CAVITY COUPLED PHOTONIC CRYSTAL ENHANCED FLUORESCENCE FOR HIGH SENSITIVITY BIOMARKER DETECTION

BY

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THESIS

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ABSTRACT

With the rise in fluorescence-based testing of biomarkers in samples, the use of dielectric optical resonators has increased dramatically. These photonic crystals make fluorophores emit more light through a process called enhanced fluorescence which in turn allows for more signal to be collected due to these biomarkers. Advances in such optical resonators can lower limits of detection for the biomarkers or allow for cheaper and more practical tests to be performed for patients. This thesis looks at the theory and the engineering design process of these one-dimensional photonic crystals such that the mechanisms of enhanced fluorescence are increased themselves. It then introduces a new design concept of a photonic crystal with a mirror beneath. The mirror is expected to increase the quality factor of the guided mode optical resonator in the photonic crystal improving the mechanism of enhanced excitation acting on the fluorophore. The mirror is also expected to improve the mechanism of enhanced extraction by redirecting the emitted light from the fluorophore back to the detection instrumentation. The last fabrication steps are performed on the mirror photonic crystals to obtain experimental results. They are analyzed through simulated and experimental reflection spectra results to find that the enhanced excitation depends mainly on the quality factor of the guided mode resonator and the Fabry-Perot reflection dip envelope caused by constructive and destructive Fabry-Perot reflections. Enhanced extraction is also found to play an important role in the performance of the mirror photonic crystals through fluorescence-based tests using Alexa Fluor 647. Lastly, the mirror photonic crystals are put into a fluorescence-based test that uses silicon photonic crystals and attempts to find the E7 antibody in serum which is common in those who have HPV-related cancers.
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CHAPTER 1: INTRODUCTION

1.1 Fluorescence

Fluorescence is a widely used method of detection in many biomedical applications due to the use of dyes in quantifying biomarkers in serum samples. By exploiting these fluorophores, concentrations of biomarkers in blood can be detected for early screening and tracking the progression of diseases in a host. As the technology of fluorescence detection is pushed forward, the ability to detect such diseases becomes more reliable, has a lower cost, lowers limits of detection leading to earlier detection, and increases the speed at which a diagnosis can be made. All of this allows patients to receive a personalized treatment plan in a reliable and cost effective way.

Fluorescence detection is particularly attractive in lab-on-a-chip and point-of-care applications over more complex detection methods due to its simpler and inexpensive experimental setups and well known concepts. First, fluorescence detection setups may use even the most common laser diodes as excitation sources to excite the fluorophore to a higher electronic state instead of using large, expensive lasers. Secondly, many fluorescence detection experiments simply need a collimated light allowing the experimental setup to be much more forgiving than other optical setups to lower the cost further. Lastly, the concepts of fluorescence may easily be described in a Jablonski diagram as detailed by Lichtman [1]. The excitation source excites the fluorophore to a higher electronic state, mostly the S\textsubscript{1} state and the higher vibrational states of S\textsubscript{1}. Any higher vibrational state quickly relaxes to the lowest vibrational state in S\textsubscript{1}. From the S\textsubscript{1} electronic state, the fluorophore emits a photon that has wavelengths larger than that of the excitation source as it relaxes to the ground electronic state, S\textsubscript{0}. These concepts can be easily
applied to the biomedical applications as the fluorophores are attached to the biomarkers of interest. Throughout this work, Alexa Fluor 647 is used that has emission and excitation spectra shown in Fig. 1.1 and is excited by 637nm solid-state laser diode to obtain the final results.

1.2 Enhanced Fluorescence

With fluorescence being a prominent method of detection, different structures have been developed to enhance the emission of photons from fluorophores through the concept called enhanced fluorescence. Enhanced fluorescence allows for even lower limits of detection while simplifying and lowering the cost of optical setups and excitation sources needed in a laboratory setting. Progressing even further allows for enhanced fluorescence to be used in non-laboratory settings making point-of-care diagnoses and lab-on-a-chip applications even more viable. In general, enhanced fluorescence is the process of enhancing the intensity of collected light through the use of two different mechanisms known as enhanced excitation and enhanced extraction. Enhanced excitation is seen when enhanced electromagnetic fields occur at the fluorophore’s position, increasing the photon density and therefore the number of excited electrons and emitted photons. Enhanced extraction is the process of directing the emitted photons towards a detection instrument to maximize the intensity of the light collected.

A common approach to enhanced fluorescence is that of metal enhanced fluorescence [2-6], which comprises methods that often use silver or gold nanoparticles to enhance the electric field around the nanoparticle itself through evanescent fields that are very large close to the nanoparticle but quickly decay as you move away. Using such a method, the peak enhancement factor that has been shown is greater than 300×. However, practical issues arise when using metal enhanced fluorescence. First of all, the distance of the fluorophores from the nanoparticle is key
for high enhancement factors. As fluorophores move away from the nanoparticle, the evanescent field intensities decrease exponentially leading to an exponentially decreasing enhanced excitation mechanism. Secondly, the fluorophore must not be too close to the metallic nanoparticle as the fluorophore will lose its energy by non-radiative means, lowering the emission of photons and therefore the mechanism of enhanced extraction. Lastly, these metallic nanoparticles are lossy due to their optical absorption, rendering high quality factor resonators difficult to make. Due to this and the emergence of fluorescence based detection of cells and biomarkers, advances in dielectric optical resonators with high quality factors have been made [7-16]. These structures, called photonic crystals (PCs), are the main topic of this thesis including the analysis of how they work, the recent progression of the PC designs used in Photonic Crystal Enhanced Fluorescence (PCEF) detection modes, and the analysis and experimental results of the new generation of cavity coupled PCs (mirror-PCs) to further increase the enhanced fluorescence.

1.3 Fluorescence-Linked Immunosorbent Assay

In order to understand the goal in designing the PCs used in PCEF and the final results, it is important to understand where and how the fluorophores are attached to the PC surface and the biomarker of interest. The current goal is detecting E7 antibodies in serum for screening of human papillomavirus related oropharyngeal cancer. In order to screen for this, an enzyme-linked immunosorbent assay (ELISA)-like sandwich assay known as a fluorescence-linked immunosorbent assay (FLISA) is performed. In order to begin the assay, a nanoplotter is used to print the antigen, protein, and control spots on the PC surface. These include the antigen of interest E7, protein A/G for onboard calibration, bovine serum albumin (BSA) for a negative control, and Alexa Flour 647 as a positive control. After using the nanoplotter, the PCs are placed in a layered acrylic microfluidic cartridge as shown in Fig. 1.2 to run the assay. The sample serum is incubated
on top of the PCs for 3 hours allowing the E7 antibodies to attach to the E7 antigens and various antibodies to attach to the protein A/G. The serum is then washed out and anti-human antibodies with fluorophores attached are flowed over the PCs to complete the FLISA process. The final result of this assay has fluorophores placed approximately 80 nm above the PC surface signaling the existence of various antibodies in the sample serum on the protein A/G spots and E7 antibodies on the E7 antigen spots. From the PC design standpoint, the positions of the fluorophores are important when looking at the enhanced electric field intensity above the PC surface contributing to the enhanced excitation mechanism.
1.4 Figures

Figure 1.1. Excitation and emission spectra for Alexa Fluor 647 used to obtain final results. The excitation peak corresponds with 651 nm and the emission peak corresponds with 667 nm. Reprinted from https://www.jacksonimmuno.com/technical/products/conjugate-selection/alexa-fluor/647.
Figure 1.2. (a) Exploded view of a 4-channel microfluidic cartridge. The clear layers represent the acrylic layers used in the structure. The blue layers show the double-sided adhesive layers used to adhere separate layers together and form the microfluidic channel. The orange blocks represent the 2 mm by 8 mm by 725 µm PCs incorporated into the microfluidic cartridge. The top gray layer is the glass coverslip to enclose the microfluidic channel. (b) Schematic view of an assembled 4-channel microfluidic cartridge. (c) Photograph of a 4-channel microfluidic cartridge while running a “dye test” to test the 3-hour incubation time needed.
CHAPTER 2: PHOTONIC CRYSTAL ANALYSIS

2.1 PC Introduction

PCs are periodic nanostructures made of alternating high and low refractive index materials. These PCs can be periodic in one-dimension (1D), two-dimensions (2D), or three-dimensions (3D) and employ various concepts to manipulate the motion of photons. The PCs used here are 1D periodic PCs that use concepts involving guided Fano resonators (GFRs) [15], diffraction gratings, total internal reflection, and Fabry-Perot (FP) reflections. These concepts allow for single peak resonances creating high quality factor (high Q) resonators that enable PC performance to overtake that of commonly used lossy metallic nanostructures, with reports of peak signal intensities greater than 8000× [13] in the ideal case.

The PCs discussed here take the non-ideal case as an approximated trapezoidal grating structure shown in Fig. 2.1a as this shape more closely matches that of the fabricated PCs. In the ideal case, the trapezoidal shape approaches a rectangular shape seen in many previous publications [7-11, 13]. The ideal case however differs enough from experimental results that the trapezoidal approximation is used throughout this thesis. This structure contains three different materials. The bottom layer is the substrate with refractive index $n_3$ which most often has been glass, silicon (Si), or the same material as the middle cavity layer of the PC. The substrate is mainly used as a foundation, but more recently it has been used to create a FP resonance in the PC to increase the enhanced extraction mechanism of enhanced fluorescence. The middle layer is known as the cavity layer as this layer is used to determine the FP cavity reflections of the device. The cavity layer, with a refractive index of $n_2$ and a cavity thickness of $t_c$, is often silicon dioxide (SiO$_2$), quartz, or a polymer. The grating structure is also part of the cavity layer as it is easy to
etch or stamp the grating structure into these materials. This grating structure is made with a period of $\Lambda$, a grating etch depth of $d$, a grating filling factor of $f_c$, and a grating angle of $\theta_c$. The final top layer is known as the slab layer or the guided mode layer, as this is where the guided Fano resonance occurs, and is made of a higher refractive index, $n_1$, material that has mostly been titanium dioxide ($\text{TiO}_2$) due to sputtering capabilities but has been silicon nitride for mid-infrared applications [15]. Because of the slab layer’s higher refractive index than those of both the background, $n_0$, and the cavity layer, $n_2$, the resonant wavelength of interest undergoes first-order diffraction to enter the top layer waveguide and becomes a guided wave through total internal reflection. This creates the GFR to produce a high Q resonator in the slab grating ultimately leading to the strong evanescent fields seen on top of the PC surface. This guided mode resonant wavelength is highly dependent on the slab thickness of $t_s$ and the incident angle of the incoming radiation, $\theta_0$, to obtain the high Q of the GFR. Like the grating structure in the cavity layer, the grating structure of the slab layer also has a period of $\Lambda$. However, the slab filling factor, $f_s$, and the trapezoidal angle approximation of the grating structure, $\theta_s$, differ from the cavity layer due to fabrication processes.

