrate shown in figure W-2. A depiction of spin-coated LOR1-A to promote adhesion of photoresist with CYTOP is shown in figure W-3. Application of photoresist S1805 and exposure by UV light using a photo mask to pattern waveguides on cured CYTOP are shown in figure W-4 and W-5 respectively. The development step after UV exposure is shown in figure W-6. Figure W-7 shows the RIE plasma etching of 35 nm deep trenches to house the gold waveguides. A 35 nm thick gold is thermally deposited in the evaporator to form gold waveguides which is shown in figure W-8. Gold lift off to obtain patterned gold waveguides in buffer oxide is shown in figure W-9, which concludes the waveguide structure on lower CYTOP cladding formed on a silicon substrate. A cleaned and salinized starting glass substrate to create the lid with microfluidic channels are depicted in figure C-1. The upper CYTOP cladding is formed by spin coating of 3 layers of A-grade CYTOP on glass substrate shown in figure C-2. Al is evaporated through E-beam to be used as a channel etch mask, as shown in figure C-3. Figures C-4 to C-8 show the bilayer lithography steps to pattern the channels on the lower CYTOP cladding. The microfluidic channels are etched using RIE plasma etch, and then Al etch mask is removed in MF321 as shown in figure C-9 and C-10 respectively. This completes the channel formation in CYTOP on a glass substrate. Both parts of the LRSPP biosensor are bonded in the wafer bonder after performing alignment, to obtain the final integrated device as shown in the center of Figure 3.1. Finally, completed wafers containing LRSPP biosensors were sent out for dicing. The precise control of the fabrication process parameters is vital to realize functional LRSPP biosensors. This includes proper application and curing of CYTOP, lithography steps, trench etching to house the waveguide structure, gold deposition and lift-off, channel etching, and wafer bonding. The purpose of controlling the process parameters is to obtain ultra-

smooth and symmetric CYTOP cladding, minimum gold roughness (1 nm or less), smooth channel walls and bottom floor, intact waveguides and rectangular channels after bonding.

3.1 Wafer Preparation

The structure of the biosensor devices consists of two 4-inch wafers; a silicon wafer to provide a structural base and a glass wafer to be used as the lid on the devices. The silicon wafer does not contribute towards sensing ability of devices. Thus, the doping of silicon wafers was not an important factor in selection. However, the physical integrity of the wafer was an important measure in wafer selection. The physical integrity depends on surface quality and flatness of the wafer. Film stress measurement tests were performed to select the best flat wafers. The selected wafers were used in subsequent fabrication steps. These wafers are subject to labeling and cleaning. The wafers were labeled by scribing a unique tracking reference number on the back of each wafer. This helps to identify the wafers throughout the fabrication process. The Piranha solution is used to clean any organic contamination from the surface of wafers. The wafer cleaning procedure is adopted from the previous work of [49]. The Piranha solution is a 4:1 mixture of sulfuric acid and hydrogen peroxide. The solution has very strong oxidation characteristics that make it a good organics removal agent. In addition, this solution has hydroxylation properties making the silicon surface highly hydrophilic by adding OH group. The wafers were dipped in Piranha solution for 10 minutes at 90°C to clean the surface and make it hydrophilic. The consequent wafer surface is ready for APTES ((3-Aminopropyl) triethoxysilane) application. APTES is an aminosilane frequently used in the process of salinization and covalent attaching of organic films to silicon and metal oxides [66]. To apply APTES, wafers were vertically placed in wafer holders and immersed in the 0.05% APTES solution. The APTES solution is a mixture of 1 ml APTES and 2-liter Ethanol. The wafers are kept immersed for 10 minutes with light agitation in the APTES solution. The wafers were then individually rinsed with ethanol using a washout bottle and dried with a N₂ gun. However, glass wafers are very fragile. Thus, extra care is required when

drying with a N₂ gun. The best practice for drying glass wafers is to place them horizontally on a particle free surface to avoid breakage. The dried wafers are followed by a dehydration step, which required baking the wafers on a hotplate at 115°C for 20 minutes. The purpose of the APTES film is to enhance adhesion between the silicon wafer and grade-A CYTOP. The adhesion is promoted due to the alkoxysilane molecules covalently bonded to carboxyl groups (-COOH) present in CYTOP grade-A [67]. CYTOP is a proprietary fluoropolymer used as cladding for biosensing devices fabricated in this project. The CYTOP is also available in grade-M and grade-S. The comparison between different grades of CYTOP is shown in Table 3.1 [68]. The grade-A CYTOP was used in this project due to transparency to light and adhesion properties.

Table 3.1 - Types of CYTOP

Type/Grade	Polymer-end Group	Application
A	-COOH carboxyl group	Plastic coating with or without special primer. Metal and Glass coating with silane coupling agent. Visible light transparency
M	-CONH ~ SiOR amino-silane coupling agent	Metal, silicone and glass coating without adhesion promoters.
S	-CF ₃ Perfluoro group	Ultra high transparency through DUV to NIR UV resistant. Non-adhesion

3.2 Lower CYTOP Cladding

The lower cladding is a stack of CYTOP created by spinning three layers of 9% CTX-809A CYTOP A-type on a silicon wafer. A total thickness of 9 µm is achieved through this step. The application procedure of CYTOP for the lower cladding is adopted from [64] [49]. As reported in [49], CYTOP can be applied using dynamic and static coating techniques. Static coating is a simple and easy method but consumes more material than dynamic coating. In addition, dynamic spin is a useful method to achieve smooth coating through even spreading of viscous materials like CYTOP. However, dynamic coating requires an automated

dispensing system attached to a spinner which was not available in the fabrication lab. Thus, a static coating method was used for the application of CYTOP for lower cladding.

The wafer was thoroughly blown with N₂ to remove dust particles before applying first later of CYTOP. The presence of any particle can cause an uneven spread of CYTOP on the wafer and may result in cracking and defects in the cladding which can be further aggravated by residual film stress. The presence of a particle can cause unbonded region on wafer during bonding process. CYTOP was poured by hand directly in the middle of the wafer. A settling time of 1-2 minutes was allowed before starting the spin coating. CYTOP was applied through the spin-coat method at 550 RPM spin speed for 10 seconds and 1500 RPM for 30 seconds, which yielded about 3 µm thickness of each layer. A resting time of 5 minutes at room temperature was allowed after each layer was coated, followed by a 10 minutes soft bake at 50°C on a hotplate. The soft baking helps to degas and evaporate solvent present in the CYTOP. Finally, to remove solvent and promote adhesion with the silicon substrate, the wafer was baked at 200°C for 4 hours using ramping steps. The details of CYTOP application are shown in Table 3.2 below.

Table 3.2 - Lower CYTOP Cladding Fabrication Steps

Activity	Description
1 st layer of CYTOP	Pour 9% CTX-809A CYTOP A-type in middle of the wafer, allow 1-2 minutes to degas bubbles, spin coat at 550 rpm for 10 s followed by 1500 rpm for 30 s
Soft bake	Allow 5 min at room temperature (RT) before baking on a hotplate at 50°C for 10 minutes
2 nd and 3 rd layer of CYTOP	Repeat above two steps
Final hard bake	Ramp from RT to 80°C, hold for 1 h, ramp to 150°C and hold for 1 h, ramp to 200°C and hold for 4 h, then ramp down to RT. The ramp rate constant at 100C/h for all steps.

There were two modifications made in the CYTOP application procedure, as mentioned in [49]. A settling time of 1-2 minutes was added between dispensing CYTOP and starting a spin coat. This step was added

to remove air bubbles which were observed in CYTOP after it was poured on the wafer. If spinning was started right away, these bubbles could cause defects in the applied layer, which appeared as holes in the CYTOP layer. The settling time allowed bubbles to escape.

In some cases, bubbles did not completely disappear after the settling time elapsed; in such cases, these bubbles were picked up using a pipette. In addition, ramp rate for final baking was decreased from 150°C/h to 100°C/h, and temperature hold steps were added for slow evaporation of solvent to get an ultra-smooth surface as shown in Figure 3.2 below. The Arithmetical Mean deviation (Ra) is the roughness parameter of concern for the optical signal. For the biosensing devices, an Ra less than 1 nm is recommended for any surface in the optical path. The Ra of Lower CYTOP cladding is 0.418 nm, which is well below the recommended threshold and ideal for Au waveguide fabrication. The green and blue squares in the figure show the maximum and minimum roughness values on the scanned surface, respectively.

Figure 3.2 - AFM Roughness Measurement of Lower CYTOP Clad Surface.

The slow evaporation of solvent allows CYTOP to settle slowly and to prevent cracks and uneven surfaces. Otherwise, a quick and fast release of solvent may create volcano-like holes and deformations in the CYTOP layers, as shown in Figure 3.3. The final bake time was also increased from 2.5 hours to 4 hours to improve adhesion [67].

Figure 3.3 - Defects in Lower CYTOP Cladding Layer Due to Quick Evaporation of Solvent and Trapped Gas.

(a) A volcano (b) A crack emerging from a volcano.

The purpose of the CYTOP cladding is to confine the LRSP. Thus, the thickness of CYTOP is a very critical parameter in the fabrication process. The minimum required thickness of CYTOP is 8 μm for this structure, as reported in [49]. Test runs were conducted in order to verify the coating thickness. A lower CYTOP cladding was fabricated on a glass wafer using the process mentioned in Table 3.2. Aluminum (Al) was used as an etch mask. Microfluidic channels were patterned, followed by photolithography and development steps. The microfluidic channels were etched in CYTOP in the MARCH JUNIPER II RIE System. RIE etching was performed through CYTOP to the glass substrate. The thickness of the lower CYTOP cladding was measured to be 9 μm using a Dektak surface profilometer. Figure 3.4 below shows the thickness of the lower CYTOP cladding at two different locations for channels of different width. Thus, a uniform cladding can be fabricated using the lower cladding process discussed in this section.

(a)

(b)

Figure 3.4 - Thickness Measurement of Lower CYTOP Cladding.

(a) At the center (b) At 1 inch from the center towards the South edge of the wafer.

3.2.1 Edge Bead Removal

An edge bead is an accumulation of the spun-on material at the edge of the wafer. The edge bead tends to build very quickly, and it can grow 10 - 30 times thicker than the applied material in the middle regions

of the wafer [69]. In the presence of the edge bead, the quality and uniformity of the films in the subsequent fabrication steps can be undermined. The edge bead can also cause a poor contact of the wafer with the mask during the exposure stage of photolithography. Thus, it is necessary to remove the edge bead before proceeding to next fabrications stages. There are several edge bead removal (EBR) methods available such as chemical dissolving, mechanical scraping, or etching. Reactive Ion Etch (RIE) was used to perform EBR using a recipe reported in [64]. Parameters used for edge bead removal using RIE are given in Table 3.3 below.

Table 3.3 - Edge Bead Removal parameters for RIE MARCH system

Parameter	Details
Power	200 Watts
Oxygen Pressure	320 mTorr
Oxygen Flow Rate	250 sccm
Time	7 minutes for a 4-inch wafer

The wafer is covered with an EBR mask, which is a 90 mm diameter stainless-steel disk, leaving wafer edges exposed to O₂ plasma for etching. It is important to note that an RIE etch at high power generates heat during EBR which may cause the wafer to get heated. In such a case, the temperature of the wafer must be carefully observed. If CYTOP is heated close to T_g , then it may become jelly-like material, and the EBR mask can damage and scratch the ultra-smooth surface of the lower CYTOP cladding. The MARCH JUNIPER II RIE System installed in the lab has chilled water flow through the etching chamber and thus overheating was not a problem during EBR.

The EBR mask may be displaced from the center of the wafer during the etch process during pumping down of etching chamber. This may result in etching the CYTOP from device region instead of only the edge bead. It is suggested to perform a brief run of 15 to 30 seconds of etching and open the lid of the

chamber to check the position of EBR mask. If the EBR mask is still in place, then full run can be completed. In case, the EBR mask has moved from the middle of the wafer, the wafer can be rotated, and a brief etch can be performed again to check the position of the EBR mask.

3.3 Waveguide Patterning

Gold (Au) waveguides are the most important part of the biosensing devices fabricated in this project. The details and important processing steps are described in this section. The Au waveguides are patterned in rectangular trenches etched into the lower CYTOP cladding using bi-layer lithography, thermal evaporation, and a lift-off process. The most critical parameters for the patterning of Au waveguides are waveguide thickness and surface roughness. The basic parameters were adapted from [64] [49] and optimized through the introduction of new processes. One of the additions is the introduction of a precise trench etch process for rectangular trenches with the depth equal to the thickness of Au waveguides. The purpose of these rectangular trenches is to provide a planar surface for subsequent wafer bonding and keeping waveguides intact during bonding process. The details of the trench etching process are discussed in section 3.3.3.

