Functionalization of Photonic Crystal Slab Biosensors

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Applied Science

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Abstract

This work describes the functionalization and testing of Si$_3$N$_4$-based photonic crystal slabs (PCS) for label-free biosensing. PCS support optical resonance modes that are sensitive to the local refractive index. Knowing that surface binding events change the local RI, analyte binding to the activated sensor can be detected.

Various functionalization recipes were tried, and one was preferred for the biosensing experiments due to its higher yield and uniformity. Additionally, thickness of the topmost sensor layer was studied to assess biosensor performance quantified through sensitivity metrics.

On the systems level, a reusable clamping system and customized microfluidic channels were designed, fabricated, and implemented on the PCS biosensors to enable device refurbishment.

Proof-of-principle biodetection experiments were carried out using the established functionalization protocol on the in-house fabricated PCS. Conjugation of streptavidin and bovine serum albumin to the sensor surface was observed through wavelength shifts of the resonant modes.
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“Nothing of me is original. I am the combined effort of everybody I’ve ever known.”

- Chuck Palahniuk

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Chapter 1

Background and Motivation

1.1 In vitro diagnostics

The ability to detect biologically relevant molecules such as enzymes, vitamins, proteins, or pathogens from fluid samples is paramount to the field of *in vitro* diagnostic biosensing. The distinguishing feature of *in vitro* diagnostics in comparison to other diagnostic methods is that the tests are carried out in a relatively controlled environment that is outside of the body. Within the scope of this thesis, the definition of *in vitro* diagnostics can be borrowed from the directive 98/79/EC of European Parliament and of the Council of 27 October 1998 [1]:

[...] *‘in vitro diagnostic medical device’ means any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, equipment, or system, whether used alone or in combination, intended by the manufacturer to be used *in vitro* for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information:

- concerning a physiological or pathological state, or
- concerning a congenital abnormality, or
- to determine the safety and compatibility with potential recipients, or
- to monitor therapeutic measures.
Specimen receptacles are considered to be \textit{in vitro} diagnostic medical devices.

[...]

Taking this definition into account, we can place the field of \textit{label-free} biosensing as a sub-field within \textit{in vitro} diagnostics. Label-free biosensing has garnered attention due is detection scheme that allows for detection of unmodified targets from a bulk fluid sample. The ability to detect unmodified targets is of significance since the state-of-the-art diagnostic schemes typically involve sample pre-processing steps such as:

1. Labelling the target with fluorescent tags [2], radioisotopes [3], or colorimetric enzyme functionalities [4] in the case that the target is plentiful but not detectable without modifications (e.g. tests for antibody, hormone, or enzyme counts; viral immunoassays)

2. Amplifying the target concentration to increase its detectability in the case that the target is low in concentration, despite being detectable without modifications (e.g. genomic assays that require polymerase chain reaction (PCR)).

Both of these sample pre-processing steps alter the physical and chemical properties of the target and can confound the diagnostic procedure. In addition, the additional steps required in tagging the ligand will complicate the assay, prolonging the lag time between the onset of symptoms and healthcare delivery.

In contrast, \textit{label-free in vitro} diagnostics have the advantage of being lower in cost and complexity compared to alternative labelled detection methods such as fluorescence in situ hybridization (FISH), immunoassay (IA), enzyme-linked immunosorbent assay (ELISA), Western Blot, or PCR [5]. One prominent \textit{label-free} detection method is refractive index (RI) based sensing, which uses changes in the local RI ($\Delta n$) that result from binding of target to immobilized capture molecules on the biosensor surface [6]. RI-based biosensors (aka \textit{optofluidic} biosensors) involve a number of technologies such as [7]:

1. Surface plasmon resonance (SPR)
In Section 1.2 the last biosensor type is detailed since the focus of this thesis is the functionalization of photonic crystal slab biosensors.

1.2 Refractive index based biosensing with photonic crystals

*Photonic crystals* are structures with periodically alternating dielectric constant ($\epsilon$) where the $\epsilon$ is modulated on a length scale similar to the operation wavelength. This results in the “crystal” structure, which is effectively a repeating pattern of dielectric constant ($\epsilon$) alternation. The periodicity of $\epsilon$ exhibited can be in 1-, 2-, or 3-dimensions as seen in Fig. 1.1 [8].

![Various dimensional periodicity in photonic crystals.](image)

This periodic index contrast serves to guide and modify the behavior of light, and desired light modulation can be achieved by altering the geometric and material properties of the structures. Some important parameters are:
• The contrast between the high-RI ($n_h$) and low-RI ($n_l$) describes the ratio of photons scattered from high-scattering and low-scattering regions of the dielectric, and is defined as $\delta = n_h/n_l$ [9].

• The unit cell parameters: slab thickness $t$ (Fig. 1.3), hole radius $r$ and lattice constant $a$ (Fig. 1.2).

![Figure 1.2: Illustration of a unit cell that makes up the square lattice of air holes in dielectric material. Important lattice parameters (lattice constant) $a$ and (hole radius) $r$ are shown on the figure.](image)

![Figure 1.3: Cross section of the photonic crystal in a 260 nm Si$_3$N$_4$ layer resting on a 2.35 \( \mu \)m SiO$_2$ layer atop a Si substrate.](image)

Previously, other groups [10] have demonstrated proof-of-concept biosensing with 1-D photonic crystals (aka distributed Bragg reflector). Despite being a simple architecture, these type of photonic...
crystals have the drawback of being sensitive to the polarization of the incoming light. The incoming waves cannot be completely stopped since the bandgaps for different directions of propagation exist at different frequencies. To completely stop light of a given frequency in all directions of propagation, 2- or 3-D modulation of $\epsilon$ is needed [11].

In contrast to 1-D photonic crystals, 2D photonic crystals can stop propagation of light in two dimensions at a given frequency. The increased symmetry of the periodic modulation also makes the device polarization insensitive, which in turn relaxes polarization constraints on the incoming light.

3D photonic crystals have been historically not investigated as much since their fabrication poses significant challenges. Nonetheless, they have been demonstrated and are able to fully block the wave propagation in all 3 directions [12].

Our devices fall under the 2D photonic crystal category. Specifically, the crystals are a square lattice (image (b) in Fig. 1.4) of air holes bored into $\text{Si}_3\text{N}_4$ substrate. By changing the geometric parameters of the lattice (lattice constant, hole radius and slab thickness) the location of the resonances can be tuned for the desired operation wavelength.

Fig. 1.4 shows different levels of the photonic crystal sensor. In Fig. 1.4 (a) shows the unit cell for the square lattice of photonic crystal, and the relevant geometrical tuning parameters. (b) shows an SEM image of the photonic crystal lattice for $r \sim 200\text{nm}$, $a \sim 980\text{nm}$, and (c) is the same photonic crystal lattice under different magnification to show the entire sensing surface (i.e. the photonic crystal mesa). The sensor integrated with a Y-junction type microfluidic channel bearing 4 mesas is illustrated in (d), and an actual picture of the finished device integrated with a single-channel microfluidic device is shown in (e). An example of a normal-incidence transmission plot is shown in (f) where light is coupled to both a TE-like and a TM-like fundamental resonance mode.

On a fundamental level, the photonic crystal sensor operation relies on the excitation of guided mode resonance (GMR) by coupling of free space radiation (FSR) into the dielectric slab guided modes. The coupling scheme can be explained by means of band diagrams, which demonstrate the
Figure 1.4: (a) Unit cell of our photonic crystal slab biosensors with geometric parameters of lattice constant $a$, hole radius $r$, and slab thickness $t$ shown in figure adapted from [13]. (b) Top-view SEM image of the crystal lattice showing air holes in Si$_3$N$_4$, 100 nm resolution. (c) Top-view SEM image of the full mesa, 10 µm resolution. (d) An illustration of the photonic crystal sensor integrated with a Y-junction microfluidic channel ready for measurement. (e) Picture of the finished sensor mounted on the measurement setup. (f) An exemplary transmission plot showing the two fundamental resonances [14].

range of FSR frequencies that can couple into the transverse guided modes. Fig. 1.5 shows the band diagram for a uniform, unpatterned dielectric slab as compared to a dielectric slab bearing a square lattice of air holes (with parameters $r=0.2a$, $a$, $t=0.5a$).

In Fig. 1.5 on the left-hand side, an unpatterned dielectric slab with $\epsilon = 12$ can be seen. The sloped line in all of the diagrams in Fig. 1.5 represents the light line with $\omega = ck$. Gray region above the light line represents the continuum of free space radiation modes and the lines represent the guided modes of the dielectric slab. Solid & dashed lines represent the doubly and singly degenerate guided modes respectively. The slab itself is described by a planar reduced zone representation as seen in the inset of the bottom left figure in Fig. 1.5. The $\Gamma$ point represents the center of the irreducible zone. At $\Gamma$, we see a 4-fold degeneracy in the solid dielectric slab (2 left pictures in Fig. 1.5).

We see 2 types of plots in Fig. 1.5 describing even modes and odd guided modes. The type of
a mode is characterized by the E-field profile with respect to the plane of the slab. The mode is categorized as an “even” mode when the E-field is confined strongly within the plane of the slab, and as an “odd” mode when the E-field is strongest in the direction normal to the slab. Since the polarization of the electric field is not purely along one direction or the other, the “even” and “odd” modes are similar to the transverse modes (transverse electric (TE) and transverse magnetic (TM)) of an optical resonator, but are referred to as TE-like and TM-like. Referring back to Fig. 1.4 (f), we see the spectral features of these two resonances: the TE-like resonances exhibit lower Q than TM-like resonances.

The continuous translational symmetry of the dielectric slab means that the FSR cannot be
coupled to the guided modes that exist within the dielectric. When this symmetry is reduced from continuous to discrete by the introduction of air holes (right hand side, Fig. 1.5), FSR can couple to to guided mode (GM) that exist above the light line. Reduction of symmetry also reduces the degeneracy. This can be seen by comparing the left and right even mode plots or left and right odd mode plots at Γ point. A clearer demonstration of the reduction in degeneracy is seen in Fig. 1.6 where the modes at the Γ point can be seen to split as the crystal hole size increases from \( r = 0 \) to \( r = 0.2a \).

![Figure 1.6: Reduction of degeneracy in the guided modes of a dielectric slab as a function of air hole radius [15].](image)

The band diagrams shown in Fig. 1.5 and Fig. 1.6 show the requirement of an alternating dielectric constant in coupling FSR to the guided modes in a dielectric to create the guided mode resonances. While the Si\(_3\)N\(_4\) layer bears the photonic crystals that can exhibit these GMR, the substrate layers underneath (Fig. 1.3) act as Fabry-Pérot etalons. Combined, the transmission spectrum exhibits resonances that appear as Fano lineshapes superimposed on a background of substrate-related Fabry-Pérot resonances. Here, Si\(_3\)N\(_4\) photonic crystal layer is responsible for the resonant Fano lineshapes while the substrate layers (both SiO\(_2\) and Si) introduce the Fabry-Pérot
A recent measurement technique known as the crossed-polarization measurement technique [16] helps remove the substrate-related Fabry-Pérot by taking advantage of the selective polarization rotation of the Fano resonances. Removal of the Fabry-Pérot in the transmission spectrum changes the resonance lineshape from Fano to Lorentzian by reducing the asymmetry of the resonance. This effect is described in Eq. 1.1 and Eq. 1.2. Eq. 1.1 describes the total transmission through a photonic crystal taking into account the resonances supported as well as the “background” Fabry-Pérot. The ratio of the resonant process and the “background” transmission process is described by the $q$ parameter in this equation. Also in Eq. 1.1 we see $\epsilon = \frac{\omega - \omega_0}{\Gamma/2}$ with $\omega_0$ as the resonance frequency and $\Gamma$ as the resonance linewidth. In this equation, $f_0$ the resonant process amplitude, and $C_0$ the background transmission amplitude. When the background process is inhibited (such as in the case of crossed-polarization measurement technique), we are in the limit of $q \to \infty$, and the expression for the resonances supported by the photonic crystals reduce to a Lorentzian lineshape (Eq. 1.2).

$$f(\omega) = f_0 \left[ 1 + \frac{(q + \epsilon)^2}{1 + \epsilon^2} \right] + C_0 \quad (1.1)$$

$$f(\omega) = f_0 \left[ \frac{1}{1 + \epsilon^2} \right] \quad (1.2)$$

### 1.2.1 Sensor performance metrics

In order to quantify sensor response and performance, it is important to define certain metrics related to sensor performance. These metrics are:

- The quality factor of the resonance: $Q = \frac{\Delta \lambda}{\lambda_0}$ where $\lambda_0$ is the resonant wavelength and $\Delta \lambda$ is the full-width at half-maximum (FWHM) (i.e. the resonant linewidth)

- Bulk Sensitivity: $S_B = \frac{\Delta \lambda \nu}{\Delta n \text{nm/RIU}}$ and
- Surface Sensitivity: \( S_S = \frac{\Delta \lambda_0 / \Delta n_{surf}}{\Delta t_{bio}} \) [RIU]

- Bulk Detection Limit: \( DL_B = \frac{k \lambda_0}{Q_{S_B}} \) [RIU]

- Surface Detection Limit: \( DL_S = \frac{k \lambda_0}{Q_{S_S}} \) [RIU/nm]

The first of these metrics, the quality factor is the metric that describes how under-damped a resonator is. A high Q improves resolution step size but also makes the light coupling more difficult. The resonance also becomes more sensitive to thermal or power fluctuations. The bulk sensitivity \( S_B \) is a metric of sensor response to a change in bulk RI of the fluid surrounding the PCS. Similarly, \( S_S \) describes the sensor response (\( \Delta \lambda \)) to a change in the surface RI, for a given thickness of biolayer (\( \Delta t_{bio} \)). The bulk detection limit \( DL_B \) [RIU] is the measure of the smallest change in bulk RI that can be detected reliably by the sensor. Finally, we have the surface detection limit \( DL_S \) [RIU/nm], which is the sensor response upon a given RI change in the layer of thickness \( \Delta t \) bound to the surface. Fig. 1.7 shows sensor response to a change in bulk RI change.

Figure 1.7: Left: resonance shift due to local RI change. Right: sensor response in terms of spectral shift due to change in the local RI (image adapted from [17]). The slope of the plot shows the sensitivity (in nm/RIU).

Both in bulk RI change and in the surface RI change are brought on by changes in the polarizability of the dielectric medium surrounding the confined resonance modes. Since the emphasis of this thesis is on surface binding, the effect of excess polarizability due to surface binding is described.

Surface binding changes the surface polarizability (\( \alpha \)) by altering the chemical composition of the dielectric surface, which induces a red-shift of the resonance frequency. Surface polarizability is
related to the local RI by:

$$\epsilon_r = n^2 = 1 + \frac{\sigma_p \alpha}{\epsilon_0}$$  \hspace{1cm} (1.3)$$

where $n$ is the local RI, $\epsilon_r$ is the relative dielectric constant of the medium, $\alpha$ is the polarizability of one biomolecule binding to the surface and $\sigma_p$ is the statistical distribution of biomolecules per unit surface area. $\epsilon_0$

If we consider an incremental shift in the dielectric constant due to biomolecular attachment to surface we get:

$$\Delta \epsilon_r = 2n \Delta n = \frac{\sigma_p \alpha_{ex}}{\epsilon_0}$$  \hspace{1cm} (1.4)$$

where in Eq. 1.4 $\alpha_{ex}$ is the excess surface polarizability due to biomolecular attachment, $\Delta \epsilon_r$ is the resultant change in the dielectric constant. In turn, $\Delta \epsilon_r$ causes a spectral shift in the resonant frequency ($\Delta \omega$) which we can describe using perturbation theory (as seen in [18] for surface perturbation and [8] for volume perturbation):

$$\epsilon \rightarrow \epsilon + \Delta \epsilon + ...$$

$$\omega \rightarrow \omega + \Delta \omega + ...$$  \hspace{1cm} (1.5)$$

Eq. 1.4 enables us to define, using the wave equation [8][19] and substituting Eq. 1.4:

$$\frac{\Delta \omega}{\omega} = - \frac{1}{2} \frac{\int \Delta \epsilon_e \left| E(r) \right|^2 dV}{\int \epsilon_r \left| E(r) \right|^2 dV} + O(\Delta \epsilon^2)$$  \hspace{1cm} (1.6)$$

$$\hspace{1cm} (1.7)$$

where in this equation $E, \omega, \epsilon$ are the unperturbed descriptions of the electric field, frequency, and the dielectric function. The terms that provide negligible contributions in the limit of $\Delta \epsilon << \epsilon$ are grouped into the higher order terms indicated by $O(\Delta \epsilon^2)$. If we take the dielectric function and the perturbation in the dielectric function to be constant throughout the perturbed region, we have Eq. 1.8:
\[
\frac{\Delta \omega}{\omega} \sim -\frac{\Delta n}{n} \cdot \frac{\int_{\text{liquid or bio}} \epsilon_r |E(r)|^2}{\int_{\text{total}} \epsilon_r |E(r)|^2}
\]

which describes the resonance shift in terms of the change in RI multiplied by what is known as the filling fraction of \( f \) – the fraction of electric field energy in the perturbed region (be it the liquid or the biolayer above the PCS).