The last parameter to consider is that of the polarization of the incident light. In this case, the transverse electric (TE) polarization is described as the electric field being parallel to the grating lines, and the transverse magnetic (TM) polarization is described as having the magnetic field vector being parallel to the grating lines as shown in Fig. 2.1a. Analysis of TE and TM polarizations has led to discovering that the TM guided modes have a much higher Q than that of TE modes [11, 14, 15]. Due to this, most of the analysis is done with the TM polarization as a higher Q will give a larger enhanced fluorescence. To add onto this, one can assume that the
polarization used is the TM polarization for the entirety of this thesis unless it is specifically stated that the TE polarization is the one of interest.

2.2 PC Theory

With the complex structure of the PC used, there is no simple closed form solution in which the design process is based. Previously a ray tracing model has been used [17] and does yield the same final answer, but the model is not as robust as classical electromagnetic theory. Also a theoretical analysis of high contrast gratings has been made [18] which has the possibility of being applied to the PCs here but would require an extremely in-depth look into theoretical concepts due to the more complex shape of the PCs. Instead, a series of steps with approximations to help with the design process are taken to find the period and the slab and cavity thicknesses given the refractive indices of the background, slab, cavity, and substrate materials given by \( n_0, n_1, n_2, \) and \( n_3 \), respectively. This design process follows that shown by Liu [15].

2.2.1 Diffraction Grating and Total Internal Reflection

The first step in the design process is to find the grating period range that is needed to have only the zeroth- and first-order diffraction. Following the equation of diffraction and Fig. 2.1b, we have

\[
k_{1mx} = k_{0x} + m \frac{2\pi}{\Lambda}
\]

where \( k_{imx} \) is the \( x \)-component of the wavevector, \( \vec{k}_{im} \), in the \( i \)th material, and \( m \) is the order of diffraction for the slab wavevectors. Following that \( k_{ix} = |\vec{k}_i| \sin \theta_i \) for the \( i \)th material, it is found that

\[
n_1 \sin \theta_{1m} = n_0 \sin \theta_0 + m \frac{\lambda_0}{\Lambda}
\]

(2.2)
where $\theta_0$ is the angle of incident radiation, $\theta_{1m}$ is the angle of transmitted radiation for the $m$th-order diffraction, and $\lambda_0$ is the free space wavelength. Equation 2.2 leads to final answers for the transmitted angle and period of the grating structure for any order of diffraction.

$$\theta_{1m} = \sin^{-1}\left(\frac{n_0}{n_1} \sin \theta_0 + \frac{m\lambda_0}{n_1 \Lambda}\right)$$  \hspace{1cm} (2.3)

$$\Lambda = \frac{m\lambda_0}{n_1 \sin \theta_{1m} - n_0 \sin \theta_0}$$  \hspace{1cm} (2.4)

If the angle of incidence is assumed to be normal, or $\theta_0 = 0^\circ$, then the equations may be simplified even further.

However to ensure the guided mode exists, first-order diffraction must be met meaning the period must be greater than the right side of equation 2.4 when $m = \pm 1$ and $\theta_{11} = \pm 90^\circ$. Likewise, second-order diffraction should not exist as some power can get lost in the second-order modes. Therefore the period must remain less than the right side of equation 2.4 when $m = \pm 2$ and $\theta_1 = \pm 90^\circ$. This leads to the condition when the incident radiation is normal to the surface of

$$\frac{\lambda_0}{n_1} < \Lambda < \frac{2\lambda_0}{n_1}$$  \hspace{1cm} (2.5)

Another influence on the range of periods available is satisfying total internal reflection inside the slab layer. This means that the transmitted first-order diffraction angle must be greater than the critical angles of both the slab/cavity and slab/background interfaces. Since the background often has a refractive index lower than that of the cavity layer, the condition can be simplified to just that of the slab/cavity interface. This gives that

$$\sin \theta_{11} > \sin \theta_{crit} = \frac{n_2}{n_1}$$  \hspace{1cm} (2.6)
giving another upper bound on the period of the grating structure. Plugging equation 2.6 into equation 2.4 and assuming normal incidence, this condition is found as

$$\Lambda < \frac{\lambda_0}{n_2}$$  \hspace{1cm} (2.7)

Even with the limit in the range, the number of periods to choose from allows for many design possibilities each with different strengths and weaknesses.

### 2.2.2 Fabry-Perot Reflection

While finding the period of the device is a good first step in the design process, the following steps are done concurrently. With the structure proposed in Fig. 2.1a, one can see that FP reflections exist in the structure for different indices of refraction. To calculate an approximate FP reflection, $R_{FP}$, the structure may be approximated as a thin film structure without the grating as seen in Fig. 2.1c and an effective cavity thickness, $t_{c,eff}$, to account for the grating structure. Using the propagation matrix approach [15, 19, 20], the incident, reflected, and transmitted electric fields are written as

$$\begin{bmatrix} E_0 \\ rE_0 \\ tE_0 \end{bmatrix} = B \begin{bmatrix} tE_0 \\ 0 \end{bmatrix}$$  \hspace{1cm} (2.8)

where $E_0$ is the electric field amplitude of an incident wave, and $r$ and $t$ are the reflection and transmission coefficients, respectively. $B$ is the backward propagation matrix defined by the reflections due to the refractive indices of the materials and the change in phase due to optical path lengths through thin films on the substrate. The backward propagation matrix can be divided into three separate matrices in the following way assuming the incident light is normal to the PC surface.
\[
B = B_{\text{slab}} B_{\text{cavity}} B_{\text{substrate}} = \begin{bmatrix} b_{11} & b_{12} \\ b_{21} & b_{22} \end{bmatrix} \tag{2.9}
\]

\[
B_{\text{slab}} = \frac{1}{2} \begin{bmatrix} \left(1 + \frac{n_2}{n_1}\right) e^{-ik_1 z t_s} & \left(1 - \frac{n_2}{n_1}\right) e^{ik_1 z t_s} \\ \left(1 - \frac{n_2}{n_1}\right) e^{-ik_1 z t_s} & \left(1 + \frac{n_2}{n_1}\right) e^{ik_1 z t_s} \end{bmatrix} \tag{2.9a}
\]

\[
B_{\text{cavity}} = \frac{1}{2} \begin{bmatrix} \left(1 + \frac{n_2}{n_1}\right) e^{-ik_2 z t_{\text{eff}}} & \left(1 - \frac{n_2}{n_1}\right) e^{ik_2 z t_{\text{eff}}} \\ \left(1 - \frac{n_2}{n_1}\right) e^{-ik_2 z t_{\text{eff}}} & \left(1 + \frac{n_2}{n_1}\right) e^{ik_2 z t_{\text{eff}}} \end{bmatrix} \tag{2.9b}
\]

\[
B_{\text{substrate}} = \frac{1}{2} \begin{bmatrix} \left(1 + \frac{n_3}{n_2}\right) & \left(1 - \frac{n_3}{n_2}\right) \\ \left(1 - \frac{n_3}{n_2}\right) & \left(1 + \frac{n_3}{n_2}\right) \end{bmatrix} \tag{2.9c}
\]

The slab thickness and effective cavity thickness are given by \(t_s\) and \(t_{\text{eff}}\), respectively, and the z-component of the wavevectors of the slab and cavity are given by \(k_1 z = \frac{2\pi n_1}{\lambda_0}\) and \(k_2 z = \frac{2\pi n_2}{\lambda_0}\), respectively. Lastly the FP reflection, \(R_{\text{FP}}\), can be found by solving for \(r\) in equation 2.8 and calculating elements in the \(B\) matrix in equation 2.9 to get

\[
R_{\text{FP}} = |r|^2 = \left|\frac{b_{21}}{b_{11}}\right|^2. \tag{2.10}
\]

The FP reflection becomes a design concern when trying to isolate the guided mode resonance from FP reflections as seen in reflection peak PCs where the guided mode resonance occurs at a reflection peak with a narrow linewidth. In such cases, the slab and cavity thicknesses should be chosen such that the FP reflection is at a minimum. Later it will be seen that FP resonances are beneficial in cavity coupled PCs where the guided mode resonance occurs at a reflection dip with a narrow linewidth. In such PCs the slab and cavity thicknesses need to be chosen such that the FP resonance is at a maximum. As a final note, the FP resonance depends on
both the slab and cavity thicknesses where the guided mode resonance depends mainly on the slab thickness. Due to this, the cavity thickness is often chosen after a slab thickness is decided upon to create the desirable FP reflection.

### 2.2.3 Guided Mode Resonance

With the knowledge of the FP reflections, a greater understanding of the guided mode resonance can be found by assuming the slab layer to be like a dielectric slab waveguide as shown in Fig. 2.1d without the grating structure [15, 19, 21]. This also assumes that the substrate is far enough away that it does not have an effect on the fields due to the first-order diffracted wave now trapped inside the slab waveguide. The final result of this analysis leads to guidance conditions for both the TM and TE cases. Taking into account that first-order diffracted wave in the TM case, the magnetic fields can be assumed to be of the form

\[
H_{0y} = A e^{-\alpha_0 z} e^{-j k_x x} \tag{2.11a}
\]

\[
H_{1y} = (B e^{-j k_{1z} z} + C e^{j k_{1z} z}) e^{-j k_x x} \tag{2.11b}
\]

\[
H_{2y} = D e^{\alpha_2 z} e^{-j k_x x} \tag{2.11c}
\]

where \( H_{iy} \) is the y-directed magnetic field in the \( i \)th layer. It is important to note that since we are in the TM case both the x- and z-directed magnetic fields are set to zero. \( A, B, C, \) and \( D \) are constants for the magnetic fields. \( \alpha_0 \) and \( \alpha_2 \) are the decay constants of the evanescent fields in the background and cavity materials, respectively, assuming total internal reflection is satisfied, and they are given by the dispersion relations in equation 2.12. Also given by equation 2.12 is the dispersion relation for the z-directed propagation constant in the waveguide, \( k_{1z} \).

\[
k_0^2 = k_x^2 - \alpha_0^2 \tag{2.12a}
\]
\[ k_1^2 = k_x^2 + k_{1z}^2 \]  \hspace{1cm} (2.12b)
\[ k_2^2 = k_x^2 - \alpha_2^2 \]  \hspace{1cm} (2.12c)

The propagation wavevector, \( k_x \), is the same in each region due to phase matching and can be solved for from the guidance conditions given the mode and the slab thickness.