3.3.1 Bi-layer Lithography

Bi-layer lithography is a process often used in microfabrication to transfer a pattern from a mask to a thin film or substrate. A sacrificial layer is created by spinning the lift-off resist, and a photo resist. An important step is to create an undercut structure by removing lift-off resist below the top photoresist layer. The undercut depends on exposure dose, soft bake temperature and time, developer type, and lift-off resist resin composition [70]. After the metal deposition, a solvent or developer is used to wash away the sacrificial layer along with deposited metal leaving behind the patterned layer of metal. The Bi-Layer Lithography process was adopted from [64] [49]. The process was applied in a class 100 clean room. The clean room is equipped with a controlled temperature between 22°C and 25°C and relative humidity between 38% and 42%. In the Bi-Layer Lithography process, the sacrificial layer was created by first

spinning MicroChem's LOR-1A lift-off resist followed by spinning S1805 which is a positive photoresist. The spectrum output range to expose S1805 with UV source is 350 nm to 450 nm. However, the LOR-1A does not have an actinic spectral response at these wavelengths but can be etched in photoresist developer [71]. In the process, a developer MF-321, which is a product of MicroChem, is used to remove UV exposed positive resist S1805 and LOR-1A underneath it. The MF-321 is a 1.91% tetramethylammonium hydroxide (TMAH) solution commonly used for development in fabrication processes. The process steps are outlined in Table 3.4.

Table 3.4 - Bi-Layer Lithography for Au Waveguides Fabrication Process

Step	Activity	Details
1	CYTOP Surface Ashing in RIE	O ₂ plasma etching of CYTOP surface for 30 seconds Power 70 W, O ₂ flow 250 sccm, O ₂ Pressure 320 mTorr
2	Edge bead removal	RIE etching of edge bead for 7 minutes
3	Inspection, imaging, and analysis	AFM scan for etched trench and background CYTOP
4	Spin coat lift-off resist	Spin LOR-1A at 1000 rpm for 10 seconds, followed by 4000 rpm for 30 seconds
5	Soft bake	180°C for 3 minutes on a hotplate
6	Spin coat photoresist (PR)	Spin S1805 at 1000 rpm for 10 seconds, followed by 4000 rpm for 30 seconds
7	Soft bake	115°C for 3 minutes on a hotplate
8	UV Exposure dose	109 ~ 109.5 mJ/cm ²
9	Develop	MF-321 for 1 minute at room temperature
10	Trench etching for Au waveguide	RIE etch into CYTOP lower cladding for 17 seconds 50 W, O ₂ flow 150 sccm and 220 mTor

The lower CYTOP cladding becomes ultra-smooth and hydrophobic after the hard bake. The lift-off resist LOR-1A does not spin consistently on the hydrophobic surface and requires a surface treatment such as RIE ashing. The ashing is analogous to roughing up the surface. it is completed in the MARCH RIE System using operational parameters described in Table 3.3 [64]. The hydrophilicity of the surface is increased after ashing, and the resultant surface enables LOR-1A to spread consistently and evenly on the ashed

CYTOP surface. The ashing may contribute towards the overall roughness of the lower CYTOP cladding. However, it is necessary to slightly roughen the CYTOP surface for good adhesion with lift-off resist and photoresist. The LOR-1A and S1805 were spun and baked according to the details given in Table 3.4. The minimum required thickness of LOR-1A for a lift-off process is 1.33 times the metal thickness. However, the recommended thickness of LOR-1A is 3 times the thickness of the metal film. Otherwise, the metal film, Au waveguides in this case, will tear-off during lift-off process [70]. Thus, for 35 nm thick waveguides, the thickness of LOR-1 lift-off resist should be 105 nm. According to the manufacturer's documentation, the thickness of LOR-1A and S1805 is 100 nm and 500 nm, respectively if the above process steps and conditions are met. To confirm the thicknesses, a test wafer was prepared using the process parameters mentioned above. The thickness and undercut measurements were checked using SEM imaging, as shown in Figure 3.5. The thickness of LOR-1A and S1805 is 105 nm and 510 nm, respectively, which meet requirements and expectations of the process. The etch rate of LOR-1A under conditions mentioned is 3 nm/s, as reported in [49]. The wafer was developed for 1 minute in MF-321 and undercut measured to be 192.7 nm, which approximately matches the reported etch rate. After the completion of Bi-Layer resist coating, the wafers are UV exposed using a Karl Suss MA6 Mask Aligner System at H-line (405 nm). Accordingly, the exposed area of photoresist S1805 becomes soluble in the developer. The exposure dose is an important parameter to precisely define feature dimensions consistent with those on the photomask. As reported in [64] [49], the optimal exposure dose is 114 mJ/cm². However, the suggested dose was adjusted to 109 mJ/cm² for optimal resolution results after a few test trials and wafers were UV exposed using the new modified dose. The wafers were developed in MF-321 developer for 1 minute after the UV exposure. The development time was modified to get the desired undercut, which is essential to fabricate straight and flat waveguides without edge defects. There were several test trials performed to get optimal development time. A short development time will yield no undercut, whereas long

development time will create undesired undercuts. The former leads to torn waveguide edges or raised edge features often called wings, while the latter may cause trenches and voids around waveguides.

(a)

(b)

Figure 3.5 - SEM Images of Bi-Layer Stack Thickness and Undercut.
(a) S1805 thickness 510 nm, LOR-1A thickness 105 nm (b) Undercut 192.7 nm.

The lithography results after the development are demonstrated in Figure 3.6 below. The test strips show that a resolution better than 1 μm is achieved. The waveguides are 5 μm wide, and the minimum gap

between waveguides occurs in triple waveguides, which is 2 μm . Thus, the achieved resolution yielded fine and crisp features.

Figure 3.6 - Bi-Layer Lithography Microscopic Images.
(a) Resolution Indicator Marks (b) Triple waveguides and gap resolution.

3.3.2 Issues with Previous Process

In the initial work, the waveguide fabrication process was adopted from [49]. In the process waveguides were patterned on the lower CYTOP cladding and bonded with glass lids containing microfluidic channels. A post bonding inspection revealed that there were void areas along the waveguide's edges. The voids were formed during the bonding process where CYTOP did not flow enough to fill the gaps during bonding. These voids disturbed the symmetrical cladding around the waveguide causing the undesired attenuation of the excited LRSPs. In addition, the waveguides were slightly deformed along the edges at the bonding interface at a few locations. In such a case, one of the possible solutions is burying waveguides in CYTOP by etching shallow trenches as discussed in [65] which is implemented in this work with modifications.

3.3.3 Trench Etching for Waveguides

After completion of Bi-Layer Lithography and development process on the lower CYTOP cladding, the trenches were etched to house 35 nm thick Au waveguide structure. The trenches were etched in the MARCH RIE system. The trench etching process is adapted from [65] [72] with modifications. To etch a trench as shallow as 35 nm in the lower CYTOP clad using an RIE etch, a precise and repeatable process is essential. A shallow RIE etch can be achieved with a combination of low RF power and a short etch time. However, for short runs, the plasma conditions in the RIE chamber may not be stable, and such a run will yield non-uniform depth. The uniformity issue is exaggerated when a 4-inch wafer is subject to such an etch. The etch rate in the MARCH RIE system depends on RF power and etch time. Although O₂ flow and pressure may also be varied, but these parameters do not affect the etch rate significantly. However, during experiments it was observed that flow rate and pressure affect the roughness and etch direction. The etch rate process was developed in three steps. In the first step, RF power and etch time were varied to determine etch time for obtaining required depth as shown in Table 3.5. The etch rate repeatability was verified in the second step as depicted in Table 3.6, and etch rate uniformity across a 4-inch wafer was checked in the third step.

Table 3.5 - RIE Trench Etching Parameters – RF Power & Time

Parameter/Sample	Sample S-1	Sample S-2	Sample S-3	Sample S-4	Sample S- 5
Power (W)	100	50	50	50	50
Pressure (mT)	300	220	220	220	220
Flow (sccm)	250	150	150	150	150
Etch Time (S)	10	10	15	25	20
Etch depth (nm)	200	20	25	43.9	29
Etch Rate (nm/s)	20.00	2.00	1.67	1.76	1.45

From Table 3.5, an etch time of 20 seconds, and 50 watts of power was determined for the 35 nm deep trench etch. The etch rates were verified using extracted RF power and etch time for consistent etch rate. Table 3.6 below shows a consistent etch rate when etching was performed for 20 seconds using 50 watts of RF power.

Table 3.6 - Etch Rate at Extracted Power and Etch Time

Wafer ID	MA 18-1	MA18-2	MA18-3	MA47-1	MA47-2	MA47-3
Etch depth (nm)	37.63	37.34	37.26	36.09	37.50	37.12
Etch Rate (nm/s)	1.88	1.87	1.86	1.80	1.88	1.86

Finally, a 4-inch wafer was used to verify the uniformity of etch rate at different locations on the wafer. The wafer was etched for 20 seconds at 50 watts of RF power. Table 3.7 shows that trench depth with a maximum variance of 3.5 nm for different locations of the wafer, however the trench depth is 4 ~ 5 nm more than required 35 nm depth. Thus, etch time was reduced to 17 seconds for the next batch of wafers to get trench depth of closer to 35 nm. The South (6'O Clock) refers to the major flat on a 4-inch wafer and may be used as a reference for other locations.

Table 3.7 - Trench Depth Uniformity Across 4-inch Wafer

Location - MA46 4-Inch Wafer	Trench Depth (nm)
12'O Clock (North)	37.26
3'O Clock (East)	40.15
6'O Clock (South)	39.41
9'O Clock (West)	40.63
Centre 1	40.81
Centre 2	39.42

After derivation of trench etching parameters, trenches were etched in the lower CYTOP cladding to accommodate the Au waveguides. The reader is reminded that the bi-Layer lithography and development have already been completed and trenches are etched in the open area after development. It is important

to verify the depth and the roughness of the etched trenches. Shallow trenches may cause topography at the bonding surface leaving defects at the bonding interface, whereas very deep trenches may create voids and gaps around the Au waveguides. Figure 3.7 below shows a line profile of trench depth and width, which are 35.14 nm and 5.31 μm respectively.

Figure 3.7 - Trench Profile.
Depth 35.12 nm (red pair of cursors), width 5.31 μm (green pair of cursors).

The results of the AFM scan for roughness inside the trench and outside in background are shown in Figure 3.8 below. The roughness inside the trench is 0.718 nm (highlighted in green), and lower CYTOP cladding background roughness outside the trench is 0.468 nm. The reader is reminded that these roughness numbers include the roughness added by the ashing process performed before bi-Layer lithography. Thus, this may be called “accumulated roughness.” The Au metallization process may further add roughness to the total roughness of waveguide surface. The 3-D view of the trench is also shown in Figure 3.8, which emerges to be approximately rectangular as desired. The trench profile was analyzed through SEM analysis, as shown in Figure 3.9. The width of the trench is found to be approximately 5 μm which agrees with the measurements of the AFM profile scan. In addition, the SEM images show a clean and smooth surface inside the trench.

(a)

(b)

Statistics										
Region	Min(nm)	Max(nm)	Mid(nm)	Mean(nm)	Rpv(nm)	Rq(nm)	Ra(nm)	Rz(nm)	Rsk	Rku
Red	8.280	12.354	10.317	9.824	4.073	0.583	0.468	3.643	-0.314	2.959
Green	-30.361	-23.187	-26.774	-27.766	7.174	0.904	0.718	6.362	-0.366	3.410
R-G	38.642	35.541	37.091	37.589	-3.101	-0.321	-0.249	-2.719	0.051	-0.452

Figure 3.8 - Trench and Background Roughness.

- (a) Trench roughness 0.718 nm (green) , background roughness 0.468 nm (red)
 (b) A 3-D view of the trench.

Figure 3.9 - SEM Image of Etched Trench in Lower CYTOP Clad.

Finally, a multi-point microscopic analysis was performed at high resolution to observe the trench profile. The images show a consistent and precise etching of the trench. Figure 3.10 below shows a Y-Junction feature with contact arm and pad magnified at 50X. The etch quality and uniformity was observed for the straight waveguide, and Y-Junction was observed at 100X magnification, as shown in Figure 3.11.

Figure 3.10 - Microscope Image of Trenched Y-Junction with Contact Arm and Pad.