### 1.3 Functionalization of PCS biosensors

Molecular recognition at the sensor surface is achieved by appropriate surface chemistry. To test our sensors we chose to carry out streptavidin immobilization and streptavidin-biotin binding assays. Basic immunoassay designs make use of the strong, specific interaction between streptavidin and biotin that is known to be the strongest non-covalent specific interaction known \( (K_d = 10^{-14} \text{ mol/L}) \). Traditionally, the antibody of interest is labelled with biotin and is immobilized onto the solid substrate via an antigen attached to the surface. A reporter-conjugated streptavidin then is allowed to react with the biotinylated antibodies. When the assay is developed or imaged, surface functionalization can be visualised [20].

Borrowing from this idea, a functionalization scheme that is amenable to visualisation as well as label-free biosensing can be developed for the \( \text{Si}_3\text{N}_4 \) photonic crystal slab (PCS) substrate at hand. For this purpose, either streptavidin or biotin can be immobilized onto the substrate, and reporter-tagged biotin or streptavidin can be used to visualize the presence of target on the surface. On the other hand, it is also possible to perform unlabelled capture experiments using the same concept and reagents that lack the reporter functionality.

The surface to be functionalized in the experiments to follow is an LPCVD \( \text{Si}_3\text{N}_4 \) surface. Surface concentration of various functional groups are as shown in Fig. 1.8 for 1500 Å of PECVD and LPCVD \( \text{Si}_3\text{N}_4 \) [21].

Of note in Fig. 1.8 is the fact that the surface lacks any kind of hydroxyl (-OH) functionality that is typically used in achieving surface immobilization on Si-based devices [22]. This is significant
because the most commonly used techniques in functionalizing SiN\textsubscript{x} surfaces are the same recipes used for functionalizing SiO\textsubscript{x} or other hydroxylated surfaces (Al\textsubscript{2}O\textsubscript{3}) and relies on the presence of surface hydroxyl groups (-OH) \cite{23}. In fact, to optimize surface functionalization, a thin SiO\textsubscript{x} layer was deposited using PECVD on the sensor surfaces (described in Sec. 2.2). The common hydroxy-group based functionalization techniques are listed in Fig. 1.9 in a table adapted from \cite{22}.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Method</th>
<th>Type of Binding</th>
<th>Nature of Film</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO\textsubscript{x}</td>
<td>Silanization</td>
<td>Covalent</td>
<td>Monolayer</td>
</tr>
<tr>
<td>SiO\textsubscript{x}</td>
<td>Esterification</td>
<td>Covalent</td>
<td>Monolayer</td>
</tr>
<tr>
<td>SiO\textsubscript{x}</td>
<td>Phosphorization</td>
<td>Covalent</td>
<td>Monolayer</td>
</tr>
<tr>
<td>SiO\textsubscript{x}</td>
<td>Layer-by-Layer</td>
<td>Electrostatic</td>
<td>Multilayers</td>
</tr>
<tr>
<td>SiO\textsubscript{x}</td>
<td>Lipid membrane</td>
<td>Electrostatic</td>
<td>Bilayer</td>
</tr>
<tr>
<td>Si-H</td>
<td>Hydrosilylation</td>
<td>Covalent</td>
<td>Monolayer</td>
</tr>
</tbody>
</table>

Figure 1.8: “Elemental concentration of 1500 Å of silicon nitride films deposited using PECVD, LPCVD, and HDP CVD methods obtained using rutherford backscattering (RBS) and hydrogen forward scattering spectrometry (HFS).” \cite{21}

In the following sections, some details on the most common functionalization techniques for -OH bearing surfaces can be found.

### 1.3.1 Silanization

The most commonly used technique in surface modification entails rendering amine group functionality to the hydroxylated substrate using a self-assembling organosilane. Based on an in-solution or gas phase deposition method, alkoxyilanes (e.g. 3-aminopropyl trimethoxysilane (3-APTMS)) can
be deposited to form Langmuir-Blodgett (LB) films. These films are monolayer films that are formed at a liquid-liquid interface (or a gas-liquid interface) which are deposited onto a solid substrate.

This process is illustrated in Fig. 1.10 where a cleaned, activated \( \text{SiO}_x \) surface is silanized [22].

![Image of silanization process](image)

Figure 1.10: Stepwise silanization of hydroxylated silicon nanowire (SiNW) surfaces bearing native \( \text{SiO}_x \) of 1.5-10nm thickness. Step 1: Cleaning and activating of the surface hydroxyl (-OH) groups. Step 2: silanization with 3-(aminopropyl)trimethoxysilane. Step 3: Further functionalization with homobifunctional crosslinker glutaraldehyde. Image from [22].

Organosilanes are thought to bond to hydroxylated surfaces by attacking the surface -OH groups, which displaces the alkyl groups of the alkoxysilanes, as seen in Fig. 1.10. The functionality achieved after the silanization and heating step is a primary amine (-NH\(_2\)). Following that, in Step 3 the primary amine can be further reacted with the homobifunctional crosslinker glutaraldehyde to confer aldehyde functionality to the substrate. Aldehyde-1° amine reaction yields a labile intermediary (a \textit{Schiff base}) which need to be reduced in the presence of a reducing agent such as NaBH\(_3\)CN (sodium cyanoborohydride). The reduction will allow for the formation of a stable 2° or 3° amine, which
will help organosilane-glutaraldehyde crosslinking as well as the immobilization of 1º amine (-NH₂) bearing proteins onto the surface via glutaraldehyde [20].

As mentioned before, silanization can be carried out in vapor phase or in solution, under anhydrous or hydrolytic organic carrier conditions. As can be expected, the choice of solvent carrier and the presence of water in the organic solvent alters the deposition properties and the layers formed. In Chapter 4, these liquid phase based deposition techniques are detailed and their differences are further explained.

Fig. 1.11 shows the common silanes used in surface silanization prior to capture agent immobilization.

Figure 1.11: Common silanes: 3-aminopropyldimethylethoxysilane (APDMES), 3-aminopropyltriethoxysilane (APTES), 3-aminopropyltrimethoxysilane (APTMS), propyldimethylmethoxysilane (PDMMS), N-(6-aminohexyl)aminomethyltriethoxysilane (AHAMTES). Image from [24].

1.3.2 Other methods

As seen from Fig. 1.9, there are many alternatives to surface functionalization apart from the most common silanization. Some of these methods as listed in [22] are listed and explained below.

- Esterification
- Phosphorization
Layer-by-Layer

Lipid bilayer

The first of these techniques, esterification, is a covalent linkage that relies on the transesterification (Fig. 1.12) on the surface where the surface acts as a primary alcohol despite being a hydroxylated silicon surface. Various ester-based surface modification techniques are available and are prevalent in the functionalization of plasmonic surfaces (e.g. gold). In the case of metallic surfaces, however, a typical chemistry is to use Biotin N-hydroxysuccinimide ester (Biotin-NHS) that will react with a surface primary amine [25]. This is in contrast to the ester chemistry applicable to silica surfaces for streptavidin-biotin assays, where the ester-linked biotin (such as biotin 4-nitrophenyl ester reportedly used by [26]) reacts with surface hydroxyl groups in the presence of a catalyst. Biotinylated surface can be then used to capture streptavidin or other avidin derivatives from solution specifically. However, hydrolytic stability of the siloxane-ester (Si-O-O-R) bonds is thought to be lower than those of siloxane (Si-O-Si) bonds conferred by silanization-type surface functionalization (described in Sec. 1.3.1).

![Figure 1.12: Illustration for the transesterification process, where R’OH demonstrates the surface hydroxyl group functionality acting as the primary “alcohol” equivalent, with R’ = Si instead of an organic side chain. R”OOR is the biotinylated ester with R” = 4-nitrophenyl and R = biotin in the functionalization outlined in [26]. Image from [27].](image)

Second covalent technique that can be an alternative to the typical silanization-based functionalization is phosphorization, which converts hydroxy functionality to maleimido functionality using multiple bifunctional linkers. Maleimido functionality is suitable to immobilize thiol-containing biomolecules such as DNA oligonucleotides or proteins. An example cites the use of
11-hydroxyundecylphosphonate as a self-assembling monolayer (SAM) over a Si nanowire surface bearing native SiOx. The surface is later functionalized with 3-maleimidopropionicacid-NHS and oligonucleotides were then immobilized on the surface [28]. In general, organophosphonates are thought to be highly stable SAMs and monolayer growth is thought to be independent of the surface (-OH) group functionalization [22], which relaxes the constraints on the surface SiOₓ type, uniformity, and thickness. However, some problems might arise in this functionalization scheme such as steric hindrance (see Linker 1 in Fig. 1.13).

Figure 1.13: Phosphorization of a native SiOₓ surface. A SAM of 11-hydroxyundecylphosphonate covers the surface, and 3-maleimidopropionic-acid-N-hydroxysuccinimide is the heterobifunctional cross-linker. Following attachment via the maleimido moiety, single stranded PNA is hybridized with a DNA strand.[28].

The other two techniques, layer-by-layer (LbL) deposition involves using the net negative surface charge of SiOₓ under acidic conditions to deposit alternating layers of positively and negatively charged polyelectrolyte multilayers. This deposition technique draws from the electrostatic interaction between the alternating charged layers. The specific deposition methodology can be based on spin-coating, dip-coating, spray coating, etc [22]. This technique is useful in determining the surface sensitivity of devices by depositing controlled thicknesses of a known material. However, in
the context of protein immobilization onto surfaces for biosensing, LbL is not commonly employed.

![Image: Biotinylated lipid bilayer capturing streptavidin, image from [29].](image)

**Figure 1.14:** Biotinylated lipid bilayer capturing streptavidin, image from [29].

Last of these techniques is the *lipid bilayer* method which functionalizes the surface with a bilayer similar to those found in the cell membrane. Such a layer covers the surface with hydrophobic head groups exposed to the liquid and the hydrophilic tails buried in the layer, as seen in Fig. 1.14. Lipid bilayers provide a more dynamic environment and can host a range of proteins ([30]). In addition, this technique can potentially be useful in minimizing non-specific molecular adsorption to the surface. Biotinylated lipid bilayers have been used in streptavidin binding assays [29]. However, this technique has added complications due to the dynamic nature of the lipid bilayer since the layer fluidity will bring about changes in the local RI that would need to be considered. In addition, the non-covalent attachment of the bilayer to the surface can cause issues in the hydrolytical stability of the sensor surface chemistry, which is highly important to reliable sensor performance.

The overall disadvantage of siloxane is that the polymer can react with an oxide surface at either amine or siloxane terminus ([31]) and that the Si-O-Si siloxane bonds are less hydrolytically stable than phosphonate bonds. At physiological pH range, siloxane bonds can easily hydrolyse. On the other hand, siloxane-based functionalities have plenty of literature available for protein immobilization onto a surface using this methodology. In order to improve the chances of the proof-of-principle experiments, the most commonly used techniques based on silanization and formation of siloxane bonds followed by the use of glutaraldehyde as a cross-linker were chosen for the surface functionalization.
Chapter 2

Fabrication of PCS devices

2.1 PCS fabrication

Figure 2.1: PCS sensor fabrication steps. Figure adapted from [13]

Fig. 2.1 illustrates the major steps in sensor microfabrication. These important steps are, in
<table>
<thead>
<tr>
<th>Fabrication Step</th>
<th>Facility</th>
<th>Equipment</th>
<th>Processing by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deposition (thermal SiO$_x$)</td>
<td>SNF</td>
<td>Tylan oxidation furnace</td>
<td>OL</td>
</tr>
<tr>
<td>Deposition (LPCVD Si$_3$N$_4$)</td>
<td>SNF</td>
<td>Tylan LPCVD</td>
<td>OL</td>
</tr>
<tr>
<td>Deposition (PECVD SiO$_x$)</td>
<td>ECTI</td>
<td>Oxford Plasmalab PECVD</td>
<td>DA</td>
</tr>
<tr>
<td>Resist spinning</td>
<td>ECTI</td>
<td>Laurell Spin Coater</td>
<td>DA</td>
</tr>
<tr>
<td>EBL</td>
<td>ECTI</td>
<td>Vistec EBPG5000+</td>
<td>DA</td>
</tr>
<tr>
<td>Development</td>
<td>ECTI</td>
<td>ECTI Wallberg Cleanroom Wetbench</td>
<td>DA</td>
</tr>
<tr>
<td>Pattern transfer (RIE)</td>
<td>SNF</td>
<td>AMT Etcher Tool</td>
<td>OL</td>
</tr>
<tr>
<td>Pattern transfer ($O_2$ Ashing)</td>
<td>SNF</td>
<td>Drytek 4</td>
<td>OL</td>
</tr>
<tr>
<td>ARC</td>
<td>ECTI</td>
<td>Oxford Plasmalab PECVD</td>
<td>RS</td>
</tr>
</tbody>
</table>

Acronyms used: Stanford Nanofabrication Facility (SNF); Deniz Aydin (DA); Ryan Schilling (RS); Costa Nicholaou (CN); Hooman Akhavan (HA); Ofer Levi (OL); and Emerging Communications Technology Institute (ECTI). Typically there was collaboration between myself and other group members (RS, CN, HA) during fabrication runs, but each would process their own samples.

Sequential order:

1. Material growth/deposition

2. Spin-coating

3. Electron beam lithography (EBL)

4. Pattern development and transfer
   
   (a) Resist development
   
   (b) High-fidelity pattern transfer to Si$_3$N$_4$ using reactive ion etching (RIE)

5. Resist strip
   
   (a) Final device cross section

2.1.1 Materials for PCS

Material growth/deposition refers to the stages of substrate preparation, and has been carried out by Dr. Ofer Levi at Stanford Nanofabrication Facility (http://snf.stanford.edu/). PCS used in this study are fabricated in approximately 260nm-thick Si$_3$N$_4$ ($n = 2 \sim 1550$ nm) deposited using the low pressure plasma enhanced vapour deposition (LPCVD) technique onto 2.35µm-thick thermally-grown SiO$_2$ on 330µm-thick Si wafers with crystal plane orientation of ⟨100⟩ (double-sided (DS)).
The individual samples are written on 15×15 mm diced sections out of the previously described Si₃N₄-SiO₂-Si (DS) substrate. As discussed in Sec. 1.2, there is a requirement of a periodic index contrast for the dielectric structure guided modes to couple to FSR and create a “photonic crystal”. In our 2-D photonic crystals, the periodically modulated layer is the topside Si₃N₄ layer. Index contrast between the high- and low-index areas of the photonic crystal \( \delta = n_h/n_l \) determines the ratio of photons scattered from the two different media, and ultimately determines the shape of the Fano resonance (described in Sec. 1.2, Eq. 1.1 and 1.2).

In the transmission spectrum for the photonic crystal, the Fano resonance due to the photonic crystal is overlayed with Fabry-Pérot noise from the Si substrate. This substrate-related noise can significantly impair measurements if no precautions are taken. A way to reduce this noise is to use an anti-reflection coating (ARC). ARC is a layer deposited on the backside of the photonic crystal, on the unpatterned SiN layer. The recipe is optimized by Ryan Schilling, and the details of the improved recipe and the detailed explanations can be found in his thesis available through T-Space [32].

The basic principle behind this coat is based on the destructive interference of waves reflected from the top surface boundary of a material and those reflected from the bottom surface boundary (see Fig. 2.2).

As seen in Fig. 2.2, there will be destructive interference established if the waves reflected from the top surface of the optical thin film (R1) are 180° out of phase with the waves reflected from the bottom surface (R2). We can see that this condition will hold if the path travelled by R2 waves \( 2 \times t \) delays them by \( \lambda_n/2 \) with respect to R1 waves, that is, \( t = \lambda_n/4 \) where \( \lambda_n \) is the wavelength of the travelling wave inside the film (i.e. \( \lambda_0/n \) with \( \lambda_0 \) being the wavelength in the external medium). According to this criterion, a simple estimate yields \( \sim 190 \) nm SiN as an ARC to reduce the substrate-related Fabry-Pérot noise at \( \lambda_0 = 1550 \) nm.

The photonic crystal patterned in Si₃N₄ is supported by a 2.35 µm-thick SiO₂ layer (\( n = 1.46 \sim 1550 \) nm) that serves to reduce the Si substrate waveguiding (\( n = 3.478 \sim 1550 \) nm) by lowering
the light confinement via index-matching. Since the refractive index (RI) of the SiO$_2$ is closer to that of Si, the confinement in the substrate is reduced.