To solve for the guidance conditions, boundary conditions on the slab/background \((z = t_s)\) and slab/cavity \((z = 0)\) interfaces will be used. The boundary conditions on both interfaces are that the tangential fields are continuous. Since we have the tangential magnetic fields, the tangential electric fields may be found, giving

\[ E_{0x} = -\frac{\alpha_0}{n_0} \frac{j}{\omega \varepsilon_0} A e^{-\alpha_0 z} e^{-jk_x x} \]  \hspace{1cm} (2.13a)
\[ E_{1x} = -j \frac{k_{1z}}{n_1^2} \frac{j}{\omega \varepsilon_0} (Be^{-jk_{1z} z} - Ce^{jk_{1z} z}) e^{-jk_x x} \]  \hspace{1cm} (2.13b)
\[ E_{2x} = \frac{\alpha_2}{n_2^2} \frac{j}{\omega \varepsilon_0} De^{\alpha_2 z} e^{-jk_x x} \]  \hspace{1cm} (2.13c)

where \( \omega \) is the operational frequency and \( \varepsilon_0 \) is the permittivity of free space. Solving for continuous tangential fields at the slab/cavity interface \((H_{1y}(z = 0) = H_{2y}(z = 0) \) and \( E_{1x}(z = 0) = E_{2x}(z = 0)\)), equation 2.14a is found. Likewise at the slab/background interface \((H_{0y}(z = t_s) = H_{1y}(z = t_s) \) and \( E_{0x}(z = t_s) = E_{1x}(z = t_s)\)), equation 2.14b is found.

\[ \frac{c}{B} = e^{-2j\tan^{-1}\left(\frac{n_1^2 \alpha_2}{n_2^2 k_{1z}}\right)} \]  \hspace{1cm} (2.14a)
\[ \frac{c}{B} e^{2jk_{1z} t_s} = e^{2j\tan^{-1}\left(\frac{n_1^2 \alpha_0}{n_0^2 k_{1z}}\right)} \]  \hspace{1cm} (2.14b)
Combining equations 2.12 and 2.14 and adding in a term for possible higher order TM$_n$ modes, the guidance condition for the TM$_n$ mode becomes

$$\sqrt{(k_0n_1)^2 - k_x^2}t_s = \tan^{-1}\left(\frac{n_2^2}{n_0^2} \frac{\alpha_0}{\sqrt{(k_0n_1)^2 - k_x^2}}\right) + \tan^{-1}\left(\frac{n_2^2}{n_0^2} \frac{\alpha_2}{\sqrt{(k_0n_1)^2 - k_x^2}}\right) + n\pi$$  \hspace{1cm} (2.15)

where $k_0$ is the wavenumber of free-space. Following a similar derivation, the guidance condition for the TE$_n$ modes is found to be

$$\sqrt{(k_0n_1)^2 - k_x^2}t_s = \tan^{-1}\left(\frac{\alpha_0}{\sqrt{(k_0n_1)^2 - k_x^2}}\right) + \tan^{-1}\left(\frac{\alpha_2}{\sqrt{(k_0n_1)^2 - k_x^2}}\right) + n\pi.$$  \hspace{1cm} (2.16)

The guidance conditions provide several insights into the device. First, a cutoff wavelength exists for the TM$_n$ and TE$_n$ modes for a specific slab thickness. As the decay constants approach zero, the wave is no longer concealed in the slab layer. Since $\alpha_2$ will hit zero first, the cutoff for the TM$_n$ and TE$_n$ modes occurs when $\alpha_2 = 0$ or $k_x = k_0n_2$. Plugging this into the guidance conditions, it is found that the slab thickness must be large enough to contain the mode of interest which in this case is the TM$_0$ mode. However, if the slab thickness becomes too large, a higher order mode will appear in the slab waveguide. An example of one such analysis along with the FP reflection analysis is in Fig. 2.2a. Secondly, the wavevector, $k_x$, may be solved for numerically for the different modes of interest and the slab thickness from the guidance conditions while showing the low and high wavelength cutoffs calculated from the dispersion relations in equation 2.12 which can be seen in Fig. 2.2b. Lastly, the wavevectors for the different modes can be used to make an estimate of the period needed for the grating by plugging $k_x$ into equation 2.1 and solving for the period at normal incidence. This estimate is included in Fig. 2.2c.
2.2.4 Concluding Remarks

The past three sections give a good starting point in the design of these PCs especially with the grating period, slab thickness, and cavity thickness, but ultimately simulations must be used to finalize the design and decide on the filling factor and grating depth of the device. General trends and concepts do exist for these parameters, however, and have been studied. A particularly well done analysis of the change in parameters is that by Pokhriyal in her PhD dissertation [22].

Some general trends include increases in the grating period and slab thickness that will red-shift the guided mode resonant wavelength. An increase in the grating depth will increase the linewidth and therefore lower the Q of the GFR. Increasing the angle of incident light will red-shift the guided mode resonant wavelength. A concept to keep in mind when designing these PCs is that any change in shape of the slab layer—whether a change to the slab thickness, period, filling factor, or grating depth—will change the Q of the resonator made by the varying steps in the grating. Therefore any change in one parameter necessitates a second look at other parameters that have been previously finalized. Throughout this thesis, a commercially available rigorous coupled wave analysis (RCWA) simulation tool (DiffractMod, RSoft) is used to characterize the PCs.

2.3 Enhanced Excitation

Now that we have a deeper understanding of how the PCs work, we can look back at the enhanced excitation and enhanced extraction mechanisms. As stated before enhanced excitation is the process of enhancing the electric field intensity and therefore the photon density in the area around the fluorophore. In turn, the fluorophores are able to excite more electrons and increase the number of emitted photons following the simple Jablonski diagram. This enhanced electric field
is caused by the evanescent fields on the surface of the PC where the FLISA process places the fluorophore.

As an example, the currently used silicon PC (Si-PC), studied more in depth in section 2.5, has a TiO$_2$ slab layer ($n_1 = 2.35$) and SiO$_2$ cavity layer ($n_2 = 1.46$) on top of a Si substrate ($n_3 = 3.87$) in air. When a 637 nm, TM-polarized plane wave is incident on the PC, some of the light will be reflected and transmitted due to the FP reflections. Some of the energy will undergo first-order diffraction and become trapped in the TiO$_2$ slab waveguide. This will cause the evanescent fields on the surface of the PC to excite more fluorophore electrons at their position. This idea may be better visualized in Fig. 2.3a. Through simulations, it can easily be seen that the electric field intensity is in fact enhanced at the approximate fluorophore position of 80 nm above the PC surface. A simulation of the currently used Si-PC is shown in Fig. 2.3b with average enhancement factors (EFs) compared to the incident plane wave at certain distances from the surface of the PC.

2.4 Enhanced Extraction

Enhanced extraction is the process of redirecting emitted photons in the preferred direction of the collection instrument. The fluorophores here are assumed to emit light in all directions, and the collection instrument collects light that is redirected back in the direction of the source. This means that much of the light will be sent through the PC or off to the sides of the PC. Taking the same Si-PC as in Fig. 2.3a, Fig. 2.4 demonstrates the idea of enhanced extraction. The collection instrument will accept the emitted photons that are sent back to it from the fluorophore. There may also be photons that are reflected back to the collection instrument due to the FP reflection of the PC itself. Lastly, a possibility exists where the TE-polarized light from the fluorophore is coupled into the PC as well if the wavelength of the TE guided mode resonance matches that of the emitted
light of the fluorophore. However, this would cause the emitted light to be trapped instead of getting reflected into the collection instrument, which would cause further complications.

2.5 Analysis of Current Si-PC

Currently, the PC used for screening the E7 antibody in serum is described by George [13]. This PC uses a Si wafer as a substrate, SiO$_2$ as the cavity with a grating structure, and TiO$_2$ for the slab layer. The experimental results including the reflection spectra for normal incidence, the on-resonance angle, and the incident angle spectra for a 637 nm source are given in Fig. 2.5. In order to fully understand the experimental results, a scanning electron microscope (SEM) image is taken of the cross-section of the Si-PC and simulation is redone so that its result matches that of the experiment and the SEM image. It is found that the parameters that match extremely well to both are $\lambda_0 = 637$ nm, $n_0 = 1$, $n_1 = 2.35$, $n_2 = 1.46$, $n_3 = 3.87$, $\Lambda = 360$ nm, $t_s = 140$ nm, $f_s = 31\%$, $\theta_s = 53^\circ$, $t_c = 780$ nm, $f_c = 20\%$, $\theta_c = 70^\circ$, and $d = 52$ nm following the definition of variables in Fig. 2.1.

Following the procedure in section 2.2, the design of the Si-PC can be decomposed completely, but here only results that compare to the experimental results in Fig. 2.5 are shown. First experimental FP reflection is compared to calculated FP reflection obtained through section 2.2.2 where the effective cavity thickness is given by $t_{c,eff} = t_c + f_c d \approx 790$ nm. Solving for the FP reflection as a function of the free-space wavelength, Fig. 2.6a is found to have an extremely close match to the background FP reflection shown in the experimental results of Fig. 2.5a. Likewise, simulation results match extremely well with the experimental results. Figure 2.6b shows the reflection spectra for both normal incidence and at the on-resonance angle. The FP background reflection matches very well, and the guided mode resonances for both normal and
on-resonance angle conditions match. Figure 2.6c shows the incident angle reflection spectra for a 637 nm source. This matches very well with the spectra found in Fig. 2.5b. Since such a match is made between the calculated, simulated, and experimental results, the simulation can be used to estimate the EF of the Si-PC at the on-resonance angle and the 637 nm resonant wavelength. This result is shown in Fig. 2.6d and shows an average EF of 15.76× at 80 nm above the Si-PC surface where fluorophores are approximately placed.