Figure 3.11 - Microscope Image Trench for Waveguides.

(a) Trench width of straight waveguide (b) Y-Junction for Mach-Zehnder interferometer.

3.3.4 Waveguide Metallization

After completion and analysis of bi-layer lithography and trench etching for Au waveguides, Au film was deposited to fabricate waveguides in the trenches using the same lithography stack. Au film can be deposited using E-beam or thermal evaporation. As concluded in [49], thermal evaporation was considered for Au film depositions. It yields stable deposition rates as well as an economical method for film deposition. The thermal evaporation can be performed by rotating the sample on a plate (like planetary motion) in the chamber or by placing the sample on a fixed lift-off jig. Initially, gold was deposited using a rotating plate method, as reported in [64]. However, the gold film thickness was not consistent due to some problems with the crystal thickness monitor. Thus, the gold film was evaporated using a lift-off jig (fixed plate), and thickness and roughness were verified to be within an acceptable range. Thus, 35 nm thick Au film was evaporated thermally using Angstrom Sciences Nexdep evaporator. The deposition was performed at vacuum pressure in the order of 10^{-7} Torr. The Au film deposition parameters are given in Table 3.8 below.

Table 3.8 - Thermal Gold Deposition Parameters

Parameter	Value
Evaporation	Thermal
Vacuum Pressure	2.3×10^{-7} Torr
Tooling Factor (TF)	46.8
Deposition Rate	$0.5 \text{ \AA/s}$
Gold film thickness	35 nm

The gold thickness and roughness are very critical for the propagation of LRSPPs. The roughness can be controlled with the deposition rate to some degree. A slower deposition rate results in the smoother gold surface, as reported in [49]. The tooling factor (TF) is the percentage difference in metal thickness realized by the sample and the Quartz Crystal Monitor (QCM). The difference in thickness occurs because the samples in the evaporator chamber are offset from the QCM detecting the metal film thickness. Tooling

factor is unique to each deposition process. After gold deposition, lift-off is performed to remove the unwanted material leaving behind the required pattern on the substrate. In this case, recessed gold waveguides in the trenches are the required structure, and the remaining gold is lifted off from all other areas. For this purpose, a photoresist stripper, PG remover is used. It is a trademark product of MicroChem. For the lift-off process, PG remover is heated to 60°C and wafer is immersed in it for 10 minutes, followed by 10 seconds ultrasonic cleaning, and these steps are repeated three times. LOR-1A peeling off slows down in metals like gold, this an extra bath may be required to ensure that wafer is clear from any residual of bi-layer stack. The PG remover attacks the LOR-1A through undercut created during the development of the bi-layer lithography after UV exposure. In initial work, more than one wafer could be immersed vertically in a beaker filled in PG remover [49] which caused PG remover waste. The vertical immersion was replaced by placing and immersing the wafer horizontally in PG remover. This allows only one wafer immersion at a time but uses only a fraction of PG remover. It was observed that the PG remover attacks the CYTOP surface at temperatures higher than 60°C and causes the gold waveguides to deform and roughens the surface of waveguides and CYTOP. Figure 3.12 shows a triple waveguide after completion of metallization and lift off. It shows that the targeted width and gap between the waveguides of 5 μm and 2 μm was accurately achieved. Figure 3.13 shows the Y-Junction crotch with required width of 2 μm . A triple waveguide, along with connecting arms and pads, is shown in Figure 3.14 to demonstrate the quality of lithography, metallization, and lift off.

Figure 3.12 - Tripple Waveguide After Completion of Metallization and Lift Off.

Figure 3.13 - Y-Junction, a Crotch with Targeted 2 μm Gap.

Figure 3.14 - Triple Waveguides Connecting Arms and Pads.

An AFM 3-D view along with a line profile scan of a recessed waveguide fabricated in a trench is shown in Figure 3.15. Note that standard surface roughness metrics are calculated based on areas as shown in Figure 3.16. The simple line scan in Figure 3.15 shows the full range of surface features which can be much larger than the average roughness. The waveguide is about 5.77 nm below the surrounding lower CYTOP cladding. This gap is expected to be filled and compensated by CYTOP during the bonding process where CYTOP is kept above T_g for several hours, allowing it to flow and fill the voids.

Figure 3.15 A Gold Waveguide Fabricated in a Trench After Lift Off.
(a) An AFM 3-D view of a waveguide (b) An AFM line profile of the waveguide.

Finally, an AFM scan to check the roughness of gold waveguide and lower CYTOP cladding after the completion of metallization and lift off is shown in Figure 3.16 below. The surface roughness of the gold waveguide and lower CYTOP cladding is 0.85 nm (area in red) and 0.46 nm (area in green) respectively, which are well within the 1 nm roughness threshold.

Figure 3.16 - Surface Roughness of Gold Waveguide a Background After Metallization and Lift Off.

A summary of metallization process is shown in Table 3.9.

Table 3.9 - Summary of Metallization and Lift Off Process for Gold Waveguides

Step	Activity	Details
1	Gold deposition	Thermal evaporation 35 nm target, actual thickness 34 nm Deposition rate 0.5 Å /s, Tooling Factor 46.8
2	Gold Lift-off	10 min dip in PG remover at 60°C, followed by 10 s ultrasonic cleaning, repeat 4 times or unless wafer is completely clean
3	Cleaning	Clean with IPA and DI wafer

One may observe that AFM images of etched trench show that trench has a round edge at the bottom as seen in Figure 3.8 (b). Thus, the sidewalls do not make perfect 90 degrees angle with bottom floor. Consequently, when gold is evaporated/deposited, the resulting waveguide takes the shape of the trench. Thus, the Au waveguide is not perfectly flat when observing a cross section view, as shown in Figure 3.15, 3-D view.

3.4 Glass Lids with Microfluidic Channels

The fabrication of glass lids containing upper CYTOP cladding and microfluidic channels is described in this section. A process to fabricate the upper cladding on a separate glass substrate independent from the lower cladding is adapted from [65] with modifications and introduction of an Al etch mask. In the previous work, as reported in [64] [49], the upper CYTOP cladding was fabricated on top of the waveguides after the introduction of an etch stop layer. However, this process faced some issues such as the inability to fully cure the CYTOP being thick stack, waveguide deformation, and cracking of cladding. Initially, in this work, upper cladding was fabricated on the lower cladding containing waveguide structures.

Nevertheless, CYTOP cracking was observed in the upper cladding, which extended into the lower cladding in some cases. Figure 3.17 and Figure 3.18 show microscope and SEM images of cracking in upper CYTOP cladding and channels. To address this issue, the upper cladding with microfluidic channels is fabricated

on a separate glass substrate, independent from waveguide structure which is on the lower CYTOP cladding. In addition, an RIE channel etching process is characterized and successfully employed yielding ultra-smooth channel walls and beds, avoiding curtaining and grass issues.

3.4.1 Issues with previous work

As reported in [64] [49] the cracking was a major problem in the fabrication of upper CYTOP cladding. In addition, waveguide deformation is also reported, and both problems arise from the same cause; the inability of CYTOP to chemically cure as reported in [65]. When hard baking of the lower cladding is performed to cure CYTOP, solvent simply evaporated and CYTOP remains vulnerable to its solvent. During the application of the upper CYTOP cladding, the new layer of CYTOP reintroduces solvent which finds ingress into lower cladding under the gold waveguides. Once the solvent diffuses into the lower cladding, it expands the CYTOP which causes the waveguides to deform or break. Subsequent baking to remove the solvent deep in the cladding layer can result in further stress and cracking in the cladding. Consequently, CYTOP cracking and waveguide deformation may appear. In the previous work of [61] [62] [63] [64] [49] microfluidic channels were etched into upper CYTOP cladding to access the gold waveguide after completion of the upper CYTOP cladding. The depth of the etched channels would extend to the depth slightly below the gold waveguides in the absence of an etch stop layer. However, an etch stop layer was introduced in the work of [49] to avoid over etching of channels. Nevertheless, an etch stop layer requires additional processing steps such as additional photo mask, bi-layer lithography, exposure, development, SiO₂ deposition, and lift off. These additional processing steps have some constraints such as baking temperature and rate due to thermal characteristics of CYTOP, gold, and SiO₂. For example, baking bi-layer stack above T_g of CYTOP causes the gold waveguides to deform, and high ramp rate may cause the thin SiO₂ film to fail [49]. The solutions to the issues have been proposed and implemented in [65] by fabricating the upper CYTOP cladding and microfluidic channels on separate substrates.

Figure 3.17 - Microscope Images of Cracks in CYTOP After Spin Coat of Upper Clad on Gold Waveguides.

(a) (b)
Figure 3.18 - SEM Images of a Crack in CYTOP Cladding.
(a) Cracks in channel (b) Crack extends out of channel

3.4.2 Glass Wafers Selection and Preparation

The purpose of the glass lid is to seal the biosensing device to contain the fluidic samples as well as to provide a structural base for the upper CYTOP cladding and microfluidic channels. A 300 μm thick borofloat glass substrate was used to fabricate the lids. The Coefficient of Thermal Expansion (CTE) of the

glass substrate is matched with the Silicon substrate to facilitate the bonding process. The glass wafers were checked for flatness, physical integrity, and surface quality. The preparation steps are identical to those mentioned in section 3.1.

3.4.3 Upper CYTOP Cladding

The upper CYTOP cladding was fabricated on glass substrates. The advantage and robustness of using separate substrates for upper and lower cladding is the use of identical processing steps and parameter values for both upper and lower cladding fabrication. Thus, the processing becomes simple, reliable, and efficient and yielding symmetrical and uniform cladding. The separation of upper and lower cladding on separate substrates eliminates the alignment of channels with waveguides. This is a tedious and challenging process in the presence of the SiO₂ layer and Al mask. Accordingly, misalignment occurs as reported in [49]. The processing steps, along with parameters described in section 3.2, are repeated for upper CYTOP cladding as well.

3.4.4 Microfluidic Channels

The fluidic channels were etched into the CYTOP upper cladding. The upper CYTOP cladding was hard baked on a hot plate at 200°C with ramping for 4 hours. The microfluidic channels were patterned on CYTOP using standard lithographic techniques. A thin Al layer was used as an etch mask. The channels were etched in the MARCH Juniper II RIE system. The Al mask was chemically etched away after the channels were etched to the desired depth. The detailed processing steps are described in subsequent sections.

3.4.4.1 Aluminum Mask Lithography

The process steps and parameter values for Al mask and lithography are adapted from [49]. After completion of upper CYTOP cladding, a layer of 20 nm thick Al was evaporated by E-beam using a tooling factor of 175, at a deposition rate of 1 Å/s. The Al layer was used as a channel etch mask; thus, roughness and thickness of the Al does not affect the quality of etch or channels. However, the thickness should be

enough to protect the covered CYTOP from etching in the O₂ plasma. After the deposition of Al, HMDS was spin coated to promote the adhesion between the Al and photo resist above it. A soft bake was performed at 80°C for 45 minutes after the application of HMDS. A photoresist, S1805 was spin-coated followed by a soft bake at 80°C for 1 hour. It is critical to start soft baking from room temperature with a maximum ramping rate of 100°C/h and ramping down to room temperature before removing the wafer from the hot plate. The Al thin film is very sensitive to abrupt thermal variations. The thin Al film deforms and crumples as a result of fast ramp rates. After the baking of photoresist, the wafer was exposed using a channel photo mask. The exposure dose was 98mJ/cm². The wafer was then immersed in MF-321 to develop for 3 minutes approximately. The MF-321 can develop Al and S1805 in the same step. The process steps are described in Table 3.10 below.