2.1.2 Pattern definition with EBL

As seen in Fig. 2.1, Steps 2 - 4.a. illustrate the fabrication steps that enable the writing and transfer of a pattern to the substrate. The process begins with Step 2., where the substrate made of the appropriate materials described in the previous section is spin-coated with $\sim380\text{nm}$ ZEP 520A (by the Nippon Zeon Corporation http://www.nanolithography.gatech.edu/ZEP520_literature_3.pdf). The devices coated with the photoresist are then patterned with the EBL as seen in Step 3 using Vistec EBPG5000+ electron beam writer in the ECTI Electron Beam Nanolithography Facility at the University of Toronto (http://www.ecti.utoronto.ca/facilities/ebeam.htm). The writing dose was 240$\mu\text{C/cm}^2$ and the beam stepping resolution 5 nm. This resolution is suitable for the 320$\mu\text{m}$-wide field-of-view (FOV) used in the self-referenced sensor designs. The stepping resolution decreases with increasing FOV because of the limitations on how widely the electron beam can be deflected.

In our experience, the feature radius in the design files and the final product have a slight discrepancy when the pattern is written with this dose of 240$\mu\text{C/cm}^2$, where the original design
radius is expanded about 10nm in the final devices. The main advantage of using this is to ensure that the patterns are clearly defined through the photoresist. Alternatively, if the pattern radius is critical, it is possible to use a smaller dose of $200\mu C/cm^2$, with the potential trade-off from thorough pattern definition in the resist layer.

Following writing, the exposed parts of the resist are removed using a developer solution. This creates the desired pattern in the photoresist layer as seen in Fig. 2.1 Step 4.a.

### 2.1.3 Pattern transfer

As seen in Fig. 2.1, Step 4.b. shows the pattern in photoresist transferred to the underlying Si$_3$N$_4$ layer through RIE. RIE is an anisotropic etching technique that allows for the transfer of the pattern to the Si$_3$N$_4$ layer.

After the pattern has been replicated into Si$_3$N$_4$, the final step involves the stripping of the resist using 20 min of O$_2$ plasma in an RIE. Both the RIE and the resist stripping have been done by Dr. Ofer Levi at Stanford Nanofabrication Facility.

### 2.2 Thin SiO$_x$ surface layer

Initial functionalization experiments have been carried out on the PCS that were fabricated in Si$_3$N$_4$ as has been done in our group previously [13]. Confirmation of surface functionalization was carried out using florescently tagged Streptavidin (http://products.invitrogen.com/ivgn/product/S32358) or Biotin (http://www.sigmaaldrich.com/catalog/product/sigma/55819?lang=en&region=CA). Initial results on the Si$_3$N$_4$ surface were highly inconsistent and even the positive results were non-uniform. Fig. 2.3 shows fluorescent results on SiN surface.

Based on these inconsistent results, it was deemed necessary to utilize a surface that is closer to the widely used surface in dielectric sensor functionalization. For instance, oxide-bearing surfaces with activated -OH groups (i.e. hydroxylated surfaces) are very common in Si-based device functionalization owing to the presence of numerous functionalization schemes [34][28][31][22][30][29]. It has been noted that the surface -OH group coverage is of pivotal importance in these schemes, and
Figure 2.3: Atto-680 biotin functionalized PCS dummies. Samples are $15 \times 15 \text{ mm} \ Si_3N_4$ ($260 \text{ nm}$) on $SiO_x$ ($2.35 \mu m$) on (330 $\mu m$) $\langle 100 \rangle$ Si.

their concentration is typically attempted to be maximized prior to functionalization through various oxidative cleaning processes such as RCA$_1$ clean [34]. To this end, coating of the SiN PCS with SiOx was proposed to increase the overall silanol (SiOH) functionality on the surface and present a
solution to the inconsistent functionalization problem.

The method chosen to deposit SiOx on our sensors was plasma enhanced chemical vapor deposition (PECVD).

The starting point was to deposit a ∼10nm layer of SiOx to see improvements on the fluorescent functionalization. To this end, a recipe for ultra-low-rate SiOx deposition was developed based on an existing ultra-low-rate deposition recipe from the Oxford Plasmalab PECVD. Details of the recipe can be found in Appendix A.3.

Figure 2.4: Cross section of a unit cell from the PCS sensors used. Left: sensors after fabrication, right: sensors after PECVD SiOx deposition.

Given the improvement in fluorescence results seen in Fig. 2.5 with the SiOx surface as compared to the results in Fig. 2.3 with the SiN surface, there is likely a causal correlation between the yield of the recipe used in functionalization and the concentration of surface -OH groups. To pursue this idea further, the effect of SiOx thickness on sensor performance was investigated in Section 2.3 in attempts to determine a suitable oxide coat thickness for optimal sensor performance. In addition, a range of PECVD SiOx thicknesses from 22nm-110nm have been tested for fluorescence with Atto 680 biotin to visualize functionalization in Fig. 2.6 and Fig. 2.7.

Fig. 2.6 and Fig. 2.7 illustrate thickness-dependent fluorescence results for functionalization. Thicker oxides appear to be correlated with stronger and more uniform fluorescence signals. This illustrates the likely causal link between surface silanol (SiOH) coverage of substrates and the yield of functionalization recipe and paves the way for top layer SiOx thickness optimization in Sec. 2.3.
Figure 2.5: Atto-680 biotin functionalized PCS dummies. Samples are: 2.5a 15×15 mm 22nm PECVD SiO\textsubscript{x} and 2.5b 170 nm of thermal SiO\textsubscript{2} both on Si\textsubscript{3}N\textsubscript{4} (260nm-thick) on SiO\textsubscript{x} (2.35\textmu m-thick) on (330\textmu m thick) ⟨100⟩ Si. Left images represent the appropriately filtered imaged.

2.3 Optimization of SiO\textsubscript{x} layer thickness

The effect of thin-SiO\textsubscript{x} coat on top of the fabricated PCS structures has been explored in this section. Change in important sensor performance metrics has been monitored in Fig. 2.8 and 2.9 for r=110nm, a=980nm design. The trend in sensor performance is practically identical for a different design (r=90nm, a=980nm), as shown in Fig. 2.10 and 2.11.

Fig. 2.8 depicts the percentage change in the resonance quality factor and the sensitivity for the r=110nm, a=980nm design. Fig. 2.8a depicts the percentage change in the Q for varying thin oxide thicknesses, starting from an initial Q of 2371 and increasing asymptotically for increasing SiO\textsubscript{x} values. Fig. 2.8b depicts the percentage change in the bulk and surface sensitivity S\textsubscript{B} and S\textsubscript{S}
Figure 2.6: Atto-680 biotin functionalized SiO$_x$ PCS dummies. Samples are 15×15 mm 22-66nm SiO$_x$ on Si$_3$N$_4$ (260nm) on SiO$_2$ (2.35μm) on (330μm) ⟨100⟩ Si.

for varying thin oxide thicknesses, starting from $S_B=162$ nm/RIU and $S_S=5.75$ nm/RIU / 9nm biolayer, and decreasing with increasing oxide thickness to $S_B=118$ nm/RIU and $S_S=4.48$ nm/RIU / 9nm at 40nm SiO$_x$. The decrease in bulk sensitivity is sharper than the decrease in surface sensitivity: a phenomenon that is quite advantageous for the surface biosensing application of these PCS. Like in many sensor devices, there is always a need to distinguish between RI changes brought on by surface binding events as compared to those brought on by bulk RI fluctuations (possibly caused by large fly-by molecules). As can be seen, above $\sim 30$nm would be a good choice of oxide
thickness to harness this effect of differential decrease in sensitivity. More importantly, however, Fig. 2.9 illustrates the change in overall sensor detection limit (DL) which demonstrates that the two competing effects for the increase in Q and decrease in S compensate for each other almost completely for below ∼40nm of SiO$_x$. This compensation is clearly visible in Fig. 2.9b for the surface DL, since the DL does not change from its original value of 7.32×10$^{-5}$ [RIU] by more than 1.6% up until 40nm of SiO$_x$ thickness for a biolayer of 9nm thickness. For the bulk DL, the compensatory effect is less visible (Fig. 2.9a) since the drop in bulk sensitivity is sharper (Fig. 2.8b), which corrupts the sensor performance more rapidly. Taking these into consideration, a range of oxide thicknesses up to approximately 40nm is shown to bring about a minimal corruption to the surface detection of the sensor and the thicker end of this 0-40 nm window can be chosen as an appropriate thickness to deposit. Depending on the molecules detected and the sensitivity required, thicker oxides can be deposited given that the reduced sensor DL will not be an issue in the detection. The initial values for bulk and surface DL are $DL_B= 2.56×10^{-6}$ and $DL_S=7.32×10^{-5}$ [RIU] (for 9nm biolayer) and they only change to $DL_B= 2.87×10^{-6}$ and $DL_S=7.44×10^{-5}$ [RIU] with 40 nm of oxide (12% and 1.6 changes respectively). For comparison, these values change to $DL_B= 3.64×10^{-6}$ and
To demonstrate the sensor performance for a different PCS design, another PCS was simulated. Fig. 2.10 depicts the percentage change in the resonance quality factor and the sensitivity for the \( r=90\text{nm}, a=980\text{nm} \) design. Fig. 2.10a depicts the percentage change in the \( Q \) for varying thin oxide thicknesses, starting from an initial \( Q \) of 5017 and increasing asymptotically for increasing \( \text{SiO}_x \) values to reach 7235 for 80nm of oxide. Fig. 2.10b depicts the percentage change in the bulk and surface sensitivity \( S_B \) and \( S_S \) for varying thin oxide thicknesses, starting from \( S_B=147 \text{ nm/RIU} \) and \( S_S=5.95 \text{ nm/RIU} /9\text{nm biolayer at } t_{ox}=0\text{nm, and decreasing with increasing oxide thickness to } S_B=74 \text{ nm/RIU and } S_S=3.59 \text{ nm/RIU}/9\text{nm at } t_{ox}=80\text{nm. Same large drop in } S_B \text{ is observed, compared to a much slower decrease in the } S_S \text{ values. Once again we see that above } \sim 30\text{nm of } \text{SiO}_x \text{ could help use the decreased bulk sensitivity effects.} \) Fig. 2.11 illustrates the change in overall sensor detection limit (DL) which demonstrates that the two competing effects for the increase in Q and decrease in S compensate for each other. This compensation is clearly visible in Fig. 2.11b for the surface DL for a regime of oxide thickness below \( \sim 50\text{nm.} \) In the 0-50nm \( \text{SiO}_x \) thickness range, the variation in the surface DL is \( 3.35\times10^{-5} \text{ to } 3.29\times10^{-5} \text{ [RIU], which yields a difference no more than } 1.8\%. \) The DL decreases to \( 3.85\times10^{-5} \text{ [RIU]} \) (\( \sim 15\% \) change) at 80nm of \( \text{SiO}_x \). Once again, the bulk DL corruptions more rapidly (as seen in Fig. 2.11a), from \( 1.36\times10^{-6} \text{ [RIU]} \) 0nm \( \text{SiO}_x \) to \( 1.47\times10^{-6} \text{ [RIU]} \) (8\% change) 40nm \( \text{SiO}_x \) and finally to \( 1.85\times10^{-6} \text{ [RIU]} \) at 80nm of \( \text{SiO}_x \) (total of 36\% change). Accordingly, approximately 40nm of \( \text{SiO}_x \) is a good choice for optimal surface detection, since it is thick enough to provide a good surface -OH group coverage, but is also thin enough to not corrupt sensor surface detection abilities. Once again, like in the previous sensor design, it is possible to deposit thicker layers if the DL range is acceptable for the analyte to be detected, given that there are no fabrication issues.
Figure 2.8: Percentage change in resonance Q and both bulk & surface sensitivity for the r=110nm, a=980nm design.
Figure 2.9: Change in both the bulk and the surface DL for the r=110nm, a=980nm design.
Figure 2.10: Percentage change in resonance Q and both bulk & surface sensitivity for the r=90nm, a=980nm design.
Figure 2.11: Change in both the bulk and the surface DL for the r=90nm, a=980nm design.
2.4 Discussion

Overall SiO$_x$ layer has been chosen as an optically-transparent, hydroxylated layer to coat the SiN PCS given its chemical compatibility with the well-known functionalization schemes. Section 2.3 provides a discussion of suitable thicknesses for this top SiO$_x$ layer given the effect of the SiO$_x$ thickness on important sensor metrics such as Q, S, and DL. Accordingly, a range of thicknesses between 20-60 nm of SiO$_x$ can be chosen for r=110nm, a 980nm design given the stable surface DL for the SiO$_x$ thicknesses in this range. For the initial proof-of-concept experiments, it might be more suitable to choose a thickness that is on the high end of this range, given the importance of good -OH group coverage on the functionalization yield (Fig. 2.6 and 2.7).

An important consideration in deciding on the oxide thickness is the anticipated shift due to biomolecules of interest. The required sensitivity for detecting streptavidin can be estimated from literature values. Scullion et al. [35] have detected as low as 60pg/mm$^2$ surface coverage with avidin as analyte. The sensor metric corresponding to this DL are Q = 3875 and $\Delta \lambda$=0.4nm for $\Delta n \sim 5 \times 10^{-2}$ [RIU], yielding $S_s = 8$nm/RIU and DL=$3.22 \times 10^{-5}$ [RIU] for avidin (corresponding biolayer thickness is close to 5-6nm). Our estimates for anticipated surface coverage is on the order of 1-10 ng/mm$^2$ (see Section 5.2.2), and the r=110nm surface DL= 7.32 - 8.5$\times 10^{-5}$ [RIU] for 0-80nm SiO$_x$ and 9nm biolayer. Since the surface coverage we are trying to detect is over 70x that cited, even the thickest SiO$_x$ coverage should still leave the sensor capable of performing the detection for streptavidin. For r=90nm designs, the surface DL= 3.35 - 3.85$\times 10^{-5}$ [RIU] for 0-80nm SiO$_x$ with a 9nm biolayer, and the full range of oxide thicknesses are therefore acceptable for the detection experiments as well.

Overall, estimates for streptavidin wavelength shift for our sensors used in Section 5.3 have Q$\sim$ 210-6600 (theoretical) and $S_B \sim 5.3$-10.7 nm/RIU for a 7nm biolayer thickness ($\sim 1$nm each 3-APTMS and glutaraldehyde; 5nm streptavidin and/or BSA) are expected to be in the range of 220-590 pm (Fig. 5.7, 5.8, 5.9), which are close to the value of 400 pm cited by Scullion et al. [35]
and others.
Chapter 3

Microfluidics: Fabrication and reuse

Microfluidic channels as seen in Fig. 3.1 are used to contain fluid volumes above the photonic crystal sensor structures, creating an optofluidic device. In this section is described the design and fabrication of microfluidic channels using replica molding (REM) soft lithography technique [36]. Section 3.1 describes the process of fabricating the molds required to make the required channel features in poly(dimethly) siloxane (PDMS), whereas Section 3.2 describes the development of a clamping system that is used to reversibly attach the fabricated channels over the sensor surface. The reversibility aspect is of particular interest since most PDMS-substrate attachment covered in the literature relies on permanent bonding of the two using O$_2$ plasma [25]. This creates a significant hurdle in the reusability of devices since the refurbishment of surface chemistry might require strong reagents (e.g. strong bases like %50 KOH) to which the PDMS channels are reactive. Therefore, clean removal or reversible attachment of the microfluidic channels onto the sensor surface is a challenge to be overcome.

3.1 Fabrication of Microfluidic Channels

3.1.1 Fabrication of SU-8 Masters for Replica Molding

The typical microfluidic channel is made of PDMS, which is purchased as a two-part elastomer kit comprised of PDMS prepolymer and a curing agent (10:1) (SYLGARD ® 184 Silicone Elastomer
Kit from Dow Corning). Before channels can be made, the “negatives” of the channel features need to be defined on a master. PDMS prepolymer is then mixed with the curing agent, and the channel is made in PDMS using the master as a mold in a heated environment that will cure the PDMS. The process of making PDMS patterns using a master is called replica molding REM [36]. In this chapter, fabrication of the master is explained prior to the fabrication of microfluidic channels.