This provides a base knowledge of how Si-PCs and the generals PCs of interest work in order to further improve the mechanisms of enhanced fluorescence. To use this knowledge to further this technology will lead to even lower limits of detection for earlier screening. This knowledge can also be used to find different biomarkers and other dyes aside from the Alexa Flour 647 used here by designing PCs with different resonances and using different excitation sources.
2.6 Figures

Figure 2.1. (a) Schematic view of a generic PC with a substrate, cavity, and slab layer. The parameters shown here are as follows: $n_0$: refractive index of the background, $n_1$: refractive index of the slab layer, $n_2$: refractive index of the cavity layer, $n_3$: refractive index of the substrate, $\Lambda$: grating period, $t_s$: slab thickness, $f_s$: slab filling factor, $\theta_s$: angle of the slab grating structure, $t_c$: cavity thickness, $f_c$: cavity filling factor, $\theta_c$: angle of the cavity grating structure, and $d$: grating depth. The blue arrow indicates the incident radiation at an angle of incidence $\theta_0$. The TE and TM polarizations follow as described. (b) View of the zeroth- ($m = 0$) and first-order ($m = \pm 1$) diffraction and total internal reflection needed which are determined by the period of the grating. (c) Thin film approximation for calculating the FP reflection of the PC assuming normal incidence. (d) Dielectric slab waveguide approximation to solve for the guidance conditions of the first-order diffracted wave due to the grating structure.
Figure 2.2. Theoretical analysis following section 2.2 for a PC in air with a TiO$_2$ slab layer and SiO$_2$ cavity and substrate layers with the following parameters: $n_0 = 1$, $n_1 = 2.35$, $n_2 = 1.46$, $n_3 = 1.46$, $t_s = 140$ nm, $t_c = 780$ nm. (a) FP reflection map for the PC with cutoff information and a suggested slab thickness for the resonant wavelength of $\lambda_0 = 637$ nm (data-point). (b) Numerical solution for $k_x$ for the given PC with low and high cutoffs for the device. (c) Suggested period for the PC based on the data-point for the TM$_0$ mode.
Figure 2.3. (a) Si-PC structure and visualization of enhanced excitation mechanism. (b) Simulation of the currently used Si-PC to find the average enhancement factors (EFs) at certain distances above the PC surface. The Si substrate is part of the simulation result but is not shown in the electric field intensity map since it is far away from the TiO$_2$ slab. Simulation parameters: $\lambda_0 = 637$ nm, $n_0 = 1$, $n_1 = 2.35$, $n_2 = 1.46$, $n_3 = 3.87$, $\Lambda = 360$ nm, $t_s = 140$ nm, $f_s = 31\%$, $\theta_s = 53^\circ$, $t_c = 780$ nm, $f_c = 20\%$, $\theta_c = 70^\circ$, and $d = 52$ nm.
Figure 2.4. Visualization of enhanced extraction mechanism. Light is lost through the transmitted wave and the light directed away from the collection instrument. The extracted light comes directly from the fluorophores and any possible reflected waves due to FP reflections.
Figure 2.5. (a) Reflection spectra of a Si-PC at normal incidence (black) and at the on-resonance angle of 3.5° (red). At the on-resonance angle, the resonant wavelength is $\lambda_0 = 637$ nm. (b) Reflection spectra of a Si-PC at $\lambda_0 = 637$ nm for varying incident angles. [13]
Figure 2.6. (a) Calculated FP reflection using the backward matrix propagation method in section 2.2.2. (b) Reflection spectra of normal incidence (black) and at the on-resonance angle (red). (c) Reflection at the resonant wavelength of 637 nm based on the angle of incidence. (d) Electric field intensity map with average EFs 10 nm, 80 nm and 150 nm above the Si-PC surface. Although the Si substrate is not shown in this picture, the simulation does include it. Simulation parameters: $\lambda_0 = 637$ nm, $n_0 = 1$, $n_1 = 2.35$, $n_2 = 1.46$, $n_3 = 3.87$, $\Lambda = 360$ nm, $t_s = 140$ nm, $f_s = 31\%$, $\theta_s = 53^\circ$, $t_c = 780$ nm, $f_c = 20\%$, $\theta_c = 70^\circ$, and $d = 52$ nm.
CHAPTER 3: ANALYSIS OF PC WITH A MIRROR UNDERNEATH

3.1 Introduction

With the high Q Si-PC from Chapter 2, new ideas must be implemented to have a greater enhanced fluorescence and lower limits of detection. One idea is to replace the Si substrate with a highly reflective material so that the mechanisms of enhanced fluorescence will be heightened. With a highly reflective surface beneath the grating structure, enhanced excitation could increase due to reflected waves having the chance to also couple into the waveguide structure. However, this effect will only help if the incident and reflected waves in the slab waveguide constructively interfere. Secondly, enhanced extraction is expected to increase drastically as the emitted photons from the fluorophore will be reflected by the mirror underneath the grating structure so that they may be collected by the detection instrument. Both mechanisms are shown in Fig. 3.1. This structure will be called a mirror-PC throughout this thesis.

3.2 Previous Work

The concept of using a mirror to better couple light into the PC and enhance the fluorescence was first introduced by Pokhriyal [12, 23]. It has been shown that using a gold thin film either on top of or below the PC grating structure exponentially increases the Q with respect to PCs without a substrate. The Q for the Si-PC is already high, however, and due to error in fabrication the linewidth of the device will be difficult to decrease as the resonance has a chance of disappearing altogether if it is too narrow. Furthermore, in the experimental setting to screen for the E7 antibodies in serum, having a slightly wider linewidth will, first, account for minor changes in device characteristics after the entire surface chemistry and FLISA processes and, second, give the device a wider on-resonance angle linewidth so that the on-resonance angle of the
E7 spots on top of the PC may still be found easily. Therefore, an attempt to lower the linewidth (less than 2 nm – 3 nm) of the PC may be detrimental to the practical aspect of the project even though the mirror-PC has the ability of having a higher Q for larger grating depths than that of the Si-PC. Due to this, the mechanism of enhanced excitation is not expected to increase significantly from the Si-PC. However the mechanism of enhanced extraction is still expected to increase the enhanced fluorescence to approximately twice that of the Si-PC due to the high reflectivity of the mirror at the fluorophore emission wavelength.

Previously an analysis of the materials, structure, and fabrication process was made by Tang giving the structure and materials provided in Fig. 3.2 [24]. The design uses aluminum (Al) as the mirror material for the PC which has a refractive index of $n_{Al} = 1.398 + j7.457$. The Al is held to the Si substrate by a titanium (Ti) layer for proper adhesion. The mirror-PCs are fabricated on 8” diameter Si wafers by a commercial silicon foundry service (Novati Technologies, Inc.) who first sputtered the Ti and Al onto the Si wafers. Then SiO$_2$ was grown by plasma enhanced tetraethylorthosilicate (PETOS), and the grating structure was made by deep-UV photolithography giving a set mirror, cavity, and grating structure for the mirror-PC wafers [24]. The next step is sputtering TiO$_2$ to create the slab waveguide for the guided mode resonance. Some preliminary experimental results are taken by Tang in Fig. 3.3 and Fig. 3.4 that show the reflection spectra of a mirror-PC and compare the fluorescent signals of a Si-PC and mirror-PC from a dye, respectively [24]. The fluorescent signal for the mirror-PC is found to be better than that of the Si-PC, which shows the idea of a mirror-PC is a worthwhile advancement. However, the reflection at the on-resonance wavelength only dips to approximately 40% where the ideal case would be a reflection dip to 0% to show that there is strong coupling to the guided mode resonator and that the FP resonance condition is met.
3.3 Analysis of Preliminary Results

An in-depth analysis is now made of the preliminary experimental results shown by Tang [24]. As with the Si-PC, the mirror-PC can be completely studied using the full analysis in section 2.2. This was done to see that the chosen period, slab thickness, and cavity thickness were such that the FP reflection was at a maximum to obtain the wanted FP resonance at 637 nm, but the result is not shown here. It is important to note that Al can be considered the substrate material, $n_3$, and stretch to infinity in the theoretical formulation since it is highly reflective and is a lossy material such that there will be no transmitted waves to the Ti layer.

Extensive simulations of the structure shown in Fig. 3.2c are made using the RCWA tool with the following parameters to match the SEM cross-sectional image: $\lambda_0 = 637 \text{ nm}$, $n_0 = 1$, $n_1 = 2.35$, $n_2 = 1.46$, $n_3 = 1.398 + j7.457$, $n_{Ti} = 2.166 + j2.938$, $n_{Si} = 3.87$, $\Lambda = 365 \text{ nm}$, $t_s = 122 \text{ nm}$, $f_s = 36\%$, $\theta_s = 60\^\circ$, $t_c = 207 \text{ nm}$, $f_c = 26\%$, $\theta_c = 90\^\circ$, $d = 76 \text{ nm}$, $t_{Al} = 93 \text{ nm}$, and $t_{Ti} = 85 \text{ nm}$. The simulation results can be seen in Fig. 3.5. It is found that these simulation results match fairly well with the experimental results in that the reflection spectra around the guided mode resonances match between the two. However, the experimental on-resonance angle (1$^\circ$) is much less than that of the simulated on-resonance angle (3.65$^\circ$), but the guided mode resonance matches well enough in linewidth, resonant wavelength, and reflection dip strength that the EF of the device can be analyzed.

Figure 3.5c shows that the average EF for the electric field intensity on a line 80 nm above the mirror-PC is only 6.24$\times$, whereas the EF for the Si-PC at the same 80 nm distance in Fig. 2.6d is found to be 15.76$\times$. However, the fluorescent signal gained in the preliminary results in Fig. 3.4 shows an increase in the total enhanced fluorescence. This leads us to believe that the mechanism
of enhanced extraction for the mirror-PC is much stronger than previously expected. Since the electric field intensity is expected to be less than that of the Si-PC by close to a factor of three, the enhanced extraction of the mirror-PC can be expected to increase the signal to $6\times$ that of the Si-PC. The overall fluorescent signal will also increase if the average electric field intensity in the evanescent fields is increased from the $6.24\times$ of the current mirror-PC. This can be done by obtaining better coupling to the guided mode resonance by having a reflection dip strength that approaches the ideal 0% instead of the 40% in current simulations.

### 3.4 Mirror-PC Trends

With a basic understanding of mirror-PCs and their preliminary results, general trends for the mirror-PCs can be found through simulations. Mirror-PCs do follow many of the same trends found for Si-PCs that were summarized by Pokhriyal in her PhD dissertation [22]. The main difference between the two analyses comes from the added FP resonance that occurs simultaneously with the guided mode resonance due to the mirror. A second difference is that the mirror causes a high reflection at wavelengths other than the guided mode resonance so the area of interest is a reflection dip as opposed to a reflection peak. All simulation results are found using the same parameters as given in section 3.3 and Fig. 3.5 unless they are denoted as the sweep parameter, and they all are results from the incident light being TM-polarized.

The first parameter to look at is the change in the device response when the angle of incidence is changed. With a general PC, as the angle of incidence increases the guided mode resonant wavelength also increases. The same is true for the mirror PCs as can be seen in Fig. 3.6. The first-order mode (at approximately 630 nm) splits into the main area of interest being the reflection dip at the largest wavelength with the order of diffraction being $m = 1$ and the reflection
dip with a larger linewidth being the order of diffraction of \( m = -1 \). There are also second (525 nm) and third-order (450 nm) modes with reflection dips that exist in the reflection map that follow the same trend but are not the resonance of interest. Unlike the general PCs, the FP resonance is seen interfering with the guided mode resonance. As the angle of incidence increases, the optical path length also increases changing the FP response of the mirror PC. This is especially noticeable at the incident angle of 6° to 9° in the first-order mode where the reflection dip disappears altogether and begins to reappear at 10°. This shows the importance of having constructive FP reflections at the resonant wavelength at the on-resonance angle.

When the period is changed, the first-order mode of the guided mode resonance acts much like that of the general PC. When the period increases, the guided mode resonant wavelength also increases as seen in Fig. 3.7. As with the incident angle reflection map, the period reflection map also contains second- and third-order modes above the first-order mode of interest due to the mirror. The FP reflections also can be seen as a contribution to the linewidth of the device as the quality factor of the resonator for all of the modes is seen changing as the period changes. This makes the design process more difficult as some choices of period will require looking at more depth into the FP reflections of the device.