Table 3.10 Upper CYTOP Cladding Processing Steps

Step	Activity	Details
1	Glass wafer preparation	Piranha clean, APTES application and CYTOP coating Parameter detail in Table 3.2
1	Edge bead removal	RIE etch; power 200 W, O ₂ flow rate 250 sccm, O ₂ pressure 320 mTorr, etch time 7 min
2	Al deposition	20nm thick Al deposited using E-beam
3	Spin coat HMDS	Spin speed 1000 rpm for 10 s, followed by 4000 rpm for 30 s
4	Soft bake	Ramped up from 25°C to 80°C, baked at 80°C for 45 min on a hotplate, and ramp down to 25°C. Ramp rate 50°C/h
5	Spin coat photoresist (PR)	S1805 spin at 1000 rpm for 10 s, followed by 4000 rpm for 30 s
6	Soft bake	Ramp up from 25°C to 80°C, bake at 80°C for 1 h on a hotplate, and ramp down to 25°C. Ramp rate 50°C/h
7	Expose	98 mJ/cm ²
8	Develop	Dip in MF-321 for 3 min
9	Channel etch in CYTOP	RIE channel etching; power 100 W, O ₂ flow rate 250 sccm, O ₂ pressure 320 mTorr, average etch rate 0.26µm/min
10	Al mask removal and cleaning	Immerse wafer in MF-321 for 2 min to remove Al etch mask, followed by dipping in Acetone, IPA and DI water 5 minutes each

3.4.4.2 Channel Etching Process

The microfluidic channels were etched into the upper CYTOP cladding which was approximately 9 μm thick. The etching was performed in an RIE O_2 plasma etcher MARCH Juniper II. The CYTOP was etched to the glass substrate level to form channels with the glass acting as an etch stop layer. This is one of the advantages of fabrication of channels in the upper CYTOP cladding on a separate glass substrate. The microfluidic channels are very important structures of the biosensing device. The channels contain the target analyte samples. Thus, it is essential for channel sidewalls and floor to be as smooth as possible to avoid any dead volume and contamination. To obtain minimum sidewall and floor roughness, RIE etching was characterized through experiments, and optimal parameters were obtained. An experiment was designed to determine channel etch rates and quality. The RF Power and O_2 flow rates were varied, and the resultant etch rates are summarized for each set of RF power and O_2 flow in Table 3.11. It was found that the etch rate depends largely on RF power. However, the O_2 flow rate also affects the etch rate.

Table 3.11 Channel etch rates in MARCH RIE

O_2 Flow (sccm)/RF Power (Watts)	75 W	100 W	150 W
250 sccm	0.177 $\mu\text{m}/\text{min}$	0.229 $\mu\text{m}/\text{min}$	0.326 $\mu\text{m}/\text{min}$
300 sccm	0.200 $\mu\text{m}/\text{min}$	0.270 $\mu\text{m}/\text{min}$	0.326 $\mu\text{m}/\text{min}$
350 sccm	0.217 $\mu\text{m}/\text{min}$	0.277 $\mu\text{m}/\text{min}$	0.355 $\mu\text{m}/\text{min}$

The channel depth for each power and flow rate combination is shown in the plot shown in Figure 3.19 below. A constant etch rate is observed over time until the glass substrate is reached.

Figure 3.19 - Depth of Etch Channel.

After the etch rates were determined, SEM imaging was performed on each sample to verify the sidewall and floor surface quality. A matrix of SEM images is shown in Figure 3.20 (a) to (i). The low RF power and flow rates yield better sidewalls and floor. At the lowest power (75W) and flow rate (250 sccm), it takes approximately 54 minutes to etch the full depth of the channel. However, accounting for the time factor, the optimal parameters used to etch channels were 100 W of power and 250 sccm flow rate since the quality of etch was not much different as seen in Figure 3.20 (a) and Figure 3.20 (b), and this saves 14 minutes of RIE etch.

(a) 250 sccm – 75 W

(b) 250 sccm – 100 W

(c) 250 sccm – 125 W

(d) 300 sccm – 75 W

(e) 300 sccm – 100 W

(f) 300 sccm – 125 W

(g) 350 sccm – 75 W

(h) 350 sccm – 100 W

(i) 350 sccm – 125 W

Figure 3.20 - SEM Images of Sidewalls and Floor of Etched Channels.

After etching was complete, the Al etch mask was removed by immersing the glass wafers in MF-321 for 2 minutes. The etch rate of Al in MF-321 is approximately 100 Å/minute as determined experimentally. Finally, the wafers were cleaned using Acetone to remove any leftover photoresist, and IPA and DI water to remove residual material and particles. The roughness inside channels was measured for each wafer at minimum of 5 locations, and the results shown in Figure 3.21 are representative of the measurements. The roughness on the channel floor is 1.55 nm, which is slightly higher for optical performance, however,

this region is not in the optical path. The floor of the microfluidic channel becomes the ceiling of the channel after the glass lid is bonded with a waveguide structure on a silicon wafer. The bonding process is described in Chapter 4:

(a)

(b)

Statistics										
Region	Min(nm)	Max(nm)	Mid(nm)	Mean(nm)	Rpv(nm)	Rq(nm)	Ra(nm)	Rz(nm)	Rsk	Rku
Red	-5.417	7.474	1.028	0.030	12.891	1.862	1.559	11.272	-0.376	2.383
Green	-3.755	3.074	-0.341	-1.111	6.830	1.343	1.097	5.841	-0.621	2.927
Blue	-3.836	4.519	0.342	-0.492	8.354	2.099	1.812	7.184	-0.422	2.059

(c)

Figure 3.21 - AFM Measurement of Etched Channel.
(a) Channel floor surface (b) Line profile of roughness (c) Channel roughness.

The depth of channels was measured at several locations of each wafer, and it was found to be approximately 9 μm as shown in Figure 3.22. The channel depth was consistent across different wafers and locations. After channel profiling and depth and surface roughness checks, the glass lids containing microfluidic channels are ready for bonding with silicon substrates containing the waveguides.

Figure 3.22 - Dektak Measurement of Channel Depth.

Chapter 4: Wafer Bonding

Upon completion of waveguides on lower CYTOP cladding and channels in upper CYTOP cladding, the wafers are bonded to realize fully functional biosensing devices.

Before the bonding process, the biosensing device exists on two separate substrates; the gold waveguides on lower CYTOP cladding fabricated on a silicon wafer, and microfluidic channels etched into upper CYTOP cladding fabricated on a glass substrate. The wafers can be bonded using indirect bonding or direct bonding methods. Indirect bonding requires an adhesive layer between two surfaces, which are subject to bonding. In a direct bonding process bulk polymer itself comprises the adhesive. The substrates containing the polymer are heated slightly above the T_g and force is applied for coupling contact. The temperature and force mutually produce the necessary flow of polymer at the interface to obtain the required contact. Ideally, the resulting bond strength may attain the cohesive strength of the bulk material [73].

The bonding interface of the fabricated biosensing devices is between the upper CYTOP cladding and the lower CYTOP cladding suggesting a direct CYTOP to CYTOP bonding approach. However, bonding methods were explored in the literature as discussed in next section before developing a bonding process.

4.1 Previous Work

There are different polymer bonding methods discussed in the literature. A voltage assisted polymer wafer bonding is reported in [74]. However, the structure does not involve any gold waveguides. In addition, the temperature used for bonding is not in the range of T_g of CYTOP being used in this project.

A direct thermal bonding approach is discussed in [73] for polymer bonding under heat and force. The work of [75] describes polymer bonding for microsystems at a temperature much higher than T_g of CYTOP, which would deform the gold waveguide structures due to the CYTOP flow under at temperatures. A channel sealing method involving molding and thermo-bonding for Teflon is discussed in [76]. However,

it involves high temperature of 260°C which is not feasible for CYTOP bonding containing gold waveguide structures. A wet CYTOP inking technique is reported in [77] in which a layer of wet CYTOP is used between two layers of cured CYTOP by applying heat above T_g . However, the work reported above did not involve gold waveguides in the structure. The presence of gold waveguides and microfluidic channels put limit on the bonding temperature and force.

In the work of [49] bonding trials on dummy wafers containing waveguides and microfluidic channels are reported. The work reported in [49] consists of two bonding trials involving channels patterned and etched in CYTOP bonded to un-patterned CYTOP on glass substrates. There were two additional accounts of bonding trials involving patterned and etched channels in CYTOP before bonding was performed. There were issues reported in bonded wafer pairs such as waveguide deformation, misalignment, unbonded channels, and channel deformation. No wafers of quality suitable for optical testing were bonded using the reported process. Nevertheless, the bonding process reported in [49] was adapted in this work with modifications. The modifications and improvements were introduced in bonding force, bonding temperature, temperature ramp rate, and bonding time.

4.2 Process Parameters

The wafer bonding is performed in AML-AWB wafer bonder. The wafer bonder has two platens, upper and lower platen with heating lamps, inside a vacuum chamber and control software on a computer. The upper platen is fixed. The lower platen can be vertically moved to bring wafers into contact as well as horizontally to align the wafers. The platens can be heated with ramping. The upper platen has two holes of about 5 mm diameter for view cameras. The camera holes are located about an inch to the left and to the right from the centre. The bonder also has an infrared (IR) camera. The wafer bonding can be controlled through process parameters such as bonding temperature, bonding force and bonding time. In addition, the vacuum pressure can be controlled in the bonder chamber to gain a better view and contact

during the alignment. The accuracy of the bonding force and pressure applied also depends on the chamber vacuum pressure.

4.3 Bonding Process

The wafers were blown with N₂ before loading them into the wafer bonder assuming that wafers are free from contaminants and were kept in clean room or transported in a sealed box or puck. The glass substrate containing channels in upper CYTOP cladding was placed on the upper platen and mounted using a spring clamp. The silicon wafer containing the gold waveguides on lower CYTOP cladding was placed on the lower platen. The chamber was closed and purged with N₂ to clean the chamber from any particles or residual. The bonder chamber was evacuated to a pressure in the order of 10⁻⁵ mbar. The wafers were then brought slowly close to each other at about 2 mm distance between them. The wafers were aligned, and the force was applied. The heating with ramping was turned on after the bonding force was applied. The initially applied force increased during the bonding process due to the expansion of CYTOP. The applied temperature and force caused the CYTOP to flow with interdiffusion of polymer chains between both surfaces leading to strong bonding. The resulting bond may achieve strength similar to the bulk CYTOP itself. The wafer pair was kept under force and heating for about 16 hours. Then heat was turned off, and the wafer was allowed to cool down inside the chamber. Once the bonded pair reached room temperature, it was taken out of the wafer bonder for inspection and further processing.

4.3.1 Aligning Wafers

Wafers were aligned inside the wafer bonder chamber. As mentioned above, the upper platen has two holes with a view camera for alignment. Therefore, the glass substrate was loaded onto the upper platen so the features could be seen on the bottom silicon wafer. The wafers were aligned using the dicing marks and channels and waveguide features. The wafers were brought to a 2 mm separation to start the alignment process. Initial alignment was performed at this distance. Then separation was decreased to 1 mm, and another coarse alignment was performed. The coarse alignment was repeated further twice at

separation of 0.75 mm and 0.25 mm, followed by a fine-tuning of alignment at 0.25 mm distance. Bringing wafers any closer could cause the wafers to stick together due to topography and misalignment, and the aligning process may need to start over again. Figure 4.1 shows a wafer pair subject to bonding. The glass substrate containing etched channels is on the top while the silicon substrate with waveguides is beneath. The edges of the fluid channels are visible as thin dark lines. The wafers were aligned, contacted, and force was applied.

Figure 4.1 - Wafer Bonding Pair Aligned in AML-AWB Wafer Bonder.
(a) A waveguide aligned in a channel (b) Dicing mark showing the alignment.

4.3.2 Process Flow and Steps

In the wafer bonding process, the wafers were loaded, aligned, the initial bonding force was applied, and heating was then turned on. The temperature was set slightly above the T_g of CYTOP, which is 108°C. The force was applied prior to applying the heat since CYTOP softens as soon as T_g is reached. Thus, applying heat before applying force can cause misalignment and deformation of channels.

In contrast, applying the force before heating brings wafers in full contact, and then heating causes the CYTOP to form a bond. The CYTOP expands and interdiffuses to form a strong bond. There were four

product quality wafer pairs bonded using the bonding process. The process steps and parameters were kept the same except the bonding temperature, which was set to 119°C initially for the first pair then decreased to 115°C for the following three pairs. The bonding temperature was decreased to compensate for temperature overshoot beyond the set point in the bonder temperature controller.

The plot in Figure 4.2 shows the behavior of CYTOP during the wafer bonding process for one of the bonded wafer pairs. As mentioned earlier, a 1000N force is applied to get perfect contact between the bonding surfaces, and then heating is applied. The temperature of both the platens was set to 115°C with a ramping rate of 1°C/minute. It took about 1.5 hours to get to the set temperature. However, an overshoot of a few degrees Celsius was observed. The maximum force reached was about 2100 N, which is due to the expansion of 18 µm thick CYTOP cladding. It was noticed that it took about 16 hours for CYTOP to fully expand at a given temperature. The heat was turned off after an elapsed time of about 17 hours when the CYTOP had reached its maximum expansion. The overall trend of the CYTOP expansion followed the applied heat and force. However, there were local minima and maxima along the force curve, which may be due to material creep caused by long term stress and heat.

Figure 4.2 - CYTOP Response During Wafer Bonding.

The process steps are summarised in Table 4.1.