In photography, “negative” of an image simply means an image where the colors are completely inverted to their complementary counterparts. Similarly, in REM, the negative of the pattern that is to be transferred to PDMS is created on a substrate using lithography. This negative relief pattern can be written via photolithography (on a photoresist), or via RIE on a solid substrate such as silicon [36]. Here, the negative relief pattern refers to a pattern that is exactly complementary to that on the final PDMS device. To create this pattern, we chose photolithography using custom designed mask (Fig. 3.2) and used Si wafers or glass slides for substrates. The photoresist chosen was SU-8 2050 [37]. Exact recipes are shown in Appendix A.1. The overall steps in defining patterns on a negative photoresist such as SU-8 is as follows [37]:

Figure 3.1: A microfluidic channel with a 375 µm tall T-junction feature mounted on a 15mm×15mm bare Si/SiOx surface. Stainless steel ferrules (from New England Small Tubing http://www.nesmalltube.com/: Type 1 - NE-1300-01, 0.025 OD x 0.017 ID, 0.500” length, s/s tube, type 304, cut, deburred, passivated) are used as fluidic ports andTygon tubing (from Cole-Parmer www.coleparmer.ca: DD-06418-02 Tygon tubing, 0.020” x 0.060”OD) is used to introduce fluids to the sensor surface.
1. Cleaning and drying of the substrates

2. Dehydrating Si substrates on a hotplate at 200°C

3. Spin coating ~ 4ml of SU-8 2050

4. Soft baking at 65°C and 95°C, followed by room temperature relaxation to reduce thermal stress on the substrate and the film

5. Exposure (using mask in Fig. 3.2) using a mask aligner

6. Post-exposure bake

7. Development of the resist, IPA rinse, drying

After these steps, the Si master is ready for casting PDMS channels. The finished Si master can be seen in Fig. 3.5.

Figure 3.2: 9 microfluidic channel designs printed on acetate to be used as a mask for SU-8 photolithography.
Mask used in exposing the SU-8 after spin-coating is the design seen in Fig. 3.2 printed on a blank acetate transparency with 20,000dpi resolution. The higher resolution alternative to this is a chrome-coated glass masks. However, these masks are considerably more expensive to obtain (commercial range $\sim 500-1000$, Advanced Reproductions, \url{http://www.advancerepro.com/}) compared to high-resolution printed transparencies (range $\sim 50-100$ for 5k, 10k or 20k DPI, CAD Art Services, \url{http://www.outputcity.com/index.htm}).

In casting microfluidic channels, it is important to ensure that the ratio of the channel height to the maximum channel width is greater than 1:10. If a channel is wider than 10x the height, it becomes liable to channel collapse [38]. In the mask design shown in Fig. 3.2, the largest feature is 5 mm wide (channel a), which would need to be at least 500$\mu$m tall by this design principle. However, this single channel is not used in experiments concerned with this thesis, and since the rest of the features are maximally 1.5 mm wide, the masters fabricated have SU-8 features that are at least 150$\mu$m tall. Three masters were fabricated for the purposes of testing. The most reliable masters were those with features $\sim 300$ and 370$\mu$m tall, with the 150$\mu$m tall master failing after the first PDMS molding. For fabricating such tall features, SU-8 spin coating and soft baking steps need to be repeated multiple times.

Based on Fig. 3.3, for 300$\mu$m thick SU-8, 421 mJ/cm$^2$ and for 370$\mu$m thick SU-8 490 mJ/cm$^2$ can be used. Soft bake at 65°C appears to require about 5 mins past $\sim 115$µm of thickness, and soft bake at 95°C can be roughly modeled as a linear trend over thicknesses. Extrapolating this trend like in determining dose, we obtain 20 and 23 mins of baking for 300 and 370$\mu$m thick SU-8 layers. These values are tabulated in Table 3.1, along with the number of spin cycles required to create layers of the given thicknesses.

Finally, the finished master is shown in Fig. 3.5.
Figure 3.3: Dose trend for SU-8 2050 used in extrapolating the approximate amount of dose required for SU-8 that is 300 and 370µm thick.

<table>
<thead>
<tr>
<th>SU-8 thickness</th>
<th>No. of spins</th>
<th>Dose [mJ/cm²]</th>
<th>Soft bake 65°C [min]</th>
<th>Soft bake 95°C [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>300µm</td>
<td>4</td>
<td>421</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>370µm</td>
<td>5</td>
<td>490</td>
<td>5</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 3.1: SU-8 thickness and relevant fabrication parameters.

3.1.2 Soft Lithography of Microfluidic Channels

To introduce fluids above the sensing surface, PDMS microfluidic channels are used. These channels are advantageous from different aspects: firstly, PDMS is chemically inert and is suitable to carry out functionalization experiments described in Chapter 4. Additionally, the material is optically transparent down to 300 nm of illumination wavelength [36].

An overview of casting channels in PDMS is presented in Fig. 3.6. Step 1 in Fig. 3.6 is the master fabrication process described in Sec. 3.1.1 and in Appendix 3.1.1. The gray structures in this figure represent the relief patterns made of SU-8. Following that, in Step 2, PDMS prepolymer and the curing agent are mixed in 10:1 ratio and are degassed to free the mixture of air bubbles. The liquid elastomer is then poured over the master in a container (e.g. petri dish) and is cured in a
Figure 3.4: Pre-exposure bake (soft bake) times for 65°C bake and 05°C bake. Data from MicroChem was plotted to illustrate the trends useful in determining the bake times suitable for thick SU-8 2050 coats above the typically used range of thicknesses (20-225 µm) [37].

Convection oven at 80°C. The cured channels can then be cut and removed from the master. Since the master produces 9 channels at a time (Fig. 3.5), excess channels can be kept on the master to
The height of a standard channel was \( \sim 300\text{–}380\mu\text{m} \), and the thickness of an average slab of PDMS is about 3 mm; this cross section is shown in Figure 3.7. Additionally, we see the relevant design parameters for the T-junction in Figure 3.8. The PDMS slab thickness is relevant in calculating the length of screws necessary to hold together the clamping system described in section 3.2. The screw length was chosen to be 12 mm, taking into consideration the structures that need to be screwed together in Fig. 3.12.

### 3.2 Fabrication and Testing of the Clamping System

The most common way to attach microfluidic channels to a sensor surface is by plasma bonding [25]. In this procedure, both the sensor surface and the PDMS microfluidic channel are plasma-treated to activate the Si-OH groups on the surface of both, which enables the formation of covalent siloxane (Si-O-Si) bonds on the surface upon contact. However, this plasma-bonding of channels to sensor
Figure 3.6: Overview of soft lithography process where a 3-channel pattern is produced in PDMS using a master. A transverse cross-section is shown.

Figure 3.7: Transverse cross-section of a T-junction channel to show dimensions.
surfaces come with some challenges: the surface activation is incredibly time-sensitive, giving the experimenter > 1 min to manually align and center the channel over the photonic crystal.

Manual alignment especially causes a problem in photonic crystal designs that are intended for self-referenced sensing. In these designs, the mesa is vertically split into two parts that will each be covered by adjacent streams of unmixing fluids as seen in the cartoon inset of Fig. 3.11. This design is intended to measure differential shifts in the resonant wavelengths by using one half as the “reference” side and the other as the “sensing” side. The resonances exhibited by these two half-circle lattices are spectrally distinct by a few nanometers. This measurement scheme allows to control for inherent measurement errors such as thermo-optic effects and bulk fluid effects.

In these self-referenced measurements, it is of utmost importance to have the etch gap that delineates the two half-mesas (shown in Fig. 3.11, width of 3.78 µm) aligned parallel to the sides of the microfluidic channel such that the split-flow boundary can be placed over the etch gap. This will allow the two halves of a photonic crystal mesa to be used simultaneously in self-referenced measurements. In these measurements, one side of the photonic crystal is chemically blocked and used as a “reference” while the “sensing” side is functionalized, and a differential measurement in
wavelength shift is observed. To be able to achieve a good alignment, mounting of the channels under a microscope is recommended; a light source should be used to illuminate the photonic crystals to visualize the etch gap alignment with respect to the channel.

Steady injection of liquids is required to create a stable split flow that will allow for this kind of alignment. This injection can be achieved by two microfluidic pumps (Standard Infuse/Withdraw Pump 11 Pico Plus Elite Programmable Syringe Pump, Harvard Apparatus) that independently control the flow-rate of the liquids flowing in a t-junction channel, centered over the etch gap. By centering the flow boundary on the etch gap, we can have the diffusion region at the interface of the two liquids spread over the gap. The movement of the boundary is shown in two snapshots in Fig. 3.9 (a) and (b). Split flow stability and diffusion region has been determined using a video of the pixel grayscale values over the flow boundary. The region of interest (ROI) is shown in Fig. 3.9 (c). Fig. 3.9 (d) shows the stability to be roughly 2-3µm and the diffusion region to be 12µm. Since the diffusion region is about 12µm with commonly used flow rates (~1 ml/min), and the etch gap about 4µm, only about 4 µm on each half of the mesa near the etch gap will be affected by the concentration gradient if using molecules as small as the food colorant dye. This number can be altered by increasing or decreasing the flow rates, and will also depend on the size of the diffusing molecule.

In addition to channel alignment issues in self-referenced sensor designs, the sensing surface becomes inaccessible for future cleaning if channels are permanently bonded to the chip surface. The result is a chip meant that needs to be discarded after each biosensing experiment is done. Given that the chip fabrication is a costly process that is also labour intensive, it is helpful to have the ability to refurbish the chip surfaces between experiments for use in multiple experiments. Prior to the development of the clamping system described in Section 3.2, multiple other methods have been attempted to remove the bonded PDMS channel from the surface. A few of these methods were:
Figure 3.9: Split flow using a T-junction microfluidic channel and colored water.

1. Piranha soak (up to 24 hours)

2. Concentrated H$_2$SO$_4$ soak (up to 48 hours)

3. Oxygen plasma treatment (5-10 × 1 min plasma)

4. A combination of 1, 2, and 3.

and none of these methods yielded good results in removing the PDMS from the oxide substrate, despite the fact that some of these methods have been claimed to refurbish sensors in the literature [39]. PDMS residue was clearly visible on the surface of chips whose channels were removed by peeling off, as seen in Fig. 3.10.

For these reasons, it was deemed necessary to work on a chip refurbishment procedure that would
Figure 3.10: Channel removal residue visible on a 15mm×15mm chip with 40nm of PECVD SiO$_2$ on Si wafer post-treatment with 48 hr Piranha soak, 24hr 30% H$_2$SO$_4$ soak, and 5 x 1min O$_2$ plasma. Allow for the reuse of the chip. A mechanical clamping system was devised as a solution for reversible channel attachment, and is described in Section 3.2.

Microfluidic channels were attached to the sensor surface using a custom designed mechanical clamping system in order for the photonic crystal surface to be freely accessible before and after the binding experiments. The purpose of a reversibly-attached microfluidic system is to make the sensing surface readily accessible for cleaning. This in turn allows the reuse of the sample for multiple experiments if a suitable surface cleaning technique is developed.

The complete system seen in Fig. 3.12 is made up of 5 major components:

1. Poly(methyl methacrylate) (PMMA) top plate to apply pressure on the microfluidic channel (3D component no 1. in Fig. 3.12a, top-view in Fig. 3.14)

2. Microfluidic channel fabricated from PDMS (3D component no 2. in Fig. 3.12, top-view and dimensions in Fig. 3.8)

3. PCS biosensor chip (15mm×15mm outline is shown in black solid line, no 3. in Fig. 3.12)
4. Heat sink (3D component is no 4. in Fig. 3.12a, top-view and dimensions in Fig. 3.13)

5. Thorlabs SM1A2 adapter with 2.5 mm diameter holes bored for screwing in the top plate (3D component is no 5. in Fig. 3.12a, side-view and dimensions in Fig. 3.15)

6. Ferrules used as fluidic ports (no 6. in Fig. 3.12a)

PMMA top plate has been designed to seal the microfluidic channel using pressure. The design seen in Fig. 3.14 has a few highlights: 7 circular holes (r=1.3mm) arranged along the periphery are for the clamping screws (screw dimensions: r=1.25mm, l=12mm). The narrow oval window near the center is opened for the laser excitation of the photonic crystals. This window (l=6.05mm, w=1mm) is designed to expose all of the mesas on a given self-referenced sensor. The window is to be centered over the center arm of the T-junction, such that the window center coincides with the split mesa center. Three circular holes (r=0.64mm) beneath the oval window are for the stainless steel
ferrules that connect the channel environment to the microfluidic tubing. The heat sink component was made from 3mm-thick stainless steel. The features of the heat sinking component as shown in Fig. 3.13 are the screw holes and the central gap that roughly exposes a 9mm×7mm area for transmission measurements. This component is attached to the Thorlabs adapter using thermal
paste that establishes thermal contact between the components.

Figure 3.13: 2D planar section of the Peltier heat sink with dimensions as shown in the figure.

The entire assembly was tested using colored water in the same way the initial microfluidic channels were tested (Fig. 3.9). A stable split flow is created in a T-junction channel using coloured water injected into one inlet of the channel using one syringe pump and plain water injected into the other inlet through another syringe pump. Once again, a stability of about 2µm is achieved and the largest diffusion region was 20µm with the dye molecules, which are estimated to diffuse 10x faster than proteins ~60kDa, roughly size of streptavidin as well as BSA. Therefore, this system appears suitable for the future self-referenced biodetection studies.
Figure 3.14: 2D planar section of PMMA top plate that is used to press down on the microfluidic channel.

Figure 3.15: 2D lateral section the Thorlabs adapter SM1A2. This component was machined to have 7 threaded holes to fit the screws that hold together the assembly (component no. 5 in Fig. 3.12)
(a) Clamping system used to pressure-bond PDMS T-junction and create a split-flow with colored water.

(b) Estimate of the diffusion region based on flow profile at different time points.

Figure 3.16: Microfluidic T-junction split-flow stability test using the PMMA clamping system.
Chapter 4

Surface chemistry of PCS biosensors

4.1 Outline of surface chemistry

The surface chemistry chosen for the biodetection experiments is one that is based on a recipe for a similar SiO$_x$ surface [39]. The SiO$_x$ surface is cleaned using Piranha solution (1:3 H$_2$O$_2$:$H_2$SO$_4$, $\sim$70$^\circ$C) to prime the surface for polymer adhesion. The purpose of this cleaning step is multifold: the solution will clean the surface from organic materials, and also increase the silanol (SiOH) groups on the surface, thereby increasing the hydrophilicity of the surface. In turn, this increased concentration of the (-OH) functionality on the surface is thought to better the adhesion of the silane polymer (3-aminopropyl trimethoxysilane (3-APTMS)), whose structure is shown in Fig. 4.1. Following the surface cleaning and activation, the self-assembling monolayer (SAM) formation is carried out in the silanization step where 3-APTMS is deposited and polymerized onto the sensor surface. The surface is then activated with the homobifunctional crosslinker glutaraldehyde that attaches to the exposed surface primary amines of the 3-APTMS through one aldehyde end, while presenting another aldehyde end for the immobilization of proteins. Finally, streptavidin is immobilized onto the now aldehyde-functionalized surface and biotin can be captured from the solution. This functionalization scheme is presented in Fig. 4.1 for clarity, and the exact recipes are shown in Appendix A.4. A depiction of the surface functional groups can be seen in Fig. 4.2.
Figure 4.1: The structural formula of 3-aminopropyl trimethoxysilane (3-APTMS), the alkoxy silane used in the self-assembling primary layer of sensor functionalization.

Fig. 4.2 shows the complete surface chemistry schematic. An unpatterned section of the surface is shown for illustration purposes. 3-APTMS is shown as crosslinked monomers on the oxide surface, enclosed by a blue box. In the green box above it is the glutaraldehyde, which serves as a homobifunctional crosslinker between the silane polymer layer beneath and the proteins above [20]. Glutaraldehyde is terminated with aldehyde functionalities (-COOH) at either terminus, and is therefore termed “homobifunctional”. At one terminus of the glutaraldehyde molecule, aldehyde
Figure 4.2: Schematic of completely functionalized surface showing streptavidin adsorbed onto the silanized sensor surface through glutaraldehyde crosslinker. BSA is used to block unreacted aldehyde groups to prevent non-specific binding of any biomolecules to the surface.

functionality enables the reaction with the alkoxy silane layer at the exposed (-NH₂) groups. This reaction yields what is known as a Schiff Base, a relatively unstable intermediary that can readily revert to the product. However, the presence of a reductive agent such as sodium cyanoborohydride (NaBH₃CN) can enable reduction of the Schiff Base into a stable secondary or tertiary amine. This process is illustrated in Fig. 4.3.

At the opposite terminus of glutaraldehyde, streptavidin molecules (shown by red S in Fig. 4.2) are immobilized via the aldehyde group reacting with streptavidin’s exposed amine (-NH₂) groups. Likewise, the reaction needs to be carried out as an immobilization through reductive amination in the presence of NaBH₃CN as seen in Fig. 4.3 for long-lasting functionalization.

As seen in Fig. 4.2, unreacted aldehyde groups are blocked with BSA (in a 1x phosphate buffered saline (PBS) containing 0.1% Tween-20 and 0.2 mg/ml BSA). Finally, biotin associates specifically
with streptavidin through strong, non-covalent interactions.