Much like increasing the period and the incident angle of light, increasing the slab thickness, \( t_s \), increases the guided mode resonant wavelength as seen in Fig. 3.8. As with the angle of incident light, the slab thickness also has a large effect on the FP reflections as adding more TiO₂ or any other material to the slab layer will increase the optical path length. This change is easily seen when the device goes from having a resonance due to constructive FP reflection to having no resonance due to destructive FP reflections in the first-order resonance and all higher
order resonances. Therefore if a higher resonant wavelength is needed, the FP reflections can move from constructive to destructive which means other parameters may need to change.

One of the main contributors to the FP reflections is the SiO$_2$ cavity thickness, $t_c$. As seen in Fig. 3.9, the cavity thicknesses where the FP reflections constructively interfere with the guided mode leave small windows for error. The cavity thickness also can determine the order mode of the FP reflections in the system. It is seen that the first-order mode is skewed compared to the higher order modes. This is due to the Al mirror affecting the evanescent field in the cavity itself. Therefore, a design using the second- or third-order mode may perform better experimentally.

Originally with a general PC, the grating depth, $d$, was specifically used to change the linewidth, or quality factor, of the guided mode resonance. For the mirror-PC, this relationship is only partially true as seen in Fig. 3.10. As the grating depth increases, the linewidth marginally increases compared to the generic PC. This means the design of the grating depth is given much more freedom than that of other parameters allowing for larger grating depths without the drawback of lowering the Q of the resonator too much. The difficulty in designing the grating depth comes from side walls in the slab layer that will form due to fabrication processes. In the simulation sweep, this is why the guided mode resonance red-shifts as the grating depth increases. It is assumed that for the same period, incident angle, and slab thickness the side walls in the guided mode layer will increase the overall effect of the slab layer, increasing the guided mode wavelength. Due to this, a smaller grating depth would be suggested over a larger one. Lastly, there is still the effect on the FP reflections of different amounts of the cavity layer material, which will adjust the final design.
Lastly the cavity filling factor, $f_c$, is analyzed because this parameter is the final controlled parameter during the fabrication process. The cavity filling factor also determines in the slab filling factor, $f_s$, and is considered in the simulation to be $f_s = 1.38f_c$ based on previous SEM images. It is found that the cavity filling factor has a small but important effect on the device characteristics as shown in Fig. 3.11. Since the cavity filling factor adds more SiO$_2$, or the cavity material, to the device as a whole, the FP resonance is changed meaning that a device fabricated with a smaller cavity filling factor than designed will need more materials to match the expected FP reflections. Also there is a slight skew to the guided mode resonant wavelength that could negatively affect the device response. It is therefore important to know the fabricated cavity filling factor of the device as this has been seen to have the highest error during the fabrication process.
Figure 3.1. (a) Expected enhanced excitation mechanism for a mirror-PC. Any resonant wave that undergoes zeroth-order diffraction initially will be reflected by the Al mirror back up to the grating structure where it may once again undergo the first-order diffraction to be trapped in the slab waveguide. Since most of the wave is trapped by the waveguide initially, however, this contribution is expected to be small. (b) Expected enhanced extraction for a mirror-PC. The mirror creates a high reflection for non-resonant wavelengths, and therefore the fluorophore emission wavelength, to increase the amount of light propagating back toward the collection instrument.
**Figure 3.2.** (a) Schematic of initial mirror-PC design by Tang. (b) Photo of a fabricated 1” × 0.5” chip. (c) SEM image of the cross-sectional view of the mirror-PC showing the TiO$_2$ slab, SiO$_2$ cavity, Al reflector, and Ti intermediate layer on top of a Si substrate. (d) SEM image of the mirror-PC. SEM parameters based on definitions from Fig. 2.1: $\Lambda = 365$ nm, $t_s = 122$ nm, $f_s = 36\%$, $\theta_s = 60^\circ$, $t_c = 207$ nm, $f_c = 26\%$, $\theta_c = 90^\circ$, $d = 76$ nm, $t_{Al} = 93$ nm, and $t_{Ti} = 85$ nm. [24]
Figure 3.3. (a) Reflection spectra of a mirror-PC for normal incidence (black) and for the on-resonance angle (red). Notice that the mirror-PC differs from the Si-PC in that the guided mode resonance is defined by a reflection dip as opposed to a reflection peak. (b) Reflection angle spectrum of a 637 nm excitation source showing the on-resonance angle to be around 1°. [24]
Figure 3.4. Intensity profile for cyanine-5 (Cy5) dye with an excitation peak at 650 nm and an emission peak at 670 nm. The three spots are excited on a mirror-PC, a Si-PC on-resonance, and a Si-PC off-resonance with a 637 nm excitation source. The mirror-PC shows an approximate EF 2× that of the on-resonance Si-PC. [24]
Figure 3.5. (a) Simulated reflection spectra of a mirror-PC for normal incidence (black) and for the on-resonance angle (red) of the mirror-PC. (b) Simulated reflection angle spectrum of a 637 nm excitation source showing the on-resonance angle to be 3.65°. (c) Electric field intensity map with average EFs 10 nm, 80 nm and 150 nm above the mirror-PC surface. Although the Ti and Si substrate is not shown in this picture, the simulation does include it. Simulation parameters: $\lambda_0 = 637$ nm, $n_0 = 1$, $n_1 = 2.35$, $n_2 = 1.46$, $n_3 = 1.398 + j7.457$, $n_{Ti} = 2.166 + j2.938$, $n_{Si} = 3.87$, $\Lambda = 365$ nm, $t_s = 122$ nm, $f_s = 36\%$, $\theta_s = 60^\circ$, $t_c = 207$ nm, $f_c = 26\%$, $\theta_c = 90^\circ$, $d = 76$ nm, $t_{Al} = 93$ nm, and $t_{Ti} = 85$ nm.
Figure 3.6. Reflection map of the angle of incident TM-polarized light on a mirror-PC showing the first- (~630 nm) and higher-order (~525 nm and ~450 nm) modes of the guided mode resonance. In the first-order mode, the resonant wavelength increases as the angle of incidence increases.
Figure 3.7. Reflection map of the mirror-PC for varying periods showing the first-order (beginning at the bottom left corner) and higher-order modes for the guided mode resonance. As the period increases, the resonant wavelength also increases in a linear fashion.
Figure 3.8. Reflection map of the mirror-PC for changing slab thicknesses showing the first- (bottom) and higher-order modes. The resonant wavelength increases as the slab thickness increases.
Figure 3.9. Reflection map for the mirror-PC for varying cavity thicknesses. The cavity thickness has a large impact on the FP reflections as shown by the first- and higher-order modes of the FP resonance. The cavity thickness needs to occur at positions of reflection minimums at the target guided mode resonant wavelength.
Figure 3.10. Reflection map for the mirror-PC with varying grating depths. As the grating depth increases, there is only a slight increase in the linewidth of the device, but smaller grating depths are preferred due to the generation of TiO$_2$ side walls red-shifting the resonant wavelength.
Figure 3.11. Reflection map for the mirror-PC with changing cavity filling factors. The change is minimal but important as the cavity filling factor can easily be fabricated differently from the designed filling factor.
CHAPTER 4: EXPERIMENTATION USING MIRROR-PCS

4.1 Introduction

This chapter looks primarily into the experimental process of testing the mirror-PCSs to find the best possible outcome of the reflection spectrum. As stated in section 3.2, several 8” diameter Si wafers have been made that include the Ti and Al sputtered on the Si wafer to make the mirror. On top of the mirror, a SiO$_2$ cavity layer has already been deposited, and the grating structure has been etched. The structural parameters for these 8” diameter wafers are given by: $\Lambda = 365$ nm, $t_c = 207$ nm, $f_c = 26\%$, $\theta_c = 90^\circ$, $d = 76$ nm, $t_{Al} = 93$ nm, and $t_{Ti} = 85$ nm as defined in Fig. 2.1. This gives a good starting point for experimental procedures as the only procedure left is the deposition of materials. This would mostly include TiO$_2$ as this forms the slab layer that controls the guided mode resonance. However, it will be seen that the deposition of more SiO$_2$ before TiO$_2$ deposition leads to a better final result due to stronger coupling into the guided mode resonator from the FP resonance.

4.2 Initial Results of Added TiO$_2$

The first experimental step taken was trying to reproduce the results found by Tang [24]. To do this, ten 1” $\times$ 0.5” chips were sent to a commercial service (Intlvac Technologies) to sputter the 122 nm of TiO$_2$ onto the SiO$_2$ cavity and grating. Since this device has a grating structure, the sputtering of the TiO$_2$ is not uniform as shown by the SEM image in Fig. 3.4. Instead the filling factor of the slab ($f_s$) becomes larger than the filling factor of the cavity ($f_c$). Also side walls are formed giving the approximate trapezoidal shape seen in the SEM images, models, and simulation results. Because of this the thickness on a flat substrate would exceed 122 nm. Therefore the
thickness asked for needs to be larger than the resulting thickness which in this case is 127 nm. Another practical issue occurs because of the surface chemistry and FLISA process. After these processes, the resonance wavelength of the device will red-shift by a few nanometers. Because of this, the chips are purposefully designed to have a resonant wavelength less than the 637 nm target wavelength, and the on-resonance angle can be found for the 637 nm target wavelength.

After the sputtering of TiO₂, the ten chips are put into a reflection setup built by Chaudhery [25] that is able to change the incident angle of the incoming radiation as seen in Fig. 4.1. The reflection setup uses a tungsten halogen lamp as a white light radiation source which passes through an optical fiber (Ocean Optics), achromatic lens, and a linear polarizer (Thorlabs Inc.) to make a collimated TM, or TE, polarized wave incident on the mirror-PC. The detection instrument along with the PC holder is set on a base rotation stage so that the incident angle of the source can be changed. The PC holder is also placed on a secondary rotation stage so that the reflected light can be directed into the collection fiber properly. Using this setup, the reflection spectra for normal incidence and the on-resonance angle of the ten mirror-PC chips can be found and compared to the results Tang found in Fig. 3.2a [24].

Figure 4.2 gives the reflection spectra of four of the ten chips that underwent the TiO₂ sputtering process. Nine out of the ten chips had failed experimental results in that there was no guided mode reflection dip at the on-resonance angle. What would be the on-resonance angle was dominated by destructive FP reflections, and the guided mode resonance simply disappeared. Figure 4.2a-c show three of the nine failed experimental outcomes due to the destructive interference. The on-resonance spectrum for each of the chips is not included simply because there is no on-resonance angle for these chips, but the nine normal incidence spectra were similar with resonances occurring around 627 nm. There was one successful chip with a guided mode resonance
close to the 637 nm target wavelength with an on-resonance angle of 4.25°. However, the reflection dip prompted by the guided mode resonance is weaker, much like that of the simulations and the previous experimental results, which overall will weaken the enhanced excitation mechanism. From these results, it is easy to see that the FP reflections are a major contributor to the mirror-PC performance and must be engineered properly for practical use.