Table 4.1 - Process Steps for Wafer Bonding

Step	Activity	Detail
1	Chamber Purging	To clean the chamber automatically maneuvered by wafer bonder
2	Wafer loading	Glass wafer loaded onto the upper platen Si wafer loaded onto the lower platen
3	Chamber vacuum	Vaccum the chamber in the range of 10^{-5} mbar
4	Wafer alignment	Align the channels on glass wafers with waveguides on Si wafer
5	Force application	Apply 1000 N force manually (equivalent Pressure 123 kPa)
6	Controlled Heating	Heating was set to 115°C for both, upper and lower plated at a ramping rate of 1°C/min
7	Bonding time	Wafers kept under force and heat for 16 hours
8	Cooling down the wafer	Cool down wafers to room temperature inside the chamber

4.3.3 Bonding Verification

After the bonding was complete, the bonding was mechanically checked by inserting a sharp edge of an Exacto knife in the bonding interface and trying to take the bond apart. The bonding interface remained intact and did not tear apart or de-bond. The wafers were visually inspected, and the bonded area was estimated to be more than 90% of the total wafer area for all 4 wafer pairs. The microscopic inspection was carried out for all bonded pairs, and excellent bonding quality was observed. Figure 4.3 shows a top view of a microscope image of a bonded wafer. The straight gold waveguides are well aligned in the channels according to the design layout. Figure 4.4 shows the waveguides inside channels and crossing a channel. The bonding was verified by injecting IPA into channels with no leakage detected. The bonding strength was also observed during dicing when no loose glass lids were reported. The device yield of these wafers was over 90%, and defective chips were found only on the 10% unbonded regions of the wafer.

Figure 4.3 – Post Bonding - Microscope Image of Straight Waveguide in Channels (Top View).

Figure 4.4 – Post Bonding - Microscope Image of Waveguides in and Accorss Channels (Top View).

Figure 4.5 to Figure 4.8 show waveguide, channels, and bond quality a higher magnification. It may be observed that waveguides are intact and do not show any sign of deformation at large. However, the waveguide near the pad connecting arm around the gap is slightly deformed as shown in Figure 4.6. This occasional deformation can be attributed to material creep during the bonding process when CYTOP was heated above T_g . The deformation may cause waveguide flattening and thinning due the expansion of CYTOP underneath them. The channels appear rectangular, and no signs of channels collapse are observed. The alignment of channels and waveguides show the successful development and implementation of the bonding process.

Figure 4.5 - Post Bonding - Y-Junction Across a Channel.

Figure 4.6 - Post Bonding – A Straight Waveguide Insdie Channel.

Figure 4.7 – Post Bonding – A Straight Waveguide and a Y-Junction Across a Channel.

Figure 4.8 - Post Bonding - A Tripple Waveguide Across a Channel.

4.3.4 Wafer Dicing and Post Dicing Checks

After completion of the bonding process and subsequent bonding verification, wafers were sent to a commercial facility for dicing. The diced chips were randomly picked from all four diced wafers for optical and biosensing testing. The facets of the chips were polished to examine the waveguide and microfluidic channel profiles after the bonding. An ultrasonic and KOH solution cleaning was also performed to remove dicing and polishing debris from the channels. The waveguide and channel cross-sections were examined using the NanoFab System. It was difficult to examine these chips under conventional SEM due to the charging of the CYTOP cladding. Thus, the NanoFab system was used which enables helium ion microscopy (HIM), an imaging technique with high resolution, strong contrast, surface sensitivity, and no charging artifacts.

A waveguide cross-section is shown in Figure 4.9. The waveguides look straight and intact. There are no signs of bent stress due to the bonding process. The width accuracy of 5 μm is achieved without any deformation, which was one of the major goals of this work. Figure 4.10 shows a cross-section of a microfluidic channel opening. The channel seems almost rectangular, and there are no signs for deformation. The height of the channel is 9.69 μm which is dimensionally accurate as expected.

Figure 4.9 – A NanoFab Image of Cross-section of Waveguide.

Figure 4.10 - A NanoFab Image of Cross-section of a Channel Opening.

4.3.5 Results and Discussion

The cutback measurement method was used to verify the optical performance of the fabricated devices. The cutback measurements were performed by Alex Krupin and Zohreh Hirbodvash at the University of Ottawa. For cutback measurements, fully cladded waveguides were used. LRSPs were excited using a 1310 nm wavelength light source. The power lost per unit length was measured in dB/mm by taking the difference between input power and output power. A photodetector was used to record the measurements. The chips were diced in three different lengths of 3 mm, 3.8 mm, and 4.8 mm to produce a cutback curve. The chips were arbitrarily chosen from all four diced wafers (MA41, MA47, MA48, and MA49). The cutback measurements curves are shown in Figure 4.11. The attenuation ranges between 3.66 dB/mm to 4.92 dB/mm as obtained from the slope of the function. The coupling loss is about 2 dB per facet, as shown Y-intercept in the function. The lower loss values could be due to thinner waveguides than the design value of 35 nm. One possibility is that the thickness of waveguides decreased due to stretch and flattening of waveguides during the bonding process. The LRSP mode output images of diced chips from different wafers are shown below in Figure 4.12 to Figure 4.15.

Figure 4.11 - Cutback Measurements Obtained from Diced Chips.

Figure 4.12 - LRSP Mode Outputs – MA41 Chips.
 (a) Waveguide length 3 mm (b) Waveguide length 3.8 mm (c) Waveguide length 4.8 mm.

Figure 4.13 - LRSPP Mode Outputs – MA47 Chips.
(a) Waveguide length 3 mm (b) Waveguide length 3.8 mm (c) Waveguide length 4.8 mm.

Figure 4.14 - LRSPP Mode Output – MA48 Chips.
(a) Waveguide length 3 mm (b) Waveguide length 3.8 mm (c) Waveguide length 4.8 mm.

Figure 4.15 - LRSPP Mode Output – MA49 Chips.
(a) Waveguide length 3 mm (b) Waveguide length 3.8 mm (c) Waveguide length 4.8 mm.

Chapter 5: Hot Embossing

The microfluidic channels and gold waveguides are the integral components of the biosensing devices presented in this thesis. The channels are etched in CYTOP cladding through an RIE etch process. The CYTOP cladding is fabricated through spin coating followed by a hard baking. As discussed in the previous chapter, the fabrication of channels in CYTOP requires Al etch mask, bi-layer lithography, development, RIE etching of CYTOP, and wet etching of Al. The process involves many resources including chemicals, processing equipment, and many lab hours to complete the process and the steps. The process of channel etching can be made simple, efficient, quick, and economical by introducing hot embossing instead of RIE etching. During this research, it was found that hot embossing can potentially be an alternative to the etching process for microfluidic channel formation. In hot embossing, a master mold or die containing the required micro features is fabricated. The master die is pressed onto a heated polymer substrate under force to transfer or emboss the stamp features on the target polymer substrate or film. Hot embossing can be implemented in three modes; plate-to-plate embossing, roll-to-plate embossing and roll-to-roll embossing [78].

A series of trials and experiments were completed in order to develop and implement a plate to plate hot embossing process to replace channels etched in upper CYTOP cladding on the glass lid. The glass substrate was replaced with a TOPAS substrate. The TOPAS, Cyclic Olefin Copolymer (COC), is a proprietary material from TOPAS Advanced Polymers. The sealing lid of the biosensing device would be a TOPAS substrate with embossed channels instead of a glass substrate containing etched channels. The major processing steps for hot embossing are the fabrication of master die and embossing the master die into the TOPAS substrate at a certain temperature with a specific force over a certain time.

In general, hot embossing is a two-step process; (1) fabrication of a die or mold comprising patterns, and (2) transferring the patterns onto a polymer film or substrate. The transfer of patterns is accomplished

through controlled force and temperature. To take advantage of hot embossing, the embossed substrate can be bonded to the wafer containing the waveguides, to form integrated biosensor. However, the bonding step which is specific to the fabrication of biosensors presented in this thesis. The processing steps of fabrication of die and then using the die to form channels via hot embossing are described in the following sections. The hot embossing steps, in general, are illustrated in Figure 5.1. The steps are illustrated as follows; (a) The embossing cycle starts with placing the die or mold and polymer substrate in platens, (b) the die and polymer are heated to embossing temperature, (c) controlled force is applied, (d) die and polymer are held under controlled heat and force (e) force is removed at de-embossing temperature and wafers are separated.

Figure 5.1 - Illustration of Hot Embossing Steps.

5.1 Fabrication of Master Die

Silicon is the workhorse of the semiconductor industry. Etching silicon using RIE processes to create different features such as micropillars, microchannels, and micro coils are widespread in fabrication labs. However, deep silicon etching (DRIE), as required in this research, must achieve three basic requirements. First, a high etch rate is required for a deep RIE etch. A low etch rate is not economical and introduces process variations. Secondly, there should be high selectivity; means that Silicon should etch at a much higher rate than the etch mask. Low selectivity limits the etch depth that can be achieved. Finally, an anisotropic etch is required throughout the etching process. If lateral etching occurs, the pattern transfer may not succeed, and the required features will not be created on die [79].

The embossing die were created using 4-inch Silicon wafers. The microfluidic channels were patterned on a Silicon wafer, and an etch mask was used to etch the silicon wafer using Deep Reactive Ion Etching (DRIE) process. The background or the field area of the silicon wafer was etched away, leaving the channels covered with etch mask during the DRIE. The final mold or die contains the raised channel pattern which is used in the hot embossing process to impress the channel profile in a polymer such as TOPAS. There were five product quality silicon die wafers fabricated, including one wafer for an advance run following the process described in this chapter. The fabrication processing steps of master die or mold are shown in Figure 5.21.

Figure 5.2- Illustration of Major Fabrication Steps of Master Die.

The major fabrications steps are illustrated as follows; (a) A 4-inch silicon wafer, (b) SiO₂ etch mask grown thermally, (c) HMDS spin-coated and baked, (d) PR S1818 spin-coated and baked, (e) light field Photo mask and exposure, (f) development in MF-321, (g) SiO₂ RIE etching in MRC, (h) Acetone and Plasma Preen to remove Acetone and organics, (i) DRIE etching of silicon in SAMCO RIE-110iP, (j) SiO₂ etch mask removed in BOE and final cleaning.

The process steps for the fabrication of silicon die are summarized in Table 5.1.

Table 5.1 – Process Steps for Fabrication of Master Die

Step	Activity	Details
1	Wafer preparation	Selection of best possible flat Si wafers and scribe wafer ID
2	RCA cleaning	3-sep cleaning, immerse wafers for 5 minutes in each solution H ₂ O:NH ₄ OH:H ₂ O ₂ in 5:0.25:1 ratio at 80°C & H ₂ O:HCL:H ₂ O ₂ in 6:1:1 at 80°C & 1% HF solution for 30 seconds to 1 minute, 2 cascade rinse in DI wafer for 5 minutes each after every step
3	SiO ₂ mask to etch Si wafer field area	Grow SiO ₂ thermally about 1.6 µm thick on the full wafer in the furnace at 1100°C, wet, takes about 4 hours
4	Dehydrate	Dehydrate on a hotplate at 200°C for 7 to 10 minutes
5	Spin coat hexamethyldisilazane (HMDS)	HMDS promotes adhesion of PR, spin at 1000 rpm for 10 s, followed by 4000 rpm for 30 s (about 150 nm thick at above parameters [80])
6	Soft bake	Soft baked at 115°C for 1 minute
7	Spin coat photoresist (PR S1818)	S1805 spin at 1000 rpm for 10 s, followed by 5000 rpm for 30 s
8	Soft bake	Soft baked at 115°C for 1 minute
9	Exposure dose	400 mJ/cm ²
10	Develop	MF-321 for 1 minute and 15 seconds
11	Hard bake	Hard bake on hotplate at 125°C for 3 minutes
12	Descum	Descum in Technics Planer Etch II Plasma Etcher for 1 minute 100 Watts, O ₂ plasma, pressure 0.3 Torr
13	RIE Etch of SiO ₂ in the Field area	29 min of RIE in MRC RIE 61 Etcher Source gases: CHF ₃ & O ₂
14	BOE dip	Removal of remaining SiO ₂ in field area 2 min 30 s dip in BOE
15	Final cleaning and removal of PR	Acetone, IPA and DI water Plasma Preen for 5 minutes using standard parameters 750 watts, 300 sccm, 5 mTorr
16	Silicon DRIE	SAMCO RIE-110iP System Etchant gases SF ₆ :O ₂ :Ar in the ratio of 14:2:20 sccm Etch time about 30 minutes

5.1.1 Mask Selection

The definitive trial of the etch mask is the fidelity of the channels pattern transfer into the silicon die over the entire etching period. Since the material used as a mask also interacts with etching process parameters, it is essential to select the right mask. Oxides, metals, and photo resists are among conventional silicon etch masks [79]. The selection of etch mask and etchant gas are tied together. Selectivity between silicon and mask etching can be estimated from etch rates for different materials as reported in [81]. The reported etch rates depend on etchants, etching conditions, and the etch system. Thus, these rates could not directly be used in mask selection process but were used a starting point. The reported etch rate of silicon, and thermal oxide are 1500 nm per minute and 29 nm per minute, respectively using SF_6 and O_2 in a Surface Technology System etch reactor. These etch rates give a selectivity of about 52. The SiO_2 was an attractive choice as a mask to etch the silicon considering the reported selectivity. However, under etching conditions used in this work, a selectivity of about 12 was experimentally determined, significantly lower than expected. This variation was due to use of different etching parameters and etching reactor configuration as discussed in the following sections. The depth of channels created using embossing process depends on the height of the raised channels on the die. A minimum depth of 20 μm was targeted for embossed channels. Thus, the thickness of the SiO_2 etch mask required was calculated to be about 1.6 μm using the selectivity of 12.