### 4.1.1 Silanization

Overall, the main deposition techniques for silane coupling agents generally fall under vapor phase deposition \[40\] or \textit{in solution} deposition techniques \[39\][41][42]. Of these techniques, in solution deposition is typically sub-categorized into two techniques based on the carrier solvent: \textit{anhydrous} and \textit{hydrolytic} deposition. \textit{Anhydrous} deposition refers to an immersion deposition technique where the polymer to be deposited is dissolved in a dry carrier solvent such as anhydrous toluene. In this deposition technique surface attachment readily occurs, but there is no cross-linking between the monomer units attached to the surface (Fig. 4.4b). In contrast, hydrolytic deposition involves the use of a “wet” organic solvent which enables the hydrolyzation of the methyl groups in the silane (Fig. 4.4a) which catalyzes polymerization of monomer units simultaneously with the deposition, thus presenting a one-step deposition process.

Table 4.1 shows various advantages and disadvantages of the commonly used covalent linkage techniques of vapour phase deposition, anhydrous and hydrolytic \textit{in solution} deposition techniques.
Spin coating and layer-by-layer assembly techniques that are not included in this comparison since these methods rely on physisorption or electrostatic interactions for coating the surface. This is in contrast to the desired, robust covalent linking to the surface functional groups.

Two silanization recipes have been considered to obtain a reliable polymer coat on the surface since silane deposition is known to come with challenges [24] [44]. First of these techniques, Recipe 1, was an anhydrous deposition technique based on a method developed by [41] for rapid anhydrous deposition of monolayer 3-APTMS films at elevated temperatures. This technique is claimed to produce highly reproducible and uniform films with very brief deposition times and without the need to post-immersion bake. However, experimental results have shown this recipe does not work well “as is” and requires modification that are likely similar to those recently cited in the literature.
<table>
<thead>
<tr>
<th>Advantages</th>
<th>Vapor Phase</th>
<th>In solution</th>
<th>Hydrolytic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reproducible</td>
<td>Most hydrolytically stable (after hydrolysis) [24]</td>
<td>Simple setup</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reproducible Smooth</td>
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<table>
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<th>Disadvantages</th>
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<th>In solution</th>
<th>Hydrolytic</th>
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<tr>
<td>Complex setup</td>
<td>Expensive</td>
<td>Moderately complex setup</td>
<td>Lower hydrolytic stability</td>
</tr>
<tr>
<td>Expensive</td>
<td></td>
<td>Requires separate hydrolyzation</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Summary of different polymer deposition techniques, comparing vapour phase deposition and in solution deposition.

by another group [45].

Figure 4.5: Filters present in the IVIS imaging system. Cy5.5 filter is suitable to image Alexa 680 and Atto 680 dyes.

<table>
<thead>
<tr>
<th>Fluorescence Filters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Excitation</strong></td>
</tr>
<tr>
<td>Set</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Figure 4.6: Fluorescence excitation and emission spectra for 4.6a. Alexa 680 and 4.6b. Atto 680.

(a) Alexa 680 fluorescent excitation/emission spectrum.  (b) Atto 680 fluorescent excitation/emission spectrum.
Images for fluorescent testing of Recipe 1 are shown in Fig. 4.7, Fig. 4.8 and Fig. 4.9. Streptavidin Alexa 680 has been used for imaging. Spectrum of Alexa 680 is shown in Fig. 4.6a. Imaging has been done at the Princess Margaret Hospital Advanced Optical Microscopy Facilities. Specifically, IVIS system has been used in the imaging of these samples. The spectrum of both Alexa 680 and Atto 680 (Fig. 4.6) matches the Cy5.5 filter (Fig. 4.5).

The fluorescence experiments on Recipe 1 has shown an approximate average success rate of ~33%, if one is to define a “successful” functionalization result to have > 50% surface area fluorescent.

EDX data for the top right image can be seen in Fig. 4.11, both in imaging mode (4.11a) and in elemental mode (4.11b).

In addition to the fluorescence imaging, scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) were performed on 44 nm of PECVD SiO$_x$ resting on 330µm (100) Si substrate. The combined SEM (Fig. 4.10) and EDX results show clumps of an ordered network of material on the surface of a 44nm SiO$_x$. Specifically the EDX results from a linescan of the polymeric network (Fig. 4.11) demonstrate that the network has buried the substrate Si signal, despite the fact that the signal is still strong. Of note is the significantly increased carbon and oxygen signals, correlating with the methoxy (-OCH$_3$) groups of 3-APTMS shown in Fig. 4.1. There is also a slight dip in the nitrogen signal correlating with the network localization. Overall, the EDX results imply the lack of a good adhesion by the 3-APTMS to the surface since there is a monomeric, reverse-attached network of 3-APTMS over the surface. This was interpreted as a failure in the polymer deposition technique, and the technique was deemed unsuitable for functionalization without significant alterations. In this context, reverse-attachment implies attachment of 3-APTMS to the SiO$_x$ surface silanol groups (SiOH) through its amine (NH$_2$) end, which was originally intended to be exposed for future functionalization. For reference, [46] describes EDX analysis principles.

This low yield has prompted the investigation of an alternative functionalization technique that utilizes a hydrolytic silane deposition method. A hydrolytic deposition technique based on that
reported for 1-D SiO$_x$ photonic crystals by Mandal et al. [39] has been adopted. Images for fluorescent testing of Recipe 2 are shown in Fig. 4.12, and Fig. 4.14. Biotin 680 © has been used for imaging.

The fluorescence experiments on Recipe 2 has shown an approximate average success rate of $\sim 82\%$, if one is to define a “successful” functionalization result to have $> 50\%$ surface area fluorescent. The positive results obtained have much more uniform fluorescent coverage than those obtained for Recipe 1. A summary of the fluorescence results is presented in Fig. 4.15 for PECVD SiO$_x$. We see an overall stronger intensity in fluorescence for the second recipe tried and also a smaller standard deviation on the mean for this recipe, indicating a more uniform fluorescence signal overall.

Different types of oxide such as PECVD, thermal oxide (Fig. 4.12, Fig. 4.14, and also Section 2.2 Fig. 2.6), and glass have all been successfully functionalized using this recipe. Based on these, Recipe 2 was chosen as the “working recipe” for the biodetection experiments in Chapter 5.

## 4.2 Discussion and Challenges

Two recipes have been tested for suitability with PCS functionalization, primarily using fluorescent maps from the IVIS imaging system. There have been attempts at characterizing the APTMS film while Recipe 1 was being troubleshooted using AFM, FTIR, and XPS but this has been extremely time consuming and difficult and has been put on halt after SEM images have been obtained of the dysfunctional silane coating process. Instead, fluorescently tagged Streptavidin (Alexa 680) and Biotin (Atto 680) have been immobilised on the surface to visualise the presence of surface functionalization for both recipes.

Overall, out of 2 recipes that tried, the hydrolytic deposition technique for APTMS (Recipe 2) seems to give more consistently positive results with fluorescent streptavidin or biotin on various oxide surfaces including PECVD SiO$_x$, thermal SiO$_x$, and glass. Despite the reported concerns on its hydrolytic stability, reliable fluorescence results led us to adopt this recipe for the proof-of-concept biosensing experiments in Chapter 5.
It is important to note some significant challenges that accompany surface chemistry work described in this chapter. Some of these challenges are listed below for reader’s consideration:

- glutaraldehyde have been found to be pH sensitive [20]

- time sensitivity of functional layers. Specifically known is the loss of 3-APTMS (surface is possibly amenable to reactivation in O\textsubscript{2} plasma—similar to other silanes such as PDMS)

- reagent quality sensitive (photobleaching of fluorescent tags, expiration of glutaraldehyde, expiration of 3-APTMS, premature polymerization of 3-APTMS if in contact with water)

- sub-optimal surface coating due to contamination of the SAM coating bath

- necessity of baking

- necessity of hydrolysis of methoxy- groups for monomer crosslinking on the surface, which contributes to layer stability in aqueous environments
(a) Negative control for Recipe 1 fluorescence experiment on 22 nm PECVD SiO$_2$. APTMS has not been deposited and the rest of the steps have been followed as per usual.

(b) Sample 1 functionalized according to Recipe 1 on 22 nm PECVD SiO$_2$.

(c) Sample 2.

Figure 4.7: Sample fluorescent images for Recipe 1 (negative control; Samples 1-2).
Figure 4.8: Sample fluorescent images for Recipe 1 (Samples 3-5).
Figure 4.9: Sample 6.

Figure 4.10: SEM images with 1µm, 500nm, 20nm and 20nm resolution (clockwise from top left).
Figure 4.11: EDX analysis of a monomeric network of 3-APTS.
Figure 4.12: Sample fluorescent images for Recipe 2 (Negative control; Samples 1-2).
Figure 4.13: Sample fluorescent images for Recipe 2 (Negative control; Sample 3-4).

(a) Negative control.

(b) Sample 3, 11nm PECVD SiO$_2$.

(c) Sample 4, 170nm thermal SiO$_2$.
Figure 4.14: Sample fluorescent images for Recipe 2 (Negative control; Sample 3-4).

(a) Negative control.

(b) Sample 3, 11nm PECVD SiO$_x$.

(c) Sample 4, 170nm thermal SiO$_x$. 
Figure 4.15: Mean grayscale intensity values for colour-separated fluorescence images for the two functionalization recipes.
Chapter 5

Demonstration of biodetection using PCS sensors

5.1 Simulation

5.1.1 FDTD

Lumerical® finite-difference time-domain (FDTD) simulation software has been used to simulate the photonic crystal structures in 3-D. FDTD refers to the computational method that utilizes a discretized version of time-dependent Maxwell’s equations called the finite difference equations to compute, in time domain, the steady-state electric and magnetic field vector components (e.g. $E_{x,y,z}$; $H_{x,y,z}$). The discretization of the system of coupled partial differential equations is achieved through the selection of an appropriate computational domain, which determines where the field components will be calculated (Fig. 5.1). Enforcing of the appropriate boundary conditions allows for the definition of the electromagnetic system of interest. This algorithm was first described by K. Yee [47] to numerically compute the transient response of a perfect conductor, but was later extended by Taflove [48] to arbitrary dielectric and conducting structures.

The computational domain is made up of unit cells called Yee cells which locate the E-field and H-field components with respect to one another in 3-D space. An example of a Yee cell is seen in Fig. 5.1. As can be seen from this figure, the E- and H-field components are spatially separated by $1/2 \times (\Delta x \text{ and/or } \Delta y \text{ and/or } \Delta z)$. In addition, the algorithm temporally separates the field components by using a leap-frog time-stepping method: in a given volume of space, E-field
Figure 5.1: A Yee cell showing the field components relative to each other, figure from [49].

components are calculated at one instant in time and the H-field values are calculated at the next instant. This allows a single run to calculate both electric and magnetic field vectors everywhere in the given volume as they evolve in time [47] [48].

Lastly, FDTD is valuable because it is able to calculate the response of a given system over a wide range of frequencies due to its time-domain-based algorithm [49]. However, despite its advantages, FDTD can be computationally intensive for structures that require fine spatial resolution since the simulation time varies in inverse proportion to the fourth power of the mesh size \(1/dx^4\) [50]. This is an important consideration when simulating biolayers relevant to this study since as can be seen in Fig. 5.2a, there are multiple layers on the order of \(\sim 1 - 5\) nm thick whose thicknesses and refractive indices have been reported. To overcome this problem, two precautions were taken: the three biolayers seen in Fig. 5.2a (thicknesses 1, 1, and 6.6 nm) were merged into a single, 10 nm biolayer (Fig. 5.2b), and non-uniform meshing was used over the computational domain. Mesh size over the entire domain was initially set to \(20\times20\times20\) nm\(^3\), but the photonic crystal unit cell section (Fig. 5.3b) was overlaid a finer mesh of \(8\times8\times8\) nm\(^3\) to resolve the biolayer.

An additional, physical reason to merge the biolayers together was the large range of reported values in the thicknesses and refractive indices of these layers, as discussed in detail in Sectionsec:s-bsa-
ads. This merging in fact accounts for the uncertainty in protein packing, as well as the uncertainties in the thickness and RI of the organic layers used in the study.

Figure 5.2: Cross section (xz-plane) of a unit cell used in FDTD simulations, illustrating the biolayer merging for simpler computation.
An important consideration in FDTD simulations is the application of boundary conditions suitable to the system modelled. For the PCS structures, an efficient method of running FDTD has been found to be simulating one-half of the unit cell and imposing symmetric and anti-symmetric periodic boundary conditions in the crystal plane ($x, y$ directions), while applying perfectly-matched layer boundary conditions in the wave propagation direction ($z$-direction). The boundary conditions for this simplified simulation domain have been illustrated in Fig. 5.3. Boundary conditions for the simulation domain shown in 3-D in Fig. 5.3a. Complementary to this, a more detailed version of the boundary conditions can be seen in Fig. 5.3b where one-half of the photonic crystal unit cell is shown in top-view. Periodicity is symmetric in the $x$ direction, but is anti-symmetric in the $y$ direction. Along $y$, this basic section of the unit cell needs to be mirrored to complete the structure to a full unit cell. When this symmetric and anti-symmetric periodic boundary conditions are applied, it is clear that an infinite photonic crystal will be created in the $xy$-plane. The perfectly matched layer (PML) boundary condition applied in the $z$ direction serves as “free space” in this simulation.

![Diagram of boundary conditions](image)

(a) Boundary conditions for the simulation domain (a unit cell of the PCS lattice) shown in 3-D.

(b) A more detailed version of the boundary conditions can be seen where one-half of the photonic crystal unit cell is shown in top-view.

Figure 5.3: FDTD simulation domain.

Figure 5.4 shows the E-field energy profiles for the fundamental TE-like GMR in an asymmetric PCS design with 5.4a $r=110\text{nm}$, $a=980\text{nm}$, $t_{ox}=80\text{nm}$ and 5.4b $r=250\text{nm}$, $a=998\text{nm}$, $t_{ox}=20\text{nm}$.
Mode profiles show interesting properties that correspond closely to those previously computed by similar asymmetric structures [13]. In these designs, the presence of the SiO\textsubscript{x} substrate underneath the SiN layer reduces the RI contrast and increases the field penetration in that direction. In addition, a similar effect is brought on by the conformal top-SiO\textsubscript{x} and the biolayers on top of the Si\textsubscript{3}N\textsubscript{4}. This thick top-SiO\textsubscript{x} and biolayer coats significantly fill the hole, thereby enabling in-plane propagation of the EM field energy to result in a confinement that is strongest inside the hole (Fig. 5.4a).

In these r=250nm designs, the mode profiles are slightly different since the top-SiO\textsubscript{x} layer is proportionately much thinner. As a result, the unit cell hole is not nearly as “filled” with this top-SiO\textsubscript{x} layer as in the r=110 nm t\textsubscript{ox}=80nm design, and we see a much weaker in-plane wave propagation and field confinement. The reduction in the field energy in the unit cell hole can be clearly seen in Fig. 5.4b, where the electric field energy $E^2$ for the r=250 nm t\textsubscript{ox}=20nm design has been plotted. Comparing this to the field energy profile for the r=110nm t\textsubscript{ox}=80nm design illustrates the stark contrast between the field confinement within the unit cell hole. Nonetheless, the effect of mode energy confinement via index-contrast remains the same. The layers whose RI are close to one another (SiN (n=2) and both bottom and top-SiO\textsubscript{x} (n=1.46) layers, as well as the biolayer (n=1.435)) enable mode penetration into a larger volume for the TE-like GMR. In this computational study, only TE-like GMR have been investigated since the biosensing experiments in the following section (Sec. 5.3) have been carried out using only this fundamental TE-like. Owing to their high Q factor, TM-like modes are more difficult to measure and therefore have not been focused on in this thesis.

In addition to obtaining the field profiles, the fill factor for the biolayer and the immersion liquid (water) have been calculated. Fill factor is the fraction of EM energy contained in a particular region over the total EM energy (Eq. 1.6). This ratio is calculated for the biolayer only ($f_b$) and for the liquid superstrate (i.e. water)$f_w$. The results are depicted in Table 5.1.1. The two competing effects in fill factor values are the increase in water fill factor with increasing radius due to a reduction
in the effective RI (see [13][32]). On the other hand, the changing oxide thickness determines how strong the planar confinement will be, with confinement increasing with increasing oxide thickness.

We note the reduced confinement in the hole in $r=250\text{nm}$ $t_{ox}=20\text{nm}$ designs compared to $r=110\text{nm}$ $t_{ox}=80\text{nm}$ designs due to reduced planar effective RI in Fig. 5.4b. We also see a slightly increased $f_b$ value for the $r=110\text{nm}$ design possibly owing to the strong field penetration into the top-SiO$_x$ layer, which leads to the slightly stronger field penetration into the biolayer on this design. In addition, we see a large field confinement in the water filling the unit cell hole, as is evidenced by the water.

Figure 5.4: Asymmetric guided resonance mode E-field energy ($E^2$) profiles in yz-cross section for two different designs for comparison.
filling factor in Table 5.1.1.