4.3 Fabry-Perot Reflection Dip Envelope

To better engineer these mirror-PCs, a new concept is introduced called the Fabry-Perot reflection dip envelope, or FP-envelope for short. As was stated before, the mirror-PCs depend on two mechanisms, the guided mode resonance and the FP reflections. The guided mode resonance is simple to engineer because it only depends on the slab thickness. The incident angle is also a variable that can be exploited to tune any PC to the appropriate resonant wavelength. The FP reflections, on the other hand, depend on the shape and thickness of both the cavity layer and the slab layer, and unlike the incident angle the FP reflections cannot be tuned after the device is built. The effect of the FP reflections on the guided mode resonance can be seen by the strength of the reflection dip of the device. Tuning the resonant wavelength by varying the incident angle also shows the effect of the FP reflections at different wavelengths for the device. This is where the concept of the FP-envelope comes into play.

Figure 4.3 shows the concept of the FP-envelope as it is applied to simulated results for the mirror-PC in previous sections \( (n_0 = 1, n_1 = 2.35, n_2 = 1.46, n_3 = 1.398 + j7.457, n_{Ti} = 2.166 + j2.938, n_{sl} = 3.87, \Lambda = 365 \text{ nm}, t_2 = 122 \text{ nm}, f_s = 36\%, \theta_s = 60^\circ, t_c = 207 \text{ nm}, f_c = 26\%, \theta_c = 90^\circ, d = 76 \text{ nm}, t_{Al} = 93 \text{ nm}, \text{ and } t_{Ti} = 85 \text{ nm}. ) \). By varying the angle of the incident light, the resonant wavelength of the device is shifted. At different wavelengths, the FP reflections
will act differently showing that the strength of the reflection dip changes. These different strengths can be put into an envelope creating the Fabry-Perot reflection dip envelope shown by the orange dashed line. In order to optimize the device, the minimum of the FP-envelope must occur on the target wavelength (637 nm) of the guided mode resonance shown by the black dashed line. Since the FP reflections depend on both the TiO$_2$ slab layer and the SiO$_2$ cavity layer, the FP-envelope can be red-shifted to the desired 637 nm by adding either TiO$_2$ or SiO$_2$ to the device.

The first idea of red-shifting the FP-envelope is adding more TiO$_2$ to the slab layer. This action affects the response of the device in two different ways, however. First it would red-shift the FP-envelope making it possible to have the minimum at the target wavelength, but it would also red-shift the guided mode resonance making the device more difficult to use since increasing the angle of incidence will only increase the resonant wavelength as well. This makes it easy to overshoot the target wavelength of 637 nm as seen in the comparison between Fig. 4.4a and Fig. 4.4b where 8 nm of TiO$_2$ is added to the simulation. The second idea is to add more SiO$_2$ to the cavity before depositing the TiO$_2$ slab layer. This would red-shift the FP-envelope with minimal effect on the guided mode resonant wavelength as seen in the comparison between Fig. 4.4a and Fig. 4.4c where 12 nm of SiO$_2$ is added to the cavity thickness in the simulation. Since it is possible to sputter SiO$_2$ onto the cavity layer before the TiO$_2$ sputtering, this allows the mirror-PCs to be optimized for the use in the PCEF project.

One final concept to note here is that of a FP cavity. If the approximate structure found in Fig. 2.1d is taken, then the optical path length between the Al mirror, $n_3$, and the background air, $n_0$, is given by

$$L_{OP} = n_1 t_s + n_2 t_{c,eff}$$  \hspace{1cm} (4.1)
where $t_{c,\text{eff}}$ is the effective cavity thickness with the grating structure which is given by $t_{c,\text{eff}} = t_c + d_{fc}$. All other variables are defined in Fig. 2.1. In order for the incident and reflected waves to constructively interfere we need

$$L_{OP} = m \frac{\lambda_0}{2}$$  \hspace{1cm} (4.2)

where $m$ is a positive integer and $\lambda_0$ is the target resonant wavelength in free-space. Through this calculation, it is easy to see that the wavelength at which the minimum of the FP-envelope occurs is close to the calculated optical path length in equation 4.1. It follows that $m = 2$ in equation 4.2 for the mirror PCs, but it may also be greater if redesigned. Due to the grating structure, however, the wavelength at the minimum of the FP-envelope does not exactly match with the optical path length given by the two equations but is close.

### 4.4 Initial Results Demonstrating the Fabry-Perot Reflection Dip Envelope

With the concept of the FP-envelope, the process of optimizing the mirror-PCs can be taken by sputtering a small amount of SiO$_2$ to red-shift the FP-envelope before sputtering the TiO$_2$ slab layer onto the mirror-PC. In this experimental process an in-house sputtering machine (Lesker PVD 75) is used. However, this sputtering machine does not have a crystal monitor in the vacuum chamber so the thickness of the sputtered material is unknown. The thickness of the SiO$_2$ and the TiO$_2$ can first be estimated by the reflection spectra and the resonant wavelength of the mirror-PC in the experimental setup in Fig. 4.1. To get an exact measurement, an SEM (Hitachi S-4800) image of the cross-section of the PC itself will be needed. The deposition rate of the Lesker is estimated to be approximately 1 nm/min, so the duration of the sputtering can also be used to estimate the thickness of the SiO$_2$ and TiO$_2$.
As mentioned previously, sputtering machines do not uniformly deposit the materials onto the grating structure of the cavity layer. Through some testing with the Lesker, it is found that the sputtering of SiO$_2$ onto the grating structure adds some of the material to the side walls of the grating structure increasing the filling factor of the cavity, $f_c$. This in turn will increase the filling factor of the slab, $f_s$, changing the device characteristics as can be seen by Fig. 3.11. Simulations show that increasing the filling factor of the slab will also red-shift the FP-envelope since more SiO$_2$ is added in general. After sputtering, an SEM image shows that the thickness added to each side wall of the grating is approximately 90% of the thickness added to the cavity thickness, making a significant impact on the filling factor of the cavity. Simulating the added SiO$_2$ thickness and filling factor, only 10 nm to 12 nm of SiO$_2$ needs to be added to the cavity. However, using the Lesker to experimentally sputter SiO$_2$ onto the cavity shows that only 4 nm to 8 nm of SiO$_2$ is needed to red-shift the FP-envelope appropriately. Another effect of sputtering SiO$_2$ onto the grating structure is to increase the linewidth of the device thereby lowering the quality factor and expected enhanced excitation. This is thought to occur because of the slight adjustments in the grating structure. Figure 4.5 shows the effect of sputtering 13 nm of SiO$_2$ before sputtering the TiO$_2$ slab layer leading to the grating parameters to change to $t_c = 220$ nm from $t_c = 207$ nm, $f_c = 32\%$ from $f_c = 26\%$, $\theta_c = 90^\circ$, and $d = 76$ nm.

Since the Lesker does not have a way of measuring deposition thicknesses, the TiO$_2$ is added in steps. First approximately 70 nm of TiO$_2$ is added to the grating with the new parameters. The reflection spectrum results for one of the samples are found in Fig. 4.5a showing the FP-envelope. The samples then have approximately 35 nm more deposited onto the slab layer making for a slab thickness of approximately 105 nm. In Fig. 4.5, the resulting reflection spectrum contains the FP-envelope and shows the red shift of the FP-envelope and guided mode resonant wavelength
due to the added materials. Ultimately too much SiO$_2$ was added to the cavity, but the results show well the effect of the added materials. Figure 4.5 also shows the larger linewidth between 6 nm and 7 nm compared to the linewidth in the previous results seen in Fig. 4.2 which was around 3 nm. This means the expected enhanced excitation will decrease due to the lower quality factor of the device, but this is necessary to obtain a FP-envelope minimum at the target wavelength of 637 nm. Ultimately, this provides a process to obtain the best possible results with the current equipment for the practical PCEF tests that will be run.
Figure 4.1. Schematic diagram of the reflection based setup used to obtain the reflection spectrum of 1” × 0.5” PC chips at varying incident angles. [24, 25]
Figure 4.2. (a-c) Reflection spectra at normal incidence of 1” × 0.5” mirror-PC chips that had no resonance at the 637 nm target wavelength (three of nine undesirable results). (d) Reflection spectra of a working mirror PC at normal incidence (black) and at the on-resonance angle (red).
Figure 4.3. Visualization of the Fabry-Perot reflection dip envelope concept. Simulated reflection spectra are shown at different angles of incidence showing each wavelength has a different reflection dip strength. The FP-envelope shown by the orange dashed line shows an approximate expected effect of the FP-reflection on the device. The dashed black line shows the target wavelength wanted for the mirror-PC. The target wavelength and the minimum of the FP-envelope need to coincide for an optimal response.
Figure 4.4. (a) Reflection spectra simulation results of the mirror-PC in section 3.3 and Fig. 3.4 at varying angles of incidence to show the FP-envelope. (b) Reflection spectra simulation results of the mirror-PC with an increase of 8 nm to its slab TiO\(_2\) thickness to show the red shift in both the FP-envelope and the guided mode resonant wavelength. (c) Reflection spectra simulation results of the mirror-PC with an increase of 12 nm to its cavity SiO\(_2\) thickness to show the red shift in just the FP-envelope. Simulation parameters: \(n_0 = 1\), \(n_1 = 2.35\), \(n_2 = 1.46\), \(n_3 = 1.398 + j7.457\), \(n_{Ti} = 2.166 + j2.938\), \(n_{Si} = 3.87\), \(\Lambda = 365\) nm, \(t_s = 122\) nm, \(f_s = 36\%\), \(\theta_s = 60^\circ\), \(t_c = 207\) nm, \(f_c = 26\%\), \(\theta_c = 90^\circ\), \(d = 76\) nm, \(t_{Al} = 93\) nm, and \(t_{Ti} = 85\) nm.
Figure 4.5. (a) Reflection spectra of a mirror-PC with 13 nm of SiO$_2$ added to the cavity layer and approximately 70 nm of TiO$_2$ for the slab layer showing the FP-envelope. (b) Reflection spectra of a mirror-PC with 13 nm of SiO$_2$ added to the cavity layer and approximately 105 nm of TiO$_2$ for the slab layer showing the red shift in the FP-envelope and guided mode resonant wavelength.
CHAPTER 5: MIRROR-PC FINAL RESULTS

5.1 Final Results

Following the process in the previous chapters, final samples are made from an 8” Si wafer previously mentioned by sputtering a small amount of SiO$_2$ and a larger amount of TiO$_2$ using the in-house Lesker sputtering machine. Since surface chemistry for the PCEF tests is expected to red-shift both the FP-envelope and the guided mode resonant wavelength, two 1” by 0.5” mirror-PC chips are made such that the FP-envelope minimum and the guided mode resonant wavelength are below the 637 nm target wavelength. In addition, the incident angle of the incoming light can be increased to obtain the 637 nm target for the guided mode resonance. The results of these two chips are shown in Fig. 5.1 using the reflection setup shown in Fig. 4.1. The thick black line indicates the reflection spectrum of the chip at normal incidence. The thick red line indicates the on-resonance reflection spectrum of the chip at the target wavelength of 637 nm. The lightly dashed lines give the reflection spectra of the chips at varying angles to illustrate the FP-envelope. For both of the chips, the minimum of the FP-envelope occurs at approximately 629 nm, and the on-resonance angle varies between 6.7° and 7°. The experimental linewidth of the devices is approximately 4.5 nm suggesting that the enhanced excitation of these mirror-PCs will be lower than that of the Si-PC with an experimental linewidth of 3.5 nm and a simulated linewidth of 1.8 nm shown in Fig. 2.5 and Fig. 2.6. As such, the quality factor of the mirror-PCs are lower than the quality factor of the Si-PCs.