5.1.2 Selection of Etchant Gases

A SAMCO RIE-110iP System was used to perform DRIE for the fabrication of silicon wafer dies. The manufacturer of the RIE system recommended the recipe to perform DRIE on silicon wafers. A combination of SF_6 , O_2 , and Ar was recommended in the ratio of 14:2:20. The recipe was tested on test wafers to verify the selectivity and etch rates. A selectivity of 12 was observed after the experimental etch using the flow rates given above. It was also noticed that increasing flow of O_2 reduced verticality of sidewalls and reduced etch rate due to passivation. Thus, the original recipe, as suggested by the SAMCO

manufacturer, was adopted for silicon wafer die tests. The DRIE etch process is described in the following sections.

5.1.3 Wafer Preparation

After the selection of the etch mask thickness and etchant gases and other processing parameters, the process to fabricate silicon die was started. Selecting a silicon wafer to be used as a die was the first step. The best possible flat silicon wafers were selected and labeled through scribing. The wafers were subject to RCA cleaning to remove any organic contaminants and particles, stripping off thin oxide and ionic contamination. The RCA clean is a standard wafer cleaning procedure completed before high temperature processing, such as oxidation and diffusion. The RCA cleaning is completed in 3 steps. In the first step, organic contaminants are removed by dipping the wafer in the solution of $\text{H}_2\text{O}:\text{NH}_4\text{OH}:\text{H}_2\text{O}_2$ in 5:0.25:1 ratio for 5 minutes at 80°C followed by 2 cascade DI water rinses in beakers of 5 minutes each. In the second step, metal and ionic cleaning is done by immersing the wafers in a solution of $\text{H}_2\text{O}:\text{HCl}:\text{H}_2\text{O}_2$ in 6:1:1 ratio at 80°C for 5 minutes and followed by 2 cascade DI water rinses in beakers of 5 minutes each. The third step is to remove the thin layer of hydrophobic oxide created in the second step. The wafers are dipped in 1% HF solution for 30 seconds to 1 minute followed by 2 cascade rinse in beakers for 5 minutes each.

5.1.4 Growing Thermal Oxide for Etch Mask

The oxide etch mask was thermally grown on silicon wafers in the furnace at Carleton University Fabrication Lab using standard wet oxidation process in the furnace. The selected wafers were placed in the boats and loaded into the furnaces. The oxide is wet grown at 1100°C and takes about 4 hours to get the thickness around $1.6\text{ }\mu\text{m}$. The oxide thickness was measured using a NanoSpec spectroscopic reflectometer and a Tencor P-1 profilometer at five distinct locations on each wafer. The oxide thickness was consistent across the wafer and found to be in the range between $1.61\text{ }\mu\text{m}$ and $1.62\text{ }\mu\text{m}$.

5.1.5 Lithography for Etch Mask

In this section, the process to etch SiO_2 from the silicon die wafer except channel is explained. The SiO_2 is a mask to protect channels while etching silicon to fabricate a die having raised channels. The etching of SiO_2 from field or background area is accomplished in a MRC RIE Etching System. However, the SiO_2 mask on channels still needs to be protected. Thus, S1818 a positive photoresist (PR) etch mask is used for this purpose. The etching recipe for SiO_2 is a combination of CHF_3 and O_2 with a flow rate of 42.5 sccm and 7.5 sccm, respectively. However, the etchant gas in this recipe like all other etchants also interacts with the PR mask and etches the targeted SiO_2 as well as the PR etch mask. It is essential for a successful RIE etch process that the PR is etched at a lower rate than the SiO_2 . The etch rates were verified for S1818 PR and SiO_2 using test samples. The average etch rates were found to be 42.5 nm per minute and 52.80 nm per minutes for S1818 and SiO_2 , respectively. Since S1818 is a very viscous PR, a primer such as HMDS is used to promote adhesion between PR and the SiO_2 layer. The wafers were dehydrated on a hotplate at 200°C for about 10 minutes before bilayer lithography. First, HMDS was spin-coated at a spin speed of 1000 rpm for 10 seconds and 4000 rpm for 30 seconds yielding about 150 nm thick layer of HMDS [80], followed by soft bake on a hotplate at 115°C for 1 minute [82]. Then, S1818 was spin-coated at a spin speed of 1000 rpm for 10 seconds and 5000 rpm for 30 seconds to produce a thickness of about 2 μm [83] and soft baked at 115°C for 1 minute on a hotplate. A five-point measurement check was performed to verify the thickness of S1818 using a NanoSpec spectroscopic reflectometer. The thickness ranged from 1.95 μm to 2.08 μm . The exposure dose was optimized to 400 mJ/cm^2 . The wafers were immersed in MF-321 for 1 minute and 15 seconds for development. Figure 5.3 shows the channels at two different locations on a silicon wafer die after the development. The channels were covered with S1818 to protect the SiO_2 underneath. The background or field area has SiO_2 only as S1818 was etched away during the development.

Figure 5.3 - Channels Shown on Silicon Wafer Die After Development.

5.1.6 SiO₂ Mask Etch in Field Area

The SiO₂ mask from the field area was etched in the MRC RIE using a combination of CHF₃ and O₂. A 1.6 μm thick SiO₂ etch mask was subject to RIE etching. However, about 1.55 μm of SiO₂ was etched in the MRC RIE etcher and remaining 50 nm was etched in Buffered Oxide Etchant (BOE). The BOE is typically used to etch thin SiO₂ films to get a uniform and smooth surface. Etching the SiO₂ solely in the MRC RIE may produce grass on the etched surface as observed in some of the test runs. It takes between 30 seconds to 60 seconds in BOE to etch 50 nm SiO₂. The wafers were dipped in BOE for about 1 minute after RIE etch to completely remove the SiO₂ mask from field areas. During the etch, process, the S1818 PR mask on the channel was also etched. The remaining 0.767 μm PR mask was removed in an acetone dip followed by cleaning dip in IPA and DI water. Finally, a 5-minute O₂ plasma etch in the isotropic Plasma Preen reactor was performed to remove any PR or any other residuals. Figure 5.4 shows microfluidic channels at two different locations on a wafer. The SiO₂ mask from the field areas has been etched away, and the silicon surface can be seen in the background which is subject to DRIE. The PR mask protecting the SiO₂ on the channels has been removed, and SiO₂ on channels still exists, and it is apparent.

Figure 5.4 - Channels on Silicon Die Wafer After SiO₂ Mask Etched from Field.

The RIE etching parameters used in the MRC etcher are given in Table 5.2.

Table 5.2 - SiO₂ Mask Etching Parameters Used in MRC RIE Etcher

Parameter	Value
RF Power	350 Watts
Pressure	150 mTorr
CHF ₃ flow	42.5 sccm
O ₂ flow	7.5 sccm

A summary of average etch rates for SiO₂ and S1818 PR are given in Table 5.3 and Table 5.4. The measurements were taken at five distinct locations on each wafer and the average etch rate was calculated to summarize the data. The major flat of the 4-inch wafer is referred as South and other positions are referred to accordingly.

Table 5.3 - Etch Rate for SiO₂ in MRC RIE Etcher

Etch Rate SiO ₂ (nm/minute)						
Wafer ID/Position	West	Centre	East	North	South	Average
MA29	54.190	52.910	53.960	55.375	52.550	53.797
MA30	53.511	52.871	54.043	53.289	54.071	53.557
MA31	54.072	52.159	52.986	53.372	52.669	53.052
MA32	53.124	50.621	50.793	51.138	52.103	51.556
MA33	52.990	50.828	51.966	51.276	52.990	52.010

Table 5.4 - Etch Rates for PR S1818 in MRC RIE Etcher

Etch Rates PR S1818 (nm/minute)						
Wafer ID/Position	West	Centre	East	North	South	Average
MA29	44.000	44.500	47.000	42.000	43.000	44.100
MA30	41.286	43.893	39.536	38.964	40.571	40.850
MA31	45.362	46.652	44.655	42.966	44.045	44.736
MA32	43.966	43.110	37.559	38.672	44.983	41.658
MA33	42.759	42.069	40.000	41.034	40.000	41.172

It was important to verify the thickness of the SiO₂ mask before DRIE of silicon is started. After etching SiO₂ from field area, AFM measurements were performed to verify the thickness and profile of the SiO₂ mask on the channels. Figure 5.5 shows the thickness of the SiO₂ mask, which is 1.528 μm . It is slightly less than the thickness measured with the NanoSpec spectroscopic reflectometer, which was 1.6 μm . The difference in thickness may be due to the fact that the AFM and profilometer measurements were done after the final cleaning with Acetone, IPA, Di water and the O₂ plasma treatment, which reduced the thickness slightly. A 3-D profile of the SiO₂ mask is shown in Figure 5.6, which appears to show a good quality mask. However, the sidewalls are not perfectly vertical. This may be due to two reasons; first, it is a natural etch profile of an MRC RIE etch for SiO₂ and secondly, the AFM may have some limitations in scanning the profile of such a large step height. Figure 5.7 shows the profilometer measurement of the SiO₂ mask. The thickness is 1.558 μm , which is very close to the measurement performed with the NanoSpec spectroscopic reflectometer. However, sidewalls are not perfectly vertical due to the same reasoning explained above. The angle of the mask sidewalls is not critical as long as the width of the mask on the top of the channel is at least equal to actual channel width and it is completely protecting the channel from etching. The channel width was measured after completion of DRIE and was found to be accurate. The details are discussed with images in the subsequent sections. The SiO₂ mask was partly

etched during DRIE of silicon and the remaining material was removed with BOE after the completion of the DRIE process.

Figure 5.5 – An AFM Scan of SiO₂ Mask on Channel.

Figure 5.6 - An AFM 3-D View of SiO₂ on Channel.

Figure 5.7 – A Profilometer Scan of SiO₂ Mask on Channel.

5.1.7 Si DRIE Etch Process for Raised Channel Formation

The silicon wafer die, or molds were created by etching the silicon in a SAMCO RIE-110iP system. As described in the Mask Selection section, the selectivity was derived from etch rates of silicon and SiO₂ using the manufacturer's recommended recipe. Under ideal conditions, silicon and SiO₂ rates were estimated to be 0.87 $\mu\text{m}/\text{min}$ and 0.05 $\mu\text{m}/\text{min}$, respectively. For the DRIE process in the SAMCO etcher, thermal contact between the wafer and carrier wafer is necessary for thermal conductivity. Thermal grease is used for this purpose. The first wafer was initially etched for 23 minutes and observed under SEM to verify the thickness of the remaining SiO₂ mask and the depth of etched silicon. The etched silicon depth was found to be 20.5 μm producing an etch rate of 0.891 $\mu\text{m}/\text{min}$. There was about 450 nm of SiO₂ mask left. To optimally use the SiO₂ mask and to obtain maximum depth, another 3 minutes of Si etching was performed followed by SEM imaging. Thus, the total depth of 21.4 μm was attained in 26 minutes.

After obtaining the required depth, which is eventually the height of the raised channels on a silicon wafer, the remaining SiO₂ mask was removed using BOE. Finally, the thermal grease was removed, and the wafer was cleaned with trichloroethylene then IPA and water. Three more silicon wafer die were processed using the same steps. However, etch time was increased in steps for each silicon die to get maximum channel height by optimally utilizing the SiO₂ mask. Table 5.5 shows the etch time and etch rates for different channel heights of the silicon wafer die.