<table>
<thead>
<tr>
<th>Design</th>
<th>$\lambda_0$ [nm]</th>
<th>$f_b$</th>
<th>$f_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>r=110nm, a=980nm</td>
<td>1574.93</td>
<td>0.0049</td>
<td>0.326</td>
</tr>
<tr>
<td>r= 250nm, a=998nm</td>
<td>1539.38</td>
<td>0.0046</td>
<td>0.265</td>
</tr>
</tbody>
</table>

5.1.2 Scatter Matrix Method: $S^4$

Biosensor designs have been simulated using a software package named Stanford Stratified Structure Solver ($S^4$) made available to us by Victor Liu at Stanford.

The structures simulated are shown in Fig. 5.5. Looking at Fig. 5.5a, we see a layer-by-layer cross section of the simulation domain. From bottom up, we have the Si substrate ($t=\infty$, $n=3.46$), SiOx ($t=2.35\mu m$, $n=1.46$), SiN ($t=260\text{nm}$, $n=2$), top-SiOx, 3-APTMS layer (Si-R-NH chains enclosed in a blue rectangle, $t\sim 1\text{nm}$ [39]; $n\sim 1.424-1.46$ [51] and [52]), glutaraldehyde layer (red “A” for aldehyde groups, enclosed in green rectangle, $t=0.7\text{nm}$-1 nm [53] and [39]; $n=1.38-1.46$ [54] and [53]), streptavidin (red “S”, $t=5-6\text{nm}$ [55] and [56], $n=\text{[1.315-1.5]}$ [57][58]), BSA (encircled “BSA”, $t=5\text{nm}$ [59], $n=\text{[1.315-1.5]}$ [60]), and biotin (blue “B”). It is important to note that BSA blocks the unreacted aldehyde groups, which means that it effectively gets incorporated into the same layer as streptavidin.

As can be seen from above, there are no consensus values on the refractive indices for the biolayers involved because the RI is highly dependent on the surface packing density, which determines the amount of water inclusion in the biolayers. This in turn determines the effective refractive index of the layers involved. Overall, there is a range in the thicknesses and RI reported for all of the layers involved in this modeling, with the range in thicknesses being a lot narrower. For this reason, the organic layers of 3-APTMS, glutaraldehyde, streptavidin/BSA, and biotin have been simulated as a single conformal layer of 7 nm thickness and $n = \text{[1.315-1.45]}$. Here, $n=1.315$ corresponds to no biolayer on the surface since this is the RI of water @ 1550nm, and $n=1.45$ corresponds to a very densely packed composite biolayer. This is simulation is intended to mimic the sensor response to the conjugation of streptavidin to the sensor surface, as well as the following BSA-blocking of the unreacted aldehydes. The simulated geometry is shown quantitatively in Fig. 5.5a and qualitatively
Additionally, it is also crucial to note that biotin does not deposit as a layer on top of streptavidin, but rather gets incorporated into the structure of streptavidin through specific interactions. Biotin bound to free streptavidin can be seen in Fig. 5.6. An implication of this postulation about biotin is that the streptavidin likely undergoes a conformational change upon biotin binding, which brings about the change in effective refractive index. Since the resonances red-shift upon biotin binding ([39][61]), the effective RI must be increasing. Therefore, biotin binding can be modeled by simply extending the biolayer RI range to \( n = [1.315-1.5] \). A range of refractive indices (\( \Delta n \)) have been simulated to obtain a range of anticipated sensor responses (\( \Delta \lambda \)) for various sensor architectures used in biotin conjugation, as seen in Figures 5.7, 5.8, 5.9.

Figure 5.5: Schematic of various layer thicknesses used in \( S^4 \) simulation structures \( S^4 \)

The experiments performed fall under one of two categories. In the first scenario, streptavidin (SA) has been conjugated to the aldehyde-functionalized sensor, followed directly by biotin immobilization. In these experiments, no blocking buffers have been used. Samples used for these experiments had the parameters: \( r=90\text{nm} \), \( a=980\text{nm} \), \( t_{\text{ox}}=40 \text{ nm} \) and \( r=250\text{nm}, a=998\text{nm}, t_{\text{ox}}=22 \).
nm. Expected sensor response ($\Delta \lambda$) is plotted for a range of biolayer RI in Fig. 5.7 for $r=90$nm architecture and 5.8 for the $r=250$nm architecture. We observe a surface sensitivity of $7.321 \, [\text{nm/RIU}]$ for a 7 nm biolayer for the $r=90$nm design for the given range of biolayer RI in Figure 5.7.

Equation of the line in Fig. 5.7 is $\Delta \lambda = A_{\text{eff}} + B$, with $A$ denoting the surface sensitivity in [nm/RIU] ($A=7.321$; $B=-9.656$). This plot enables us to both determine the surface sensitivity ($A \, [\text{nm/RIU}]$) as well as biolayer sensitivity ($A/\Delta t_{\text{bio}}$) and read off the effective refractive index of the bound layer. The sensor response for $r=250$nm is plotted in 5.8, and the linear fit parameters for the line in Fig. 5.8 are ($A=10.682$; $B=-14.503$). We see an increase in the sensitivity with the increasing hole radius, as reported before [13].

The second scenario for the biosensing experiments is as follows: streptavidin is conjugated to aldehyde functionalized sensors, blocking of unreacted aldehydes by incubation with BSA, followed by specific association of biotin onto streptavidin. This experiment has been carried out on a sensor architecture with parameters $r=110$nm, $a=980$nm, $t_{\text{ox}}=80$ nm. Simulation of anticipated shifts for this sensor design is seen in Figure 5.9, and the fit parameters are ($A=5.295$; $B=-7.19019$).
Figure 5.7: Simulation of possible range of resonance shifts for composite biolayer of 7 nm thickness on r= 90 nm, a=980 nm, t_{ox}= 40 nm design, given the RI range for bound biolayer. Based on Figures 5.7, 5.8, 5.9, we can determine the surface sensitivity and the Q factor of the resonances as well as the expected shifts. These values are listed in Table 5.1 to serve as a guideline for the experiments to follow. Here we find the expected wavelength shift (in pm), expected biolayer sensitivity (in nm/RIU for an estimated 7nm-thick biolayer) and the expected Q.

<table>
<thead>
<tr>
<th>Design</th>
<th>n_{bio} = [n_i - n_f]</th>
<th>Δλ [pm]</th>
<th>S_{bio}[nm/RIU]</th>
<th>Q</th>
</tr>
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<tr>
<td>r=250nm</td>
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<td>10.7</td>
<td>210</td>
</tr>
<tr>
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<td>0-1326pm</td>
<td>7.3</td>
<td>6600</td>
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<td>0-488pm</td>
<td>5.3</td>
<td>3360</td>
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<td>r=110nm</td>
<td>1.315-1.5</td>
<td>0-753pm</td>
<td>5.3</td>
<td>3360</td>
</tr>
</tbody>
</table>

Table 5.1: Summary of anticipated sensor responses to streptavidin, streptavidin/BSA, streptavidin/BSA/biotin association. Streptavidin association is modelled as a range of biolayer RI that goes from 1.315 (n_{water}@1550nm) up to 1.45. Meanwhile, streptavidin/BSA, streptavidin/BSA/biotin or streptavidin/biotin is modelled as a range from 1.315 to 1.5.
Figure 5.8: Simulation of possible range of resonance shifts for composite biolayer of 7 nm thickness on r= 250 nm, a=998 nm, $t_{ox} = 22$ nm design, given the RI range for bound biolayer.

5.2 Surface detection calculations

5.2.1 Estimate RI change due to SA and BSA adsorption

*Refractive index increment*, or *specific RI*, denoted by $\alpha = \frac{dn}{dc} \text{ RIU ml/g}$, is a measure of how the RI of a polymer, biopolymer, small molecule or protein solution changes with changing concentration [62]. In our case, we are interested in calculating the expected refractive index change of streptavidin (SA) and bovine serum albumin (BSA) protein solutions as they adsorb to the surface, so we need to obtain $\alpha_{SA}$ and $\alpha_{BSA} @1550nm$. On this note, it is important to note that $\alpha$ is a parameter that is wavelength dependent. To calculate $\alpha$ at the desired wavelength, $\alpha$ at another wavelength needs to be known. Perlmann-Longsworth formula shown in Eq. 5.1 can then be used to calculate...
Figure 5.9: Simulation of possible range of resonance shifts for composite biolayer of 7 nm on r=110 nm, a=980 nm, $t_{ox}=80$ nm design, given the RI range for bound biolayer.

streptavidin (SA) and bovine serum albumin (BSA) refractive index increment [63].

\[
\left( \frac{dn}{dc} \right) = \left( \frac{dn}{dc} \right)_{578nm} \times \left( 0.94 + \frac{20000nm^2}{\lambda^2} \right)
\]  

(5.1)

Using some consensus values from the literature @578 nm (0.188 RIU ml/g for SA and 0.19 RIU ml/g for BSA [64] [63]), we obtain @1550 nm: $\alpha_{SA}=0.1783$ ml/g and $\alpha_{BSA}=0.1802$ ml/g.

The refractive index increment can be more explicitly defined as a line integral of the concentration dependent refractive index increment over a range of concentrations (Eq. 5.2):
\[
\Delta n = \int_{c_1}^{c_2} \frac{dn}{dc} dc
\]
\[
(n_2 - n_1) = \alpha \Delta c = \alpha (c_2 - c_1)
\]

where \(n_2\) is the RI of the protein solution, \(n_1\) is the RI of the adsorbate layer, \(c_2\) is the concentration of protein in solution, and \(c_1\) is the protein concentration in the adsorbate layer. For rough estimates, all of these quantities are assumed to be constant. For streptavidin, we have a \(c_2=100\mu g/ml\) solution, \(c_1=0.6\) g/ml. Based on these, we obtain a \(\Delta n_{SA} = 10.7 \times 10^{-2}\) RIU. Looking at Table 5.4, we see that both \(r=250\)nm and \(r=110\)nm designs have an RI change very close to this value for SA association (\(6.10 \times 10^{-2}\) and \(9.08 \times 10^{-2}\) RIU respectively). Similarly for BSA using \(c_2=0.2\)mg/ml and the same \(c_1\) value as before, we calculate \(10.92 \times 10^{-2}\) RIU. The total shift due to SA and BSA in Fig. 5.15 is 752pm, which yields \(\Delta n = 18.5 \times 10^{-2}\) [RIU]. It is reasonable for this total shift to be larger than that calculated purely for a densely packed monolayer of BSA.

When the difference in two index increments is taken, we are left with an approximate value for BSA-related index change of \(\Delta n = 9.42 \times 10^{-2}\) [RIU], which is in excellent agreement with the predicted value. Overall, we see that the surface concentration of both SA and BSA is likely lower than the assumed 0.6 g/ml.

### 5.2.2 De Feijter’s Formula: Streptavidin and BSA surface density

In this section we relate the experimentally observed shifts in resonance to surface mass density by using de Feijter adsorption relations to quantify surface detection limits. Since streptavidin and BSA have both invoked a sensor response close to the estimated values, we saw it fit to estimate their surface mass densities to quantify sensor performance. Biotin has not been detected with good accuracy nor precision, therefore its surface density has not been estimated.

The first of these molecules, streptavidin, is a \(\sim 60\) kDa tetrameric protein capable of binding biotin at 4 sites with extremely high affinity, with an association constant of \(K_a \sim 10^{15}\) [65] [66].
In the biodetection experiments, streptavidin solution concentration was \( \sim 100 \text{ug/ml} \). The second, bovine serum albumin (BSA), is a 66.5 kDa protein that is typically used as a blocking agent in immunoassays to prevent non-specific interactions of biomolecules in solution with the solid phase (generally the assay surface) \[67\]; in our experiments its concentration was 0.2mg/ml.

According to De Feijter et al. \[68\], the refractive index of proteins adsorbed onto a solid phase can be estimated as a linear function of the solute concentration. This enables us to calculate the approximate amount of adsorbed molecules per unit surface area (\( \Gamma \) in [ng/mm\(^2\)]) as follows:

\[
\Gamma = \frac{\bar{h}_1 (\bar{n}_1 - n_2)}{a}
\]

where \( \bar{h}_1 \) is the estimated thickness of the adsorbed layer, \( \bar{n}_1 \) is the estimated refractive index of the adsorbed layer, and \( n_2 \) is the refractive index of the adsorbate in solution. The variable \( a = \frac{dn}{dc} \) is the refractive index increment of the solute/adsorbate in [ml/g] calculated in Section 5.2.1. For the following calculations, we will use the measured effective refractive index for the biolayers in place of \( \bar{n}_1 \) and a literature consensus value for the layer thickness \( \bar{h}_1 \).

For streptavidin we can choose the following parameters for use in Eq. 5.3:

- an adsorbed layer thickness \( h_1 \) of 5 nm \[56\]
- 100 ug/ml streptavidin solution (in 1 x PBS) \[39\] with an estimated RI of \( n_2 \sim 1.32 \) (very close to \( n_{\text{water}} \) @ 1550 nm, 1.315)
- an adsorbed streptavidin layer RI of \( n_1 \sim 1.391 \). This is an average of the measured \( n_{\text{eff}} \) for streptavidin in Section 5.3.2
- \( \left( \frac{dn}{dc} \right)_{1550\text{nm}} = 0.1783 \text{ ml/g} \) based on Eq. 5.1

we obtain \( \Gamma_{\text{SA}} = 1.99 \text{ ng/mm}^2 \) for maximal streptavidin surface density, which is in fact in very good agreement with previously reported experimental surface densities for streptavidin monolayers (1.6 - 9.2 ng/mm\(^2\))\[69][70][71\].
Similarly, for the SA/BSA composite layer, we can compute the total surface density using the following parameters:

- an adsorbed layer thickness \(h_1\) of 5 nm [59];
- treating SA/BSA adsorption from solution as a simultaneous, competitive event; an estimated RI of \(n_2 \sim 1.32\) for the hypothetical SA/BSA solution [72]
- an adsorbed composite layer RI of \(n_1 = 1.5\) from measurement data in Section 5.3.2
- \(\left(\frac{dn}{dc}\right)_{1550nm} = 0.18\) ml/g based on Eq. 5.1; a value close to both \(\alpha_{SA}\) and \(\alpha_{BSA}\)

yields \(\Gamma_{SA+BSA} = 5\) ng/mm\(^2\) for the maximal surface density of the composite biolayer. This yields an average \(\Gamma_{BSA} = 3\) ng/mm\(^2\) assuming an average \(\Gamma_{SA} = 1.99\) ng/mm\(^2\). Literature values report BSA coverage of 1.2-1.8 ng/mm\(^2\) [72]. We see that the shift due to BSA being roughly twice that due to streptavidin in Fig. 5.15, and the surface density calculations correlate exactly with the factor of two surface coverage by BSA compared to SA. This is believed to point at the presence of multilayers of BSA.

The resultant values for surface mass detection limits of individual sensors used in Section 5.3.2 are listed in Table 5.2.

<table>
<thead>
<tr>
<th>Design</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\Gamma_{SA}) [ng/mm(^2)]</td>
</tr>
<tr>
<td>(r=250)nm, (a=998)nm, (t_{ox}=22) nm</td>
<td>1.57</td>
</tr>
<tr>
<td>(r=110)nm, (a=980)nm, (t_{ox}=80) nm</td>
<td>2.41</td>
</tr>
</tbody>
</table>

Table 5.2: Summary of calculated surface coverage from sensor response to various biolayers.

### 5.3 Experiments

#### 5.3.1 Methods

Experiments were carried out on custom designed photonic crystals (design by Ryan Schilling, details of the design processes and the optimal configurations can be found in his thesis [32]). The photonic
crystal sample is mounted reversibly with a T-junction type microfluidic channel whose design is seen in Fig. 5.10. In Figure 5.10a we see the design parameters and in 5.10b we see the actual mask printed on acetate paper from the design. The main difference between the two images is that the angular corners have been rounded off to prevent bubbles injected into the channel from being permanently trapped.

Figure 5.10: Layout of the microfluidic channel used in these measurements. The T-structure and the calculated dimensions allow for laminar flow from two inlets.

The PCS assembled in the reversible clamping system with the T-junction microfluidic channel, tubing, bent ferrule inlets, and the temperature control components is seen in Figure 5.11. The system is seen mounted on the transmission setup.

The tunable laser source (TLS) used in characterization is a Photonetics Tunics PRI tunable laser diode source whose operation wavelength is the 1480 - 1590 nm range. The maximum wavelength stepping resolution is about 1 pm and the maximum power output is 3mW. For the measurements in Section 5.3.2, 5 pm resolution and 1 mW power were used during acquisition. The transmitted power was detected with a Newport 1835-C optical meter which takes the output from a Newport 818 IR photodetector (Germanium, 7801800 nm, BNC Connector).
The functionalization experiments follow the following overall procedure (in chronological order):

1. Measurement preparation: IR laser is collimated and aligned in x, y, and z coordinates on both upper and lower paths (Fig. 5.12 components 1, 2, 3, 4, 10 are on the upper path and 6-9 are on the lower path).