The two 1” by 0.5” mirror-PC chips are sent to be diced by another commercial service (American Precision Dicing, Inc.) to run more tests. They are diced into a total of twenty 2 mm by 8 mm chips as this size is used in the PCEF tests in screening for E7 antibodies in serum samples.
One of these samples is used to find the incident angle spectrum using the line scanner setup as described by Chaudhery in his dissertation and seen in Fig. 5.2 [25]. Figure 5.3 shows the results from the angle scan. The result from the angle scan may seem counter-intuitive but still shows the on-resonance angle. The reflection at the on-resonance angle should theoretically be at a minimum. However, the line scanner setup that is used for the angle spectrum does not have an emission filter that only allows the 637 nm resonant wavelength to be captured. The experimental setup is also strongly influenced by the evanescent fields produced at on-resonance. These experimental results are of the resulting fluorescent signal generated. One can also think of the result as having the evanescent fields dominating the collected signal so it is not the far-field reflection spectrum of the mirror-PC. As such, the experimental results here simply show the on-resonance angle for the mirror-PC itself, which is approximately 6.43° agreeing with the reflection spectrum results in Fig. 5.1.

One of the 2 mm by 8 mm mirror-PC chips has an SEM image made of the device cross-section in order to compare experimental results with simulated results. The SEM image can be seen in Fig. 5.4a. One important structural difference between this mirror-PC cross-section and previous PC cross-sections is that the slab thicknesses on top of the grating and in the dips of the grating are notably different. These are denoted as shown in Fig. 5.4b as $t_{s}^{\text{top}}$ and $t_{s}^{\text{dip}}$, respectively, and all other parameters are the same as previously stated. The new mirror-PC is found to have the structural dimensions of: $\Lambda = 365$ nm, $t_{s}^{\text{top}} = 130$ nm, $t_{s}^{\text{dip}} = 150$ nm, $f_{s} = 43\%$, $\theta_{s} = 67^\circ$, $t_{c} = 211$ nm, $f_{c} = 26\%$, $\theta_{c} = 84^\circ$, $d = 77$ nm, $t_{Al} = 93$ nm, and $t_{Ti} = 85$ nm based on the SEM image. The SEM image also shows the imperfections caused by sputtering SiO$_2$ before the TiO$_2$ guided mode layer because each period of the mirror-PC is slightly different from the neighboring periods. This is mainly thought to be caused by the sputtering of the very thin
layer of SiO$_2$ making the grating structure rougher. With a rougher grating the quality factor of the resonator should decrease as well since some resonances will be slightly off of the desired resonant wavelength. However, this structure can still be simulated and the proper refractive indices for the materials can be found based on the experimental results. The material properties that match the experimental results are found to be: $n_{\text{air}} = n_0 = 1$, $n_{\text{TiO}_2} = n_1 = 2.15$, $n_{\text{SiO}_2} = n_2 = 1.46$, $n_{\text{Al}} = n_3 = 1.398 + j7.457$, $n_{\text{Tl}} = 2.166 + j2.938$, and $n_{\text{Si}} = 3.87$. The TiO$_2$ refractive index is much lower than the expected 2.35 in previous simulations, but the decrease in refractive index is key for the experimental results and the simulations to agree. It is assumed at this point that the in-house sputtering machine has a TiO$_2$ with a refractive index of 2.15.

The simulated results following these parameters are shown in Fig. 5.5. The reflection spectrum shown in Fig. 5.5a matches well with the experimental results presented in Fig. 5.1 for the normal incidence spectrum, the on-resonance angle spectrum, and the FP-envelope generated by varying the angle of incidence. At the on-resonance angle, the guided mode resonance has a linewidth of 3.6 nm comparing well to the 4.5 nm received in the experimental results. Figure 5.5b shows the simulated far-field angle spectrum for the target wavelength of 637 nm. The on-resonance angle is found to be 6.815° matching the experimental results as well. Lastly, Figure 5.5c shows the electric field intensity map to estimate the EF above the mirror-PC surface. The EF at the target 80 nm above the surface is found to be approximately 6.31× that of the incident wave. This is still approximately a third of the Si-PC shown in Fig. 2.6. This is to be expected however since the quality factor of the mirror-PC is approximately half that of the Si-PC and the FP-envelope is not at a minimum at the 637 nm target wavelength. After surface chemistry, the mirror-PC is expected to have a slightly larger EF due to the red-shifting expected from the process. Overall, the expected contribution of the enhanced excitation mechanism for the mirror-PC is
about half of what the enhanced excitation mechanism contribution for the Si-PC produces. This contribution can easily be matched by redesigning the mirror-PC such that the quality factors of the two match. A higher quality sputtering machine with TiO$_2$ closer to the 2.35 refractive index and a more uniform sputtering of SiO$_2$ is also expected to increase the quality factor of the resonator.

5.2 Fluorescence Test Results

Even though the expected contribution of the enhanced excitation mechanism is less than that of the Si-PC, the enhanced extraction mechanism is expected to be more due to the mirror. In order to test this, Alexa Fluor 647 fluorophores are printed onto Si-PCs and mirror-PCs to find the resulting fluorescent signal. First the PCs are cleaned and undergo a silanization process. The Alexa Fluor 647 is then printed onto the PCs using a nanoplotter. Different Alexa Fluor 647 concentrations are used to test the limits of detection for the mirror-PC. The concentrations of Alexa Fluor 647 that were used in this test are 10 $\mu$g/mL, 1 $\mu$g/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, and 100 pg/mL. After the printing of spots, the PCs are then scanned using the line scanning instrument shown in Fig. 5.2 to obtain the fluorescent signals. First the on-resonance angle for the Alexa Fluor spots are found by adjusting the angle of the incident light until the intensity of the spots are at their brightest. Since any surface chemistry is expect to shift the on-resonance angle, this angle will be different from the on-resonance angle of a bare mirror-PC. At the on-resonance angle, a scan is made of all the spots being careful not to photobleach the fluorophores. After the on-resonance scan, an off-resonance scan is made in the same area. A set of scans for one such mirror-PC is shown in Fig. 5.6 showing the on-resonance and off-resonance scans. At first look, it is easy to see that most of the spots are undetectable. In fact, the two columns seen in the image are the 10 $\mu$g/mL and 1 $\mu$g/mL Alexa Fluor concentrations. The 100 ng/mL can
barely be seen on the mirror PC, but no other concentrations have a large enough signal. The same process is done for a Si-PC, but the 100 ng/mL concentration on the Si-PC is undetectable. Because of the detectability issues of the lower concentrations, only the 10 µg/mL and 1 µg/mL concentrations are analyzed.

Figure 5.7a-b show the comparison between the mirror-PC and the Si-PC for the Alexa Fluor spots with a concentration of 10 µg/mL. Several mirror-PCs and Si-PCs are scanned and quantified by their on-resonance signal-to-noise ratio (SNR) and their enhancement factor (EF) between the on-resonance spots and off-resonance spots. The SNR is defined as the average intensity of the spot divided by the average intensity of the background for the on-resonance scan, or

$$SNR = 10 \log_{10} \left( \frac{\text{average spot intensity}}{\text{average background intensity}} \right).$$

(5.1)

The EF is defined as the average difference between the spot intensity and background intensity for the on-resonance scan over the average difference between the spot intensity and background intensity for the off-resonance scan, or

$$EF = \frac{[\text{average spot intensity} - \text{average background intensity}]_{\text{on--res}}}{[\text{average spot intensity} - \text{average background intensity}]_{\text{off--res}}}. \quad (5.2)$$

Following the definitions set by equations 5.1 and 5.2, the average SNR for the mirror-PC 10 µg/mL Alexa Fluor spots is 7.41 dB whereas the average SNR for the same spots on the Si-PC is 2.53 dB. The average EF for the mirror-PC 10 µg/mL Alexa Fluor spots is 8.36 whereas the average EF for the same spots on the Si-PC is 8.29. The same process is done for the 1 µg/mL spots on the mirror-PCs and Si-PCs, and an example image for the on- and off-resonance spot scans is shown in Fig. 5.7c-d for both PCs. The average SNR for the mirror-PCs obtained with the
1 µg/mL concentration is 0.90 dB, and the average EF for the mirror-PCs 1 µg/mL spots on the mirror-PCs is 5.49. The average SNR and EF for the 1 µg/mL Alexa Fluor spots on the Si-PCs are 0.55 dB and 4.62, respectively. From these values, it is easy to see that the mirror-PCs perform slightly better than the Si-PCs in this fluorescence test despite their lower quality factor in the guided mode resonance. Ultimately this shows the overall improvement due to the enhanced extraction due to the mirror. It is expected that these signals will only increase from mirror-PCs with FP-envelope minimums at the target resonant wavelength and guided mode resonators with a higher quality factor.