Table 5.5 - Silicon Etch Rates in SAMCO RIE System

Wafer ID	Time Etched (min)	Etch Depth (μm)	Etch Rate (μm/min)
MA30	26	21.4	0.823
MA31	33	29.6	0.897
MA32	35	28.5	0.814
MA33	30	26.6	0.887

The plot in Figure 5.8 shows the overall etch depths and etch rates at different etch times. The purpose of this plot is to show that optimal etch time for this process is 33 minutes. After 33 minutes, the etch rate decreases indicating that the SiO₂ mask is completely consumed during DRIE process and silicon is being etched from the top of the channels along with background silicon yielding almost no or very little additional depth.

Figure 5.8 - Silicon DRIE Etch Rates

The silicon die was inspected and analyzed using SEM, profilometer, AFM, and optical microscope to observe channel profile, the verticality of channel sidewalls, channel height, etch quality, roughness, and width. Figure 5.9 shows channels at two different locations after the DRIE etch. The legend represents 10 μm. The height of the channels is 21.4 μm after 26 minutes of silicon etch. Figure 5.10 (a) shows MA33 etched for 30 minutes producing 26.6 μm raised channels. A curtaining effect on the sidewalls can be observed as a result of deeper and longer DRIE etch. Curtaining is an RIE artifact which appears as vertical striations on etched structures such as sidewalls of microfluidic channels. Figure 5.10 (b) features a curved channel which shows the ability of the process to perform DRIE of different shapes of channels. A channel sidewall before BOE clean is shown in Figure 5.11. There is still some SiO₂ mask left on the top of the channel. The sidewall is perfectly vertical at 90°, which shows the ability of the DRIE etch process to perform vertical etches. However, the SEM image is slightly blurred due to the charging of the CYTOP in the SEM.

Figure 5.9 - SEM Images of 21.4 μm Raised Channels After Silicon DRIE Etch.

**Figure 5.10 - SEM Images of 26.6 μm Raised Channels After Silicon DRIE Etch.
(a) Showing curtaining for deeper etch (b) A curved channel.**

Figure 5.11 - SEM Image of Channel Sidewall.

The channel heights were measured using a profilometer BRUKER model DekTak XT to verify the silicon etch depth. The height of a channel on MA30 is shown in Figure 5.12, which is about 21.4 μm as expected. The channel height of MA33 is shown in Figure 5.13, which is 26.6 μm . The verticality of the channel, especially from the left side, seems compromised. The accuracy of the channel profile measurement depends on the diameter of the profilometer tip. In addition, the tip is diamond-shaped which may not accurately measure the verticality of the sidewall. However, there is a probability that all the channel walls are not perfectly vertical on a 4-inch wafer. There are some variations expected in the etch profiles of channels depending upon their location on the wafer. This aspect may be explored further in future studies. The surface roughness of the raised channel is a crucial parameter. This roughness can be transferred to the channel bottom as a result of embossing. The roughness of one of the channels is shown in Figure 5.14, which is 0.346. Finally, Figure 5.15 shows a microscope image of a 250 μm wide channel after DRIE etch was complete. It is a bit difficult to focus at two different surfaces with a height difference

of 26.6 μm at high magnification such as 20X as in this image. The DRIE process was able to realize the width of the channel almost accurately. A 250 μm wide channel appears to be between 242.8 μm and 252 μm wide.

Figure 5.12 - Profilometer Scan of 21.4 μm Raised Chanel.

Figure 5.13 - Profilometer Scan of a 26.6 μm Raised Channel.

Figure 5.14 - AFM Roughness Measurement of Channel Surface After DRIE.

Figure 5.15 - Microscope Image of a Channel Showing Width Post DRIE Etch.

5.2 TOPAS Substrate

CYTOP and TOPAS have similar properties. Both of the materials are a glass-clear amorphous polymer with high transmissivity and high resistance to common solvents used in microfluidics, including alcohol, acids, bases, and water [84] [85]. CYTOP has a refractive index of 1.34 whereas the refractive index of TOPAS ranges from 1.49 to 1.53 depending upon norbornene content. The T_g of CYTOP is 108°C which matches with T_g of TOPAS grade 5010. However, T_g of TOPAS ranges from 65 to 178, depending on grade [86]. The refractive index of borofloat glass used in this research to fabricate lids and upper CYTOP cladding is 1.47 [87]. Thus, the TOPAS substrates can be used as lids and upper cladding containing embossed microfluidics channels. However, a TOPAS substrate may also be used as lower cladding as well to provide a structural base to support the final device. Thus, a complete biosensing device can be fabricated using TOPAS substrates without using a silicon wafer. The possibility of TOPAS to replace glass lids and upper CYTOP cladding is explored in next sections. However, the idea can be extended further to fabricate complete biosensor using TOPAS. It is available in substrates of different thickness and diameters. The TOPAS substrates can also be customized through injection molding. The customised TOPAS wafers are expensive. Thus, pre-fabricated TOPAS substrates were used in the embossing experiment having a thickness of 1.5 mm and a diameter of 115 mm. The refractive index is 1.53, and T_g is 142°C. The CTE is in the order of $10^{-6}K^{-1}$, which is in the range of silicon and borofloat glass substrates.

One may ask that why TOPAS was used instead of CYTOP for hot embossing, although CYTOP has been used as the cladding material for the fabrication of integrated biosensors reported in this work. Firstly, CYTOP was not available in substrate or wafer form, it was available only in liquid form for spin coating and dip coating. TOPAS was available in wafer form with customizable thickness and diameter. Secondly, an alternative material was investigated which can potentially be used to develop a simple, robust and economical hot embossing process for microfluidic channels. CYTOP might be used for hot embossing by

spin coating on a substrate to form embossed thick layer suitable for embossing. However, significant additional work would be required on hot embossing of CYTOP in order to develop a repeatable process.

5.3 Embossing Process

After the fabrication of silicon die wafers and provision of TOPAS substrate, the hot embossing was performed in the AML-AWB wafer bonder. The embossing process model was adapted from [88] [89]. The hot embossing process is a 4-step process; (1) heating the die and polymer substrate to embossing temperature which is slightly above the T_g of the polymer, (2) isothermal molding by embossing the microfluidic structures pattern at embossing temperature under controlled force, (3) cooling the mold and polymer substrate to demolding temperature which is slightly below T_g of polymer, and finally (4) demolding by removing the force and separating the die from polymer substrate. The polymer experiences two phases of deformation in the complete process; the first phase is stress concentration and strain hardening that occurs in heating and embossing steps and the second phase is stress relaxation and deformation recovery that occurs in cooling and demolding stages [90].

To perform hot embossing in the wafer bonder, the silicon wafer die and TOPAS substrates were loaded onto upper and lower platens respectively. The hot embossing was done in a moderate vacuum pressure in the order of 10^{-3} Torr. The moderate vacuum pressure helps to accurately apply controlled force at the desired rate. A very high vacuum, however, may cause the silicon die to stick to the TOPAS polymer when cooling down. After reaching the desired vacuum, the die and TOPAS substrate were heated slightly above T_g , which is 142°C . A few minutes were allowed before force was applied to allow the system to reach thermal equilibrium. Then the force was applied slowly to ensure good control. An embossing time was allowed for TOPAS to flow and to fill in the features. Then heat was turned off, wafers were cooled to demolding temperature and force was slowly removed. Finally, die, and TOPAS substrates were cooled to

room temperature and removed from the wafer bonder. The embossing process steps are summarised in Table 5.6.

Table 5.6 - Process Steps for Hot Embossing

Step	Activity	Parameter
1	Mounting wafers on platens	Silicon die on top and TOPAS substrate on lower platen
2	Vaccum Pressure	In the order of 10^{-2} mbar
3	Embossing temperature (Heat platens with ramping)	Embossing temperature slightly higher than T_g (142°C) Varied for 5 trials from 143°C to 162°C for optimization
4	Time for thermal equilibrium	Varied from 5 minutes to 15 minutes for optimization
5	Bring wafers in contact	Make sure wafers are in good contact
6	Apply force	Forced varied from 1.5 kN to 2 kN
7	Time for force application	30 seconds
8	De-embossing temperature	De-embossing temperature slightly lower than T_g (142°C) Varied for 5 trials from 120°C to 132°C for optimization
9	Remove force	Completely remove force to make sure die and TOPAS substrate are not in contact
10	Cool down	About 25°C
11	Remove wafers from bonder	Wafer may stick together, separate them carefully

The hot embossing process cycle is shown in Figure 5.16. The plot is constructed from wafer bonder data from the embossing process of MA44. The force and temperature curves show the important points during the hot embossing process.

Figure 5.16 - Hot Embossing Process Cycle for MA44.

5.3.1 Embossing Trials

There are number of variables that may be tuned to optimize the embossing process. However, the glass transition temperature T_g , embossing force, and hold time are important parameters for a hot embossing process with polymers. Thus, the user has the option to control the following parameters;

1. Embossing temperature
2. Embossing force
3. Holding time
4. Demolding temperature

There were five embossing trails conducted by varying the embossing parameters to develop an optimized hot embossing process. The silicon die with different channel heights were used in the trials to obtain embossed channels to their respective depths. The user-controlled parameters to optimize the hot embossing process are shown in Table 5.7.

Table 5.7 - Summary of User-controlled Parameters for Optimization of Hot Embossing Process

Parameter	Trial 1-MA38	Trial 2-MA43	Trial 3-MA44	Trial 4-MA45	Trial 5-MA51
Vacuum Pressure	2×10^{-2} mbar	2×10^{-2} mbar	2×10^{-2} mbar	2×10^{-2} mbar	2×10^{-2} mbar
Embossing temperature	152°C	152°C	162°C	143°C	157°C
Thermal equilibrium time	5 min	5 min	5 min	5 min	15 min
Applied Force	2 kN	1.5 kN	1.5 kN	1.5 kN	1.5 kN
Time to exert force	30 sec	30 sec	30 sec	30 sec	30 sec
De-embossing temperature	132°C	132°C	120°C	120°C	125°C

For the first embossing trial, the MA32 silicon die was used. The die has 28.5 μm raised channels; thus, the depth of the channel was expected to be the same. Embossing was done at 152°C, which is 10°C above T_g , and 2 kN force was applied. The resulting depth was measured using a profilometer and found to be 27 μm deep, which is about 1.5 μm less than the expected depth. The sidewalls of the channels were not perfectly vertical, but vertical at the bottom and slightly curved at the top as shown in Figure 5.17.

In the 2nd trial, the embossing force was reduced to compensate for sidewall recovery by allowing the microcavities to be filled at the top of channel producing vertical sidewalls. The MA31 die with 29.6 μm raised channels was used which produced the channels with the almost same depth as the die. However, the sidewalls have the same profile, vertical at the bottom and slightly curved at the top.

Figure 5.17 - Channel Depth of Embossed Wafer – MA38 – Trial 1.

In the 3rd embossing trial, an attempt was made to fix the sidewall issue by increase embossing temperature by 20°C above T_g and reducing the de-embossing temperature by 12°C from previous trials. The idea was that at high embossing temperature TOPAS would become softer to fill the sidewalls microcavities at the top of channels. The decrease in de-embossing temperature would allow TOPAS to recover and orient properly as it was heated much above T_g during embossing cycle. The trial resulted in the accurate depth and producing side walls straighter than previous trials but not completely vertical, as shown in Figure 5.18.

Figure 5.18 - Channel Depth of Embossed Wafer – MA44 – Trial 3.

The embossing temperature was set to almost at T_g during the 4th trial. The change in embossing temperature was based on the idea that at T_g , TOPAS would just start to become soft and pushing the die at such temperature may result in the straight wall since the polymer is not soft enough to flow long distance and it can rearrange orientation quickly to form straight walls. The profilometer scan did not show any improvement in the verticality, although accurate depth was attained. During 5th trial, the embossing temperature was increased 13°C above T_g , whereas the de-embossing temperature was set to 125°C, which is 5°C above the value used in the previous 2 trials. Almost the same results were seen in terms of sidewalls; however, the required depth was obtained as expected, as shown in Figure 5.19. The SEM imaging was performed to analyze the embossed channels on the TOPAS substrates. Figure 5.20 (a) shows a curved channel and a part of the channel focusing on the side wall at high magnification. The sidewalls profile looks vertical at the bottom and curved edges on the top. Figure 5.21 shows a comparison of embossing done at two different temperatures. The sidewalls seem more vertical for the embossing done at higher temperatures as seen in Figure 5.21 (a).