2. A red laser diode (633 nm) is used to visually check the alignment and facilitate locating mesas on the photonic crystal. This laser (no. 11 in Fig. 5.12) is directed into the optical path of the IR via removable mirrors (no. 12, 13 in Fig. 5.12).

3. Thorlabs Glan-Laser calcite polarizers with a theoretical extinction ratio of (100 000:1), no. 2 and no. 7 in Fig. 5.12), are crossed to achieve an experimental extinction ratio ≥ 30,000.
Extinction ratio is the ratio of transmitted power when both polarizer 1 (no. 2 in Fig. 5.12) and polarizer 2 (no. 7 in Fig. 5.12) are at 0° over the transmitted power when polarizer 1 is at 0° and polarizer 2 at 90°. This can be denoted as:

\[ E = \frac{P_{0^\circ,0^\circ}}{P_{0^\circ,90^\circ}} \]  \hspace{1cm} (5.4)

4. Surface preparation: The surface of the photonic crystals have been cleaned with 1:5 Piranha solution prior to thin oxide deposition.

5. The sensing surface of the devices are deposited with PECVD SiOx of 20-80 nm thickness (Section 2.3).

6. Samples are cleaned again with 1:3 Piranha solution.

7. Sensing surfaces are coated with 3-APTMS in solution with 95% EtOH (see Appendix A.4 for full recipe).

8. Oven-cured samples are functionalized with glutaraldehyde

9. Following this, samples are manually aligned with the microfluidic channels and secured in the clamping system. The seal is tested with microfluidic pumps prior to sample mounting, with flow rates up to 1-1.2ml/min. The assembled, unmounted system can be seen in Fig. 5.11.

It is important to note that the maximum transmitted power when polarizers are un-crossed \( P_{0^\circ,0^\circ} \) in Eq. 5.4) is approximately 0.300 mW with 1 mW input power. Approximately 66% of the input power is lost due to the free-space coupling scheme of this transmission setup.

Measurements have been carried out using the setup in Fig. 5.12. The major components are enumerated in this figure are: (1) tunable IR laser (range 1480-1590nm) mounted with a collimating lens; (2) the first polarizer; (3) a lens that focuses the laser on the sample; (4) a beam splitter that splits the beam into horizontal and vertical components (50% each) ; (5) the sample stage, (17) an x, y, z stage; (6) a 45 ° mirror; (7) the second polarizer; (8) focusing lens to focus light from
the sample on the lower detector; (9) lower-arm photodetector; (10) upper-arm photodetector. The red laser (11) is used to track goodness of alignment. This laser is directed into the optical path of the IR using two mirrors, (12) and (13) – 13 is shown to be not mounted in the figure. The imaging components are (14), the CCD camera (QIClick from QImaging) and (15), a microscope (OLYMPUS SZ-60).

The sample stage enabled us to vertically locate the PCS exactly at the focused beam waist (≈80μm-wide), as well as give us the degree of freedom to either measure one-half of a mesa at a time, or to measure both halves at the same time by focusing the beam at the center of the etch gap. The experiments in Section 5.3.2 were carried out with the laser focused on only one half of a
5.3.2 Results

Figure 5.13: Measurement of streptavidin conjugation on r= 250 nm, a=998 nm, t_{ox} = 22 nm design.

Fig. 5.13 was obtained with an r= 250 nm, a = 998 nm design that was deposited with a 22 nm thick PECVD SiOx layer. Approximately 196 pm shift is observed due to streptavidin post-conjugation after washing with 5 ml PBS buffer with a resonance with Q=196. Going back to Fig. 5.7 for the expected sensor response, we see that this corresponds to an n_{eff}=1.376 and a Δn = 6.10 × 10^{-2} [RIU] for the bound streptavidin layer compared to 1.315 for water (@ 1550nm). The shift correlating to the range of n_{eff} = [1.315, 1.45] calculated through S4 simulations was found to be 0 - 986 pm. The measurement data shows a shift of 196±5 pm which falls nicely within this range. Assuming n=1.43 correlates with a densely packed monolayer of streptavidin (n=1.45 @

m2a.
Table 5.3: Comparison of anticipated wavelength shifts with regards to relative mass proportions of streptavidin (SA), bovine serum albumin (BSA), and biotin (B).

<table>
<thead>
<tr>
<th></th>
<th>Expected GMR shift ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta \lambda_{\text{BSA}}$</td>
<td>1.11</td>
</tr>
<tr>
<td>$\Delta \lambda_{\text{SA}}$</td>
<td>$\frac{1}{1.11}$</td>
</tr>
<tr>
<td>$\Delta \lambda_{\text{B}}$</td>
<td>$\frac{1}{2}$</td>
</tr>
</tbody>
</table>

1340nm from [73]), the maximum expected shift is 775pm, and the observed shift demonstrates an approximate 25% surface coverage by streptavidin based on the sensor response.

However, in Fig. 5.13 we also see an extreme 1019±5 pm shift due to biotin binding. A simple way to compare the expected relative shifts of these layers can be devised based on the ratios of the molecular weights of the relevant biomolecules, and the binding stoichiometry where applicable. Accordingly, Eq. 5.5 describes the expected relative shifts of due to bovine serum albumin (BSA) vs. streptavidin (SA). Similarly, Eq. 5.6 describes the expected relative shift of biotin vs. SA assuming on average 2 available binding sites for biotin per SA molecule:

\[
\begin{align*}
\frac{\Delta \lambda_{\text{BSA}}}{\Delta \lambda_{\text{SA}}} &= \frac{MW_{\text{BSA}}}{MW_{\text{SA}}} \sim \frac{66.5 \text{kDa}}{60 \text{kDa}} \\
\frac{\Delta \lambda_{\text{B}}}{\Delta \lambda_{\text{SA}}} &= \frac{2 \times MW_{\text{B}}}{MW_{\text{SA}}} \sim \frac{2 \times 244 \text{Da}}{60 \text{kDa}}
\end{align*}
\] (5.5)

and accordingly, we find in Table 5.3 the expected ratio of shifts.

A second experiment of direct biotin association following streptavidin (SA) conjugation to aldehyde functionalized sensors is shown in Figure 5.14. Here we see an interesting discrepancy in the sensor response to SA association: there is a 100pm blueshift in the GMR location after 1hr SA incubation on the aldehyde functionalized sensors. There are two possible causes of this: the measurement uncertainty on this experiment is extremely large due to low-resolution scan (100pm step size), so the peak location is cited with an extreme error. Alternatively, we see in this figure a removal of the previously adsorbed layers (3-APTM and GA) which results in this -100±100 pm shift in the resonance. Later, when the channel is incubated with biotin (1hr), we see a large red-shift in the resonance (430±5 pm) that is most likely due to NSB of biotin to the now-bare substrate.
Figure 5.14: Measurement of streptavidin and biotin conjugation on r= 90 nm, a=998 nm, $t_{ox} = 40$ nm design.

A likely scenario in this kind of response is the lack of good cross-linking by the 3-APTMS which renders it hydrolytically unstable. As a result, the hour-long incubation with aqueous streptavidin solution causes the polymer to gradually peel off, which increases the NSB by biotin onto the bare substrate. We observe that the biotin response is lower than that seen in the previous experiment (Fig. 5.13), which could mean that the NSB of biotin is lowered. This too could be explained by the removal of the underlying organic layers; bare SiOx is less likely to have copious amounts of organic adsorbates than various organic layers.

This postulate, when compared to the biotin-related wavelength shift in Fig. 5.15 of 200±5 pm after BSA-blocking, appears to hold water. Overall, this would mean that the most significant NSB
by biotin is on organic layers rather than inorganic, and the application of a blocking buffer such as 0.1% Tween-20, 0.2mg/ml BSA used in Fig. 5.15 significantly reduces this non-specific binding. However, we also see from Table 5.3 that the expected biotin shift should be 123x less than that for streptavidin, which would be on the order of 2pm. This means that there is a significant need in empirically determining the concentration/composition of the blocking buffer in order to be able to reliably measure unlabelled biotin as intended.

It is still possible to determine the effective RI of the adsorbed layer of biotin in Fig. 5.14. The baseline peak location is taken as the average of the spectral locationa of the post-glutaraldehyde and post-streptavidin resonance peaks due to the low resolution scan. This yields a biotin-related shift of 480pm, which corresponds to $n_{eff}=1.385$ and $\Delta n = 6.95 \times 10^{-2}$ [RIU]. However, this experiment should not be considered as a reliable demonstration of surface binding because of the clearly apparent problems associated with it.

Finally, we observe in Fig. 5.15 a shift of 255±5 pm due to streptavidin association, corresponding to $n_{eff} = 1.406$ and $\Delta n_{S} = 9.08 \times 10^{-2}$ RIU (compared to 1.315 for water). The shift correlating to the range of $n_{eff} = [1.315, 1.45]$ calculated through S4 simulations was found to be 0 - 488pm. The measurement data shows a shift of 255 pm which falls nicely within this range. If $n=1.43$ is a dense, homogeneous monolayer of streptavidin, the expected shift is 382pm, and the observed shift demonstrates an approximate 67% surface coverage.

In the same figure, we see a very large shift due to BSA blocking of aldehyde groups unoccupied by streptavidin (497±5 pm), which indicate that there must be a large number of unreacted aldehyde groups on the surface. Given that streptavidin and BSA are roughly the same size (66.5kDa:60kDa), their relative shifts should be a 1.11:1 ratio when they are competing for the same binding sites. However, we see approximately a 2:1 ratio for $\frac{\Delta \lambda_{BSA}}{\Delta \lambda_{SA}}$. This might be due to an overabundance of remaining aldehyde groups for BSA to attach to, as well as an overestimate of the SA surface coverage.

When we consider SA and BSA related shifts together, we see that $\Delta \lambda_{BSA}=752±5$ pm, which
falls within the reported composite biolayer range \( n_{eff} = [1.315, 1.5] \rightarrow \Delta \lambda = 0-752 \text{pm} \). We in fact see that the composite biolayer RI is \( n_{eff} = 1.5 \), in excellent agreement with accepted values for streptavidin-containing biolayers [58] [74].

Finally, we see a shift due to biotin in this experiment of about 200±5 pm, much less than those reported in previous experiments. This likely implies that the presence of the blocking buffer has reduced the NSB by biotin. However, given the 255±5 pm shift due to streptavidin, the expected biotin shift is approximately 2±5 pm. We see two orders of magnitude discrepancy between the predicted range of shifts and the measured. Overall, there seems to be certain necessary steps that need to be taken in order to detect biotin reliably by the current sensors, as discussed in Section 5.4.

When all the shifts are considered additively, the total shift in Figure 5.15 is 953 pm, which yields \( n_{eff} = 1.538 \) or \( \Delta n = 0.223 \) [RIU]. This \( n_{eff} \) (or \( \Delta n \)) is due to the adsorption of streptavidin (SA), BSA, and biotin all together. Once again, the effective refractive index is close to those accepted for streptavidin/biotin-containing biolayers (\( n=1.5-1.55 \)) [58] and [74].

<table>
<thead>
<tr>
<th>Design</th>
<th>( \Delta \lambda_S )</th>
<th>( \Delta \lambda_B )</th>
<th>Q</th>
<th>( \Delta n_{SA} ) [RIU]</th>
<th>( \Delta n_{BSA} ) [RIU]</th>
<th>( \Delta n_B ) [RIU]</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r=250 \text{nm}, a=998 \text{nm}, t_{ox}=22 \text{nm} )</td>
<td>196 pm</td>
<td>-</td>
<td>196</td>
<td>6.10 \times 10^{-2}</td>
<td>-</td>
<td>13.8 \times 10^{-2}</td>
</tr>
<tr>
<td>( r=90 \text{nm}, a=980 \text{nm}, t_{ox}=40 \text{nm} )</td>
<td>-100 pm</td>
<td>430 pm</td>
<td>970</td>
<td>-</td>
<td>-</td>
<td>7 \times 10^{-2}</td>
</tr>
<tr>
<td>( r=110 \text{nm}, a=980 \text{nm}, t_{ox}=80 \text{nm} )</td>
<td>255 pm</td>
<td>200 pm</td>
<td>1112</td>
<td>9.08 \times 10^{-2}</td>
<td>18.5 \times 10^{-2}</td>
<td>22.3 \times 10^{-2}</td>
</tr>
</tbody>
</table>

Table 5.4: Summary of experimental results for biodetection experiments with different photonic crystal sensors.

<table>
<thead>
<tr>
<th>Design</th>
<th>( n_{eff}(SA) )</th>
<th>( n_{eff}(SA+BSA) )</th>
<th>( n_{eff}(SA+BSA+B) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r=250 \text{nm}, a=998 \text{nm}, t_{ox}=22 \text{nm} )</td>
<td>1.376</td>
<td>-</td>
<td>1.453</td>
</tr>
<tr>
<td>( r=110 \text{nm}, a=980 \text{nm}, t_{ox}=80 \text{nm} )</td>
<td>1.406</td>
<td>1.5</td>
<td>1.538</td>
</tr>
</tbody>
</table>

Table 5.5: \( n_{eff} \) determined from the biodetection experiments.

### 5.4 Discussion

FDTD simulations demonstrate how the electric field distribution and energy confinement is affected by oxide-coating and functionalizing the photonic crystal surface. We see overall that these conformal
Figure 5.15: Measurement of streptavidin conjugation on r= 110 nm, a=998 nm, $t_{ox} = 80$ nm design. Approximately 255 pm shift is observed after washing with 5 ml PBS buffer post-conjugation.

layers reduce the refractive index contrast between the liquid and the SiN, which allow for field penetration into these layers. In the r=110nm design with a very thick (80nm) SiOx coat, we see the holes of the photonic crystal almost filled with oxide, which increases the in-plane mode confinement and result in a strong field localization inside the hole. In the r=250 nm design, the oxide coat is much thinner (20nm), which results in weaker in-plane confinement and increased field penetration into the liquid.

The results produced here demonstrate the first reported use of defectless 2-D photonic crystal slabs in biodetection experiments by the use of unlabelled streptavidin and bovine serum albumin (BSA). We have succeeded in detecting streptavidin conjugation to our biosensors reliably with the
first proof-of-principle experiments, determining an average effective RI of \((1.391\pm0.015) @ 1550\text{nm}\) which yields an average \(\Delta n = (8 \pm 2) \times 10^{-2} \text{ [RIU]}\) with respect to \(n_w = 1.315 @ 1550\text{nm}\). This result is in line with the widely accepted RI for streptavidin-containing biolayers with \(n=1.315 - 1.5\) [57][58] in the NIR range (1550 -1137 nm).

Additionally, we have been able to account for BSA adsorption to the surface. Since BSA and streptavidin are competing for the same glutaraldehyde binding sites, if the total shift due to streptavidin and BSA were considered to be due to the binding of one composite biolayer, the effective RI of this composite layer can be estimated to be 1.5, again close to RI estimates for SA-containing biolayer [58]. We then estimate a \(\Delta n = 15 \times 10^{-2} \text{ [RIU]}\) with respect to \(n_w = 1.315\) based on a single measurement. The surface density estimates in Section 5.2.2 yield an average \(\Gamma_{SA} = 1.99 \text{ ng/mm}^2\) for streptavidin and \(\Gamma_{BSA} = 3 \text{ ng/mm}^2\) for BSA. We postulate that there might be a multi-layer adsorption by BSA given the two-fold surface mass density value of \(\Gamma_{BSA}\) compared to the expected values.

Of note are the large wavelength shifts due to both SA and BSA that enable simple detection without the need to implement the self-referencing scheme. There remain some key issues for a robust biosensor platform. Notably, the biotin association to streptavidin has not been detected reliably by these sensors yet. BSA as a choice of blocking agent does not seem to prevent the excess non-specific adsorption by biotin, despite the BSA apparently forming multilayers on the surface. A new blocking buffer should be empirically determined.

Overall, the measurements are clouded by what appears to be hydrolytic instability and non specific absorption issues. To overcome these hurdles, the surface chemistry needs to be strengthened. In addition, detection of a small molecule such as biotin with an anticipated shift of roughly \(2\pm1 \text{ pm}\), it will be necessary to (1) use sensors with significantly improved surface sensitivities, and/or (2) employ measurement techniques capable of reliably detecting this signal.
Chapter 6

Conclusions and Future Work

6.1 Conclusions

The work described in this thesis contributes to the use of 2D photonic crystal as biosensors. We have demonstrated the biosensing capability of our custom-made devices through unlabelled, unmodified streptavidin conjugation, and we are likely able to detect other large proteins (∼60kDa) by the current sensor architectures without any further optimization. We have been able to quantify the approximate effective refractive index of the bound streptavidin and streptavidin (SA)/bovine serum albumin (BSA) composite layers using our preliminary measurements. Estimates for surface coverage by SA, BSA, and biotin were given. Despite our efforts, biotin binding to streptavidin has not yet been reliably observed with our current sensors.

Coating the sensor surface with a thin SiO$_x$ layer has not only provided us with an adhesion layer for the functionalization, but has also increased in-plane the field localization. Owing to this, a very high sensitivity to analytes in the holes of the photonic crystal can be achieved. This effect can be harnessed by using a small hole radius and a thick oxide coat (such as r=110nm, t$_{ox}$=80nm) and by integrating a particle trapping system in the future. Taking advantage of this index matching effect to confine electric fields can pave the way to interesting applications in localized sensing.

In this work we have also demonstrated the utility of a novel reusable system for device refurbishment. A clamping system has been designed, fabricated, and integrated with the current optical transmission setup to facilitate surface functionalization and future self-referenced biosensing work.
This system has been tested and is shown to be able to hold steady split-flows with 2μm stability with excellent reliability.

6.2 Future Work

6.2.1 Surface chemistry

In the measurements carried out, there have been issues that require to be addressed immediately to better the sensor performance. These issues are the non-specific binding (NSB) of biotin in extreme quantities to the sensor surface and the hydrolytic stability of the 3-APTMS layers. Empirical optimization of the blocking buffer is required for reduce the amount of non-specific binding (NSB) before biotin can be detected reliably. Incubating the samples with excess streptavidin might pose a solution to the biotin-related NSB problem. Simultaneously with this, a better step-resolution in the tunable laser source used in the transmission setup is required to detect the estimated picometer-range shift due to biotin binding to streptavidin. On the other hand, the 3-APTMS layers’ stability can be improved by adopting an anhydrous deposition technique that has implemented the reported improvements [45].

There is also the alternative to adopt a different chemistry to improve sensor performance. The current chemistry has been chosen to due to its inexpensive availability and the simplicity of the chemical structures of the reagents involved that minimize considerations such as steric hindrance in modelling the sensor response.

There exist reliable chemistries reported based on ester-linked biotins (e.g. Biotin N-succinimidyl ester) immobilized onto 3-APTMS surfaces. These chemistries involve the detection of avidin-like large specific binders, which could easily be detected by the current sensor architectures.

6.2.2 Self-referenced biosensing measurements

Self-referenced sensing based on the split-mesa designs are to be carried out to demonstrate differential binding of an analyte to the surface. The current surface chemistry and sensor setup is amenable to performing these experiments given that an appropriate glutaraldehyde block can be de-
terminated to block completely the “referencing” side of the sensor in a T-junction split-flow system.

Avidin-family proteins can then be differentially detected on the “sensing” side as they bind to the active aldehyde sites.
Bibliography


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[38] Stanford Microfluidics Foundry. Basic design rules.


Contributions

Conferences


Appendix A

Recipes

A.1 SU-8 mold recipe

For microfluidic channel molding, either silicon or glass substrates can be used. Two recipes presented below are for these two substrates respectively.

A.1.1 SU-8 molds on 4” Si wafers

No “seed” layer required for Si wafers.

1. Cleaning with 1:5 Piranha solution, dIH2O rinse

2. (Optional) Re-cleaning with IPA and dIH2O (if not immediately used after piranha step above)

3. Dehydrate Si substrate on 200°C hotplate for 5-10 min

4. Treatment with O2 plasma for 30 s to increase the surface Si-OH (silanol) group concentration

5. Spin coating using Microchem SU-8 2050 (Recipe 21) One spin yields ~ 75 um thick layer

6. Soft bake at 65 °C on hotplate for 5 min

7. Soft bake at 95 °C on hotplate for 10 min

8. (if doing multiple layers) Repeat steps 4 and 5 as many times as necessary to achieve desired feature height (i.e. channel height). We most commonly used 5 x spins to achieve 375 um tall
channels that were designed to be 1mm wide, with aspect ratio of 3:8. Aspect greater than 1:10 are required to prevent channel collapse

A.1.2 SU-8 molds on 2” x 3” glass slides

1. Cleaning with Acetone, isopropyl alcohol (IPA), and dIH2O

2. Dehydrate glass slides on

3. “Seeding”: Spin coating a base layer using Microchem SU-8 25 (Recipe 1) One spin yields ~ 25 um thick layer [75]

4. Soft bake seed layer at 65 °C on hotplate 3 min [75]

5. Soft bake seed layer at 95 °C on hotplate 7 min [75]

6. Maskless exposure of the seed layer

7. Spin coating layer 1 using Microchem SU-8 2050 (Recipe 1) One spin yields ~ 25 um thick layer

8. Soft bake layer 1 at 65 °C on hotplate 3 min

9. Soft bake layer 1 at 95 °C on hotplate 7 min

10. Spin coating layer 2 using Microchem SU-8 2050 (Recipe 21) One spin yields ~ 75 um thick layer

11. Soft bake layer 2 at 65 °C on hotplate 3 min

12. Soft bake layer 2 at 95 °C on hotplate 9 min

13. Exposure
A.2 Additional design images

A.3 PECVD SiOx deposition recipe

Recipe for the deposition of PECVD SiO$_x$ at 300°C using the Oxford Plasmalab PECVD at the Emerging Communications Technology Institute at the University of Toronto is found in Table A.3. The deposition rate for the recipe is found to be $\sim$9.7-11.0 nm/min.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber Clean</td>
<td>1300</td>
<td>80% CF4/O2 at 150 sccm</td>
<td>200 (RF)</td>
<td>15 min</td>
</tr>
<tr>
<td>Pump Down</td>
<td>0</td>
<td>0 sccm</td>
<td>0</td>
<td>3-10 min</td>
</tr>
<tr>
<td>Preheat</td>
<td>600</td>
<td>N2 at 1000 sccm</td>
<td>0</td>
<td>3 - 5 min</td>
</tr>
<tr>
<td>N2</td>
<td>1500</td>
<td>N2 at 1000 sccm</td>
<td>100 (RF)</td>
<td>30 sec</td>
</tr>
<tr>
<td>Deposition</td>
<td>500</td>
<td>5 % SiNH4/N2 30 sccm; N2O 700 sccm</td>
<td>50 (fwd RF)</td>
<td>2-10 min</td>
</tr>
<tr>
<td>Pump Down</td>
<td>0</td>
<td>0 sccm</td>
<td>0 W</td>
<td>1 min</td>
</tr>
</tbody>
</table>

A.4 Functionalization recipes

Anhydrous recipe for surface functionalization, adapted from [41] and [39].

- Thin-layer SiOx deposition on PCS according to recipe in Appendix A.3.
- 15 min CH2Cl2 soak, drying with N2
- Cleaning in NH3 :H2O2 :H2O (1 :1 :5 volume ratio) 70°C for 20 min, drying with N2
- dI H2O wash, drying with N2

- Deposition of 1 wt % of 3-APTMS in anhydrous toluene  60°C for 4 min (thought to yield -NH2 functionality)

- Toluene wash, dry with N2

- Immersion in 1 x PBS [1 x PBS is 10 mM phosphate buffer, 137 mM NaCl, and 2.7 mM KCl], pH 7.4, containing 10 mM glutaraldehyde and 10mM sodium cyanoborohydride room temperature (RT) for 2h (to give aldehyde functionality)

- Immersion in 100 ug/ml streptavidin (plain, Alexa 680 conjugated, or hydrazide conjugated) in 1x PBS, pH 7.4 for 1h

- Immersion in 1x PBS containing 0.1% Tween-20 and 0.2 mg/ml BSA for 30 min

- Immersion in 100 ug/ml biotin (plain or Atto 680 conjugated) in 1x PBS, pH 7.4 for 1h

Hydrolytic recipe for surface functionalization mainly based on [39].

- Thin-layer SiOx deposition on PCS according to recipe in Appendix A.3.

- H2O2:H2SO4 (1:3) soak for organic clean (“Piranha clean”) as well as surface activation

- Deposition of 2 % of 3-APTMS in 95% EtOH (5% H2O) RT for 10 min (thought to yield -NH2 functionality)

- Rinse with EtOH

- Cure  80°C for 2h

- Immersion in 1 x PBS [1 x PBS is 10 mM phosphate buffer, 137 mM NaCl, and 2.7 mM KCl], pH 7.4, containing 10 mM glutaraldehyde and 10mM sodium cyanoborohydride room temperature (RT) for 2h (to give aldehyde functionality)
• Immersion in 100 ug/ml streptavidin (plain, Alexa 680 conjugated, or hydrazide conjugated) in 1x PBS, pH 7.4 for 1h

• Immersion in 1x PBS containing 0.1% Tween-20 and 0.2 mg/ml BSA for 30 min

• Immersion in 100 ug/ml biotin (plain or Atto 680 conjugated) in 1x PBS, pH 7.4 for 1h
Appendix B

Simulation scripts

B.1 S^i scripts

--This is a comment. To run, >s4 onesi.lua

a = 1
real_a = 0.98 -- vary this real lattice const
r = 0.090 -- vary this real radius
r_norm = r/real_a
Th = 0.260 -- vary this nitride thickness
Th_norm = Th/real_a
ThOxide1 = 0.040 -- vary this oxide thickness
ThOxide_norm1 = ThOxide1/real_a
ThOxide2 = 2.35 -- vary this oxide thickness
ThOxide_norm2 = ThOxide2/real_a

t_aptms = 0.0007 -- 0.7 nm APTMS monolayer
t_glut = 0.0007 -- 0.7 nm glutaraldehyde
t_strep = 0.007 -- 7 nm biolayer
t_biotin = 0.001 -- 1 nm biotin

t_bsa = 0.00145 -- 1.45 nm BSA

t_s_norm = t_strep/real_a

num_points = 500 -- number of sample points

num_threads = 3

num_blocks = math.ceil(num_points/num_threads)

start_lambda = real_a/1.57

end_lambda = real_a/1.59

for delta_strep = 0, 0.4, 0.04 do

--create text file
xprecision = 10;
yprecision = 10;

fstr = "%."..tostring(xprecision).."f ..%."..tostring(yprecision).."f\n"

fp=io.open("bio-r .. r*1000 .. ",a", . real_a*1000 .. ",delta_str" .. delta_strep .. ", conformal" ..

S = S4.NewSimulation()
S:SetLattice({a,0}, {0,a})
S:SetNumG(49)

S:AddMaterial("air", {1,0}) -- real and imag parts
S:AddMaterial("water", {1.729225,0})
S:AddMaterial("silicon_nitride", {4,0})
S:AddMaterial("dlc", {2.56,0})
S:AddMaterial("silicon_oxide", {2.1316,0})
S:AddMaterial("silicon", {12,0})
S:AddMaterial("APTMS",{2.027776,0}) -- APTMS n=1.424
S:AddMaterial("glut",{1.89200025,0}) -- glut n=1.3755
S:AddMaterial("strep",{(1.315+delta_strep)^2,0}) -- strep n=1.4-1.45
S:AddMaterial("biotin",{(1.315)^2,0}) -- biotin n=1.4
S:AddMaterial("BSA",{2.4649,0}) -- BSA n=1.57

-- Water/biological layers above

S:AddLayer('waterabove', 0 , 'water')
-- S:AddLayer('BiotinAbove',t_biotin/real_a,'biotin')
S:AddLayer('StrepAbove',t_strep/real_a,'strep')
-- S:AddLayer('GlutAbove',t_glut/real_a,'glut')
-- S:AddLayer('APTMSAbove',t_aptms/real_a,'APTMS')
S:AddLayer('pc_oxide', ThOxide_norm1, 'silicon_oxide')
S:AddLayer('pc_slab1', Th_norm-ThOxide_norm1-t_s_norm, 'silicon_nitride')
S:AddLayer('pc_slab2', t_s_norm, 'silicon_nitride')
S:AddLayer('pc_slab3', ThOxide_norm1, 'silicon_nitride')

-- S:SetLayerPatternCircle('BiotinAbove','water',{0,0},r_norm)
S:SetLayerPatternCircle('StrepAbove','water',{0,0},r_norm-ThOxide_norm1-t_s_norm)

-- S:SetLayerPatternCircle('GlutAbove','water',{0,0},r_norm)

-- S:SetLayerPatternCircle('APTMSAbove','water',{0,0},r_norm)

S:SetLayerPatternCircle('pc_oxide','water',{0,0},r_norm-ThOxide_norm1-t_s_norm)

S:SetLayerPatternCircle('pc_slab1', 'silicon_oxide', {0,0}, r_norm)
S:SetLayerPatternCircle('pc_slab1', 'strep', {0,0}, r_norm-ThOxide_norm1)
S:SetLayerPatternCircle('pc_slab1', 'water', {0,0}, r_norm-ThOxide_norm1-t_s_norm)

S:SetLayerPatternCircle('pc_slab2', 'silicon_oxide', {0,0}, r_norm)
S:SetLayerPatternCircle('pc_slab2', 'strep', {0,0}, r_norm-ThOxide_norm1)

S:SetLayerPatternCircle('pc_slab3', 'silicon_oxide', {0,0}, r_norm)

-- Layer of oxide
S:AddLayer('below', ThOxide_norm2, 'silicon_oxide')

-- Substrate layer
S:AddLayer('Substrate', 0, 'silicon')

S:SetExcitationPlanewave(
{0,0}, -- incidence angles
{1,0}, -- s-polarization amplitude and phase (in degrees)
{0,0}) -- p-polarization amplitude and phase
-- Turn on smoothing for better convergence behavior w.r.t. NumG S:EnableLanczosSmoothing()

S_sweep = {} 
freq = {} 
forward = {} 

for j = 1, num_blocks do 

-- Populate array for frequency sweep 
for i = 1, num_threads do 
S_sweep[i] = S:Clone() 
freq[i] = start_lambda - (start_lambda - end_lambda) * (i + j*num_threads)/num_points 
S_sweep[i]:SetFrequency(freq[i]) 
end 

-- Solve in Parallel 
S4.SolveInParallel('Substrate', unpack(S_sweep)) 

for i = 1, num_threads do 
forward[i] = S_sweep[i]:GetPoyntingFlux('Substrate',0) 
fp:write( string.format( fstr, freq[i], forward[i] ) ) 
end 
end 

io.close( fp ) 

end
Appendix C

Acronyms

**AFM** atomic force microscopy

**3-APTMS** 3-aminopropyl trimethoxysilane .................................................. 13

**ARC** anti-reflection coating ................................................................. 21

**BSA** bovine serum albumin ............................................................... 80

**CN** Costa Nicholaou

**DA** Deniz Aydin

**DS** double-sided ................................................................................. 20

**DL** detection limit .................................................................................. 28
**EBL** Electron beam lithography .................................................... 20

**ECTI** Emerging Communications Technology Institute

**EDX** energy-dispersive X-ray spectroscopy ........................................ 59

**ELISA** enzyme-linked immunosorbent assay ..................................... 2

**EM** electromagnetic

**FISH** fluorescence in situ hybridization ........................................ 2

**FDTD** finite-difference time-domain ................................................ 70

**FTIR** fourier transform infrared spectrometer

**FWHM** full-width at half-maximum .................................................... 9

**FSR** free space radiation ................................................................. 5

**FOV** field-of-view ........................................................................... 22
GA glutaraldehyde

GM guided mode

GMR guided mode resonance

HA Hooman Akhavan

HFS hydrogen forward scattering spectrometry

IA immunoassay

LbL layer-by-layer

LB Langmuir-Blodgett

LPCVD low pressure plasma enhanced vapour deposition

NaBH$_3$CN sodium cyanoborohydride

NSB non-specific binding
OL Ofer Levi

PBS phosphate buffered saline ................................................................. 55

PCR polymerase chain reaction ................................................................. 2

PCS photonic crystal slab ........................................................................... 12

PDMS poly(dimethyl) siloxane ................................................................. 36

PECVD plasma enhanced chemical vapor deposition ................................... 25

PMMA Poly(methyl methacrylate) ............................................................... 47

PML perfectly matched layer ................................................................. 73

RBS rutherford backscattering ................................................................ x

REM replica molding ................................................................................. 36

RI refractive index ................................................................................. 2
**RIE** reactive ion etching

**RS** Ryan Schilling

**RT** room temperature

**SA** streptavidin

**SAM** self-assembling monolayer

**SEM** scanning electron microscopy

**Si$_3$N$_4$** silicon nitride

**SiN$_x$** silicon nitride

**SiNW** silicon nanowire

**SNF** Stanford Nanofabrication Facility
\[ \text{SiO}_x \] silicon dioxide

\[ \text{SiO}_2 \] silicon dioxide

\[ S^4 \] Stanford Stratified Structure Solver

\[ \text{SPR} \] Surface plasmon resonance

\[ \text{TE} \] transverse electric

\[ \text{TLS} \] tunable laser source

\[ \text{TM} \] transverse magnetic

\[ \text{XPS} \] X-ray photoelectron spectroscopy