Figure 5.19 – Channel Depth of Embossed Wafer - MA51 – Trial 5.

(a)

(b)

Figure 5.20 - SEM Images of Embossed Channels - MA44.
(a) A curved channel (b) A higher magnification view of the sidewall.

(a)

(b)

Figure 5.21 - SEM Images of Embossed Channels.
(a) Almost vertical sidewall - MA44 relatively high temperature
(b) Curved sidewalls - MA38 relatively low temperature.

5.4 Embossing Results

As mentioned earlier, the embossing process can be divided into two parts; (1) fabrication of die and (2) transferring die features on TOPAS substrate through hot embossing. The first part, fabrication of die is successfully completed using a repeatable process. The protruding microfluidic channel with accuracy has been fabricated. The hot embossing process can produce the required channel depth, but it needs improvement in terms of filling the channel sidewalls at the top to obtain rectangular channels. The user-controlled parameters were varied to see the resultant embossed channels on the TOPAS substrate. The embossing temperature appeared to most sensitive during these trials. Consequently, slightly straighter walls were achieved at higher embossing temperature as compared to embossing done at low temperature. It worked better for narrower channels than wider channels. The sidewall curvature was more obvious on wider channels. In general, the channels are wider at the top than at the bottom. Table 5.8 shows the channel depth, and width at the top and the bottom of channels of TOPAS embossed wafers at different locations. The difference between top and bottom width of channel confirms that wafer (MA51) embossed at high temperature as well as narrow channels have straighter sidewalls.

Table 5.8 - Channel Profiles of Embossed Wafers

Channel Profiles of Embossed Wafers					
Wafer ID	Location	Depth (μm)	Top Width (μm)	Bottom Width (μm)	Delta (Top/Bottom %)
MA38	Centre West	26.60	392.40	227.90	72.18
MA43	Centre West	30.00	375.90	217.30	72.99
MA44	Centre South	29.20	281.20	206.80	35.98
MA44	Centre West	29.60	276.40	202.00	36.83
MA44	Centre East	28.40	283.40	213.80	32.55
MA44	Centre	28.50	251.80	199.90	25.96
MA 51	Centre	21.27	260.40	209.00	24.59

The improvement in the de-embossing step may solve this issue, as reported in [91]. However, there is a need to investigate further into mechanical, thermal, and chemical properties of TOPAS to develop a hot embossing process to get perfect channel walls. The fidelity of the hot embossing not only depends on the parameters discussed in the previous section but also depends on the thickness of the polymer substrate, the orientation of the polymer chain of the substrate, and the orientation of channels with respect to polymer chains. In addition, compression molded and injection molded TOPAS substrates behave differently during hot embossing [89]. Thus, this topic requires further research and investigation considering the different variables in the context of material properties, dynamics, and the behavior subject to heating and stress, as mentioned above.

5.5 Bonding Trial of Embossed TOPAS Substrate

The purpose of formation of the microfluidic channel through hot embossing on TOPAS substrate is to replace the channel etching process in CYTOP cladding on the glass lid. Thus, an attempt was made to replicate the bonding process mentioned in the previous chapter using a TOPAS substrate with embossed channels. After the embossed substrate was completed, a trial was performed to bond the TOPAS substrate with a silicon wafer containing the gold waveguide on lower CYTOP cladding. A layer of M-grade CYTOP was spin-coated for improved adhesion and bonding between CYTOP and TOPAS [92]. The wafer bonding was performed using the process described in Wafer Bonding chapter. The key point on the bonding process is T_g of polymers being bonded. In CYTOP-CYTOP bonding, T_g of both films is identical. Hence, no complication arises. However, in this trial, T_g of TOPAS used in this research is 142°C where is T_g of CYTOP 108°C. Thus, it is difficult to perform bonding for material having two different glass transition temperatures (T_g). The bonding was performed by heating TOPAS and CYTOP to 147°C and 120°C respectively. All other bonding parameters were kept the same as mentioned in the Wafer Bonding chapter.

Nevertheless, bonding between CYTOP and TOPAS did not occur. It was observed that upon contact, the temperature at the bonding surface was not favorable for bonding because of difference in T_g of polymers. Due to the heat transfer between TOPAS and CYTOP, there was not enough heat for TOPAS to become soft enough to flow and diffuse with CYTOP. The lower temperature CYTOP coated wafer decreased the TOPAS surface temperature. It became more like a hot embossing situation where TOPAS was being pressed or extruded into CYTOP lower cladding. The possible solution could be heating up CYTOP and TOPAS substrates above T_g of both and holding the temperature for a longer time to ensure both materials are softened and are able to fuse together. However, the higher temperature may cause the CYTOP to move longer distances and waveguides may deform as a result. Thus, a more feasible option for future work is to use TOPAS and CYTOP with matching T_g .

Chapter 6: Conclusion and Future Work

The fabrication of an integrated LRSPP waveguide biosensor was pursued and accomplished in this research work. The work reported in this thesis was based on the previous work of [60] [61] [62] [63] [64] [49]. The contribution made through this work includes the integration of discretely fabricated waveguide structures and microfluidic channels through a bonding process in a wafer bonder. The alignment of microfluidic channels to corresponding waveguides was performed in the wafer bonder, which avoided the misalignment problem due to an opaque Al mask as reported in [49]. This process also fixed the cracking issue completely, frequently reported in previous work, due to ingress of CYTOP solvent into lower cladding while fabricating upper cladding on the same substrate. The quality of the interface between the waveguide and bonded top cladding was improved by characterizing and implementing a process to form an ultra-shallow trench to obtain recessed gold waveguides in CYTOP and present a planar process for bonding. The wafer bonding process was significantly modified from previous work to obtain intact waveguides and microfluidic channels without compromising their integrity and producing over 90% yield. An RIE channel etching process was characterized and successfully employed yielding ultra-smooth channel walls and beds, avoiding curtaining and grass issues. A hot embossing process to form the microfluidic channel on TOPAS substrate was introduced. Embossing die using 4-inch silicon wafers through deep reactive ion etching (DRIE) process with protruding channels were fabricated, and up to 29 μm deep microfluidic channels were attempted.

The optical measurements show that MPA of the fabricated LRSPP devices ranges between 3.66 dB/mm and 4.92 dB/mm depending on the chip, which is below the theoretical value of 7.2 dB/mm in any case. The low losses are likely due to the reduced thickness of gold waveguides during the bonding process. In future work, the reduction in the gold thickness needs to be explored further and characterized. Thus, the reduction in thickness may be compensated to get the final thickness of 35 nm.

The process of hot embossing using TOPAS substrate requires improvements to be considered in future work. Although the fabrication of die was completed with the desired profile having channels raised to 29 μm , but the resulting embossed channels lacked desired verticality of walls near the top surface. A further study of TOPAS properties is needed to successfully implement a hot embossing process.

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Appendix A:

Process steps for the fabrication of LRSPP Biosensors

Process	Step	Description	Process Details
Au waveguides on CYTOP lower cladding on Si wafer	1	Piranha clean	Dipped wafers in Piranha solution for 10 min at 90°C. It is a 4:1 mixture of H ₂ SO ₄ and H ₂ O ₂ .
	2	APTES ((3-Aminopropyl) triethoxysilane) application	Immersed wafers in 0.05% APTES solution for 10 min, followed by dehydration step 115°C for 20 minutes. It is a mixture of 1ml APTES and 2-litre Ethanol.
	3	Spin coat CYTOP for lower cladding	Spun 9% CTX-809A CYTOP A-type at 550 rpm for 10 s and 1500 rpm for 30 s and perform soft bake on a hotplate at 50°C. Repeated the above step 2 more times followed by final hard bake at 200°C for 4 hours with ramping.
	4	CYTOP Surface Ashing	O ₂ plasma RIE etching of CYTOP; power 70W, O ₂ flow rate 250 sccm, O ₂ pressure 320 mTorr for 30 s
	5	Edge bead removal	RIE etching of edge bead; power 200W, O ₂ flow rate 250 sccm, O ₂ pressure 320 mTorr, etch time 7 min
	6	Inspection, imaging, and analysis	AFM scan for etched trench and background CYTOP for roughness, and profile.
	7	Spin coat lift-off resist	LOR-1A (MicroChem) spun at 1000 rpm for 10 s, followed by 4000 rpm for 30 s
	8	Soft bake	180°C for 3 min on a hotplate
	9	Spin coat photoresist (PR)	S1805(MicroChem) spun at 1000 rpm for 10 s, followed by 4000 rpm for 30 s
	10	Soft bake	115°C for 3 min on a hotplate
	11	UV Expose	109 mJ/cm ²
	12	Develop	Dip wafer in MF-321 for 1 min
	13	Inspection, imaging, and analysis	AFM scan of the opened area after development in the etched trench for roughness and profile.
	14	Trench etching for Au waveguide	RIE trench etch into CYTOP lower clad; power 50W, O ₂ flow 150 sccm and 220 mTor, for 17 s, etch rate 2.3 nm/s
	15	Au deposit	Deposition by thermal evaporation, 35 nm gold

	16	Au Lift-off	10 min dip in PG remover at 60°C, followed by 10 s ultrasonic treatment, repeat 4 times or unless wafer is completely cleaned
	17	Cleaning	Clean with IPA and DI wafer
	18	Inspection, imaging, and analysis	AFM scan of waveguide and CYTOP for roughness and waveguide profile. Optical imaging to see waveguide gold quality and integrity
Channel formation in CYTOP upper cladding on the glass wafer	1	Glass wafer preparation	Followed steps 1 to 3 for Piranha clean, APTES application and CYTOP coating on glass wafers
	2	Edge bead removal	RIE etching of edge bead; power 200W, O ₂ flow rate 250 sccm, O ₂ pressure 320 mTorr, etch time 7 min
	3	Al deposition	20nm thick Al deposited using E-beam evaporation
	4	Spin coat hexamethyldisilazane (HMDS)	HMDS promotes adhesion of PR, spun at 1000 rpm for 10 s, followed by 4000 rpm for 30 s
	5	Soft bake	Ramped up from 25°C to 80°C, baked at 80°C for 45 min on a hotplate, and ramp down to 25°C. Ramp rate 50°C/h
	6	Spin coat photoresist (PR)	S1805(MicroChem) spun at 1000 rpm for 10 s, followed by 4000 rpm for 30 s
	7	Soft bake	Ramped up from 25°C to 80°C, baked at 80°C for 1 h on a hotplate, and ramp down to 25°C. Ramp rate 50°C/h
	8	Expose	98 mJ/cm ²
	9	Develop	Dip in MF-321 for 3 min
	10	Channel etch in CYTOP	RIE channel etching; power 100W, O ₂ flow rate 250 sccm, O ₂ pressure 320 mTorr, average etch rate 0.26µm/min
	11	Al mask removal and cleaning	Dipped wafer in MF-321 for 2 min to remove Al etch mask, followed by dipping the wafer in Acetone, IPA and DI water 5 minutes each
	12	Inspection, imaging, and analysis	AFM scan of channel beds for roughness. Profilometer and Dektak to check channel depth and profile.
Bonding of Si and glass substrates containing waveguides and microfluidic channels	1	Wafer Loading and alignment	Glass wafer containing etched channels in CYTOP loaded to the upper platen and Si wafer containing Au waveguides on CYTOP cladding loaded to lower platen. The wafers were aligned under vacuum.
	2	Force	A 1000 N force was applied for bonding

	3	Heating	Heating was set to 115°C for both, upper and lower plated
	4	Bonding time	Wafers kept under force and heat for 16 hours
	5	Inspection and analysis	Optical microscopic inspection to check waveguides and channel integrity and bonding quality.
Dicing Resist	1	Vapor priming hexamethyldisilazane (HMDS)	Applied in the oven at 95°C
	2	Spin coat thick photoresist	SPR-220 (MicroChesm) spun at 1000 rpm for 30 s, followed by 2300 rpm for 10 s
	3	Bake	Held at 25°C for 20 min after a spin, ramp to 100°C, bake for 20 min at 100°C on a hotplate, ramp down from 100°C to 25°C, ramp rate 50°C/h

Appendix B:

Silicon and SiO₂ Etch Rates Derivation from Test Samples

Sample ID	Etch Rate (nm/min)	
	Silicon	SiO ₂
MA25-1-3M	789	65
MA25-2-5M	948	55
MA25-3-7M	853	51
MA25-4-10M	900	50

From the table above the Silicon etch rates can be averaged to be 0.87 $\mu\text{m}/\text{min}$ and SiO₂ rate tend to decrease as etch time increase thus it stabilizes around 0.05 $\mu\text{m}/\text{min}$.