5.3 PCEF Preliminary Test Results

From the previous section, the currently made mirror-PCs show only a slight increase in performance from the Si-PCs currently in use. A preliminary PCEF test was run on these mirror-PCs to find if this increase in performance can be carried into the detection of E7 antibodies. The mirror-PCs and Si-PCs for comparison are again cleaned and undergo a silanization process. Then the protein A/G, antigen E7, and negative control BSA spots are printed onto the PCs using a nanoplotter as with the fluorescence test. These spots include protein A/G spots with concentrations of 100 µg/mL, 25 µg/mL, and 12.5 µg/mL, E7 spots with a concentration of 456 µg/mL, and BSA spots. Now however the PCs have serum introduced to them that has a concentration of 1 ng/mL of E7 antibodies for the 3 hour incubation time as mentioned in section 1.3. Then the FLISA process is performed to attach the fluorescently-labeled antibodies to the capture antibodies of the serum. After this process, the PCs can once again be scanned using the line scanning instrument in Fig. 5.2.
Figure 5.8 shows one result of a mirror-PC following the PCEF process with an on-resonance and an off-resonance scan. The same process is performed on a Si-PC to be able to compare results as shown in Fig. 5.9. From the images, it is easy to see that the background is much brighter on the mirror-PC than anticipated, and that both images include satellite spots as a result of the nanoplotter. The SNR and EF can still be found for the protein A/G and E7 spots and compared to the SNR and EF of the Si-PC. For the protein A/G spots, the SNRs and EFs are found to be similar across the different concentrations. As such the SNR for the protein A/G is found to be 8.15 dB for the mirror-PC spots and 11.45 dB for the Si-PCs following equation 5.1. The EF for the protein A/G can then be found by equation 5.2 to get 6.43 for the mirror-PC spots and 4.04 of the Si-PC. This suggests that the EF factor for the mirror-PC is better even though the background noise is worse. The spots of interest however are the E7 spots. The SNR for the E7 spots are found to be 0.98 dB for the mirror-PCs and 5.82 dB for the Si-PC. This means that the mirror-PCs are expected to raise the limit of detection for the E7 antibodies as the background noise is much more than the Si-PC. The EF for the E7 spots can also be averaged to obtain 4.49 on the mirror-PCs, and the EF of the E7 spots on the Si-PCs become 15.55. This shows that the mirror-PCs ultimately perform worse than the Si-PCs in the preliminary PCEF test.

Immediately introducing the mirror-PCs into the PCEF tests may not give an accurate end result however. Previously, a lot of work has been done toward optimizing the PCEF processes for the Si-PCs specifically. Optimizing the concentration of the printed proteins may lead to better results than those obtained in the preliminary data. Also one might notice the spread in spot size in the mirror-PC versus the Si-PC. Due to the deeper grating structure in the mirror-PC, the proteins do spread more along the grating direction. This however should not affect the concentration of the printed proteins as the proteins will bind to the surface as normal and any unbound proteins
are washed away during the assay process. Overall however, it would be best to redesign the mirror-PCs to have a greater quality factor to see an improvement.
5.4 Figures

Figure 5.1. Final reflection spectrum results of two 1” × 0.5” mirror-PC chips showing the response at normal incidence (black), at the on-resonance angle (red), and various increasing angles to show the FP-envelope (dashed lines).
Figure 5.2. Line scanning instrumentation schematic used to find the angle spectrum for PCs and the results from fluorescence and PCEF tests. The instrument uses a 70 mW solid state laser (AlGaAs) at 637 nm that is focused to a 6 μm by 1 mm line at the PC. It then collects the reflection (without the emission filter) or fluorescent signal (with the emission filter) of the sample. It is also capable of changing the incident angle of the laser and scanning across the PC with motorized stages as described by Chaudhery. [25]
Figure 5.3. Intensity of collected light for a 2 mm by 8 mm mirror-PC excited by a 637 nm solid state laser in Fig. 5.2 at varying incident angles. Due to experimental setup, this provides the user with the on-resonance angle for a bare mirror-PC at approximately 6.43°. The reflection peak occurs due to the fluorescent signal, or evanescent fields, being collected by the collection instrument.
Figure 5.4. (a) SEM image of the cross-section of a mirror-PC giving the parameters of: $n_{\text{air}} = n_0 = 1$, $n_{\text{TiO}_2} = n_1 = 2.15$, $n_{\text{SiO}_2} = n_2 = 1.46$, $n_{\text{Al}} = n_3 = 1.398 + j7.457$, $n_{\text{Ti}} = 2.166 + j2.938$, $n_{\text{Si}} = 3.87$, $\Lambda = 365$ nm, $t_{s}^{\text{top}} = 130$ nm, $t_{s}^{\text{dip}} = 150$ nm, $f_s = 43\%$, $\theta_s = 67^\circ$, $t_c = 211$ nm, $f_c = 26\%$, $\theta_c = 84^\circ$, $d = 77$ nm, $t_{\text{Al}} = 93$ nm, and $t_{\text{Ti}} = 85$ nm. (b) Schematic structure of a mirror-PC with the slab thickness ($t_s$) being split between the slab thickness on top of the grating ($t_{s}^{\text{top}}$) and the slab thickness in the dips of the grating ($t_{s}^{\text{dip}}$). All other dimensions hold with Fig. 2.1.
Figure 5.5. (a) Simulated reflection spectrum for the mirror-PC showing the response at normal incidence (black), at the on-resonance angle (red), and various increasing angles to show the FP-envelope (dashed lines). (b) Simulated reflection angle spectrum for the mirror-PC with an on-resonance angle of 6.815°. (c) Simulated electric field intensity map for the mirror-PC on resonance showing a 6.31x EF for 80 nm above the PC surface. Simulation parameters: $\lambda_0 = 637$ nm, $n_{air} = n_0 = 1$, $n_{TiO_2} = n_1 = 2.15$, $n_{SiO_2} = n_2 = 1.46$, $n_{Al} = n_3 = 1.398 + j7.457$, $n_{Ti} = 2.166 + j2.938$, $n_{Si} = 3.87$, $\Lambda = 365$ nm, $t_{s\text{top}} = 130$ nm, $t_{s\text{dip}} = 150$ nm, $f_s = 43\%$, $\theta_s = 67^\circ$, $t_c = 211$ nm, $f_c = 26\%$, $\theta_c = 84^\circ$, $d = 77$ nm, $t_{Al} = 93$ nm, and $t_{Ti} = 85$ nm.
Figure 5.6. Fluorescence test results of Alexa Fluor 647 on a mirror-PC. The concentration of the two notable columns from left to right are 10 µg/mL and 1 µg/mL. Two scans are taken of the same area: one where the Alexa Fluor spots are (a) on-resonance and where they are (b) off-resonance.
Figure 5.7. On- and off-resonance scans of Alexa Fluor 647 spots on (a) a mirror-PC with a concentration of 10 µg/mL, (b) a Si-PC with a concentration of 10 µg/mL, (c) a mirror-PC with a concentration of 1 µg/mL, and (d) a Si-PC with a concentration of 1 µg/mL. Intensity values in these images are used to find the SNR and EF for the mirror and Si-PCs.
Figure 5.8. PCEF test result on a mirror-PC with different protein A/G concentrations for a calibration curve, E7 spots with a concentration of 456 µg/mL, and BSA for a negative control. Images are of (a) an on-resonance scan and (b) an off-resonance to find the SNR and the EF for the mirror-PCs.
Figure 5.9. PCEF test result on a Si-PC with different protein A/G concentrations for a calibration curve, E7 spots with a concentration of 456 µg/mL, and BSA for a negative control. Images are of (a) an on-resonance scan and (b) an off-resonance to find the SNR and the EF for the Si-PCs and compare them to the mirror-PCs.
CHAPTER 6: CONCLUSIONS

6.1 Mirror-PCs

Overall, the mirror-PC results are not as favorable as expected when compared to that of the Si-PCs in current use in the PCEF project. Through enhanced fluorescence, the mechanisms of enhanced excitation and enhanced extraction act slightly different from what was previously expected. Enhanced excitation was originally thought to increase once a mirror is placed behind the resonant cavity, which is in general true. However, the limiting factor in the PC design process for enhanced excitation is how small a linewidth one can fabricate. If the quality factor of the resonator increases, the evanescent field strength on the surface of the PC will also increase. For the mirror-PC specifically, this can be obtained simply by lowering the grating depth as described in section 3.4. It is also seen in Fig. 3.9 that using a FP mode greater than the first order currently used will decrease the linewidth of the mirror-PC. This is particularly attractive because the Al mirror can interfere with the evanescent fields and lower the quality factor of the guided Fano resonator, so putting some space between the resonator and mirror will make for a better device. Lastly, the FP-envelope needs to be at a minimum at the target resonant wavelength. The strength of the reflection dip is a big consideration in the fabrication of mirror-PCs as any destructive interference will lower the strength of the evanescent fields, which acts as a drawback to this design. In summary, the mechanism of enhanced excitation is mainly determined by the guided mode slab layer of the PCs. Once the mirror is added behind the structure however, the FP reflections must constructively interfere with the incident wave. Using the concept of the FP-envelope helps to visualize, design, and fabricate this device to satisfy this need to obtain the best possible results. The second mechanism of enhanced fluorescence is that of enhanced extraction.
Enhanced extraction with the mirror-PC was initially thought to increase the fluorescent signal to 2× or at most 3× that of the Si-PC in current use, which is found in general to be true. Through fluorescence tests, the enhanced extraction is seen to increase the signal compensating for its loss due to the lower quality factor resonator and hence the lower enhanced excitation. This becomes useful when a mirror-PC is designed to have a similar evanescent field strength as the Si-PC currently in use because the overall enhanced fluorescence should increase.

6.2 Future Work

Any design can be further optimized or fabricated more precisely. The mirror-PC is no different. As mentioned previously, the mechanism of enhanced excitation can be increased by fabricating a more precise mirror-PC. The in-house Lesker sputtering machine is found to have two critical issues. First, the sputtering of a very thin layer of SiO₂ makes the grating structure rougher than it needs to be, increasing the linewidth of the resonator. Second, the refractive index of TiO₂ using the Lesker is found to be 2.15. This is much lower than the 2.35 seen from the mirror-PCs that were sputtered at the outside company of Intlvac. In order to make the mirror-PCs on the 8” Si wafers currently with the grating structure, it is suggested Intlvac sputter these with 5 nm of SiO₂ and then the 127 nm of TiO₂ to make the slab layer. The 5 nm of SiO₂ is an estimate but should be within 4 nm of the needed amount of SiO₂ to be added to the cavity layer. This in turn should give a FP-envelope minimum at the target resonant wavelength. One important advantage in following this process that was not mentioned before is that the on-resonance angle during the fluorescence and PCEF tests is much easier to find because the quality factor of the resonator is lower. Practically this can speed up the line scanning process and possibly eliminate some failed scans due to photobleaching.
The other option in using the mirror-PCs is to completely redesign them to have a lower linewidth and a larger cavity thickness. Separating the resonator from the lossy Al mirror will help in increasing the evanescent fields around the slab layer, so a second- or third-order FP resonance is suggested. It will also help to decrease the grating depth as this will decrease the linewidth of the device overall. As seen in the final results, the spot intensities do increase in brightness well so decreasing the linewidth should effectively make the mirror-PCs perform better than the Si-PCs. The issue with this solution is the precision needed for the fabrication of the device. Simply putting a mirror underneath the PCs makes the PCs more difficult to make with the correct amount of SiO$_2$ and TiO$_2$ for both the guided mode and FP resonance. Decreasing the linewidth of the device will further require more precision in the fabrication of mirror-PCs. Also, this process will cost more as the grating structure will have to be remade.

If the mirror-PCs are still made, many more PCEF tests need to be run. Much work has already been done in generating dose-response curves and statistics using the Si-PCs in current use. The mirror-PCs will need to go through the same process to develop their own statistics as well as make sure the results can be repeatable. This includes tests for photobleaching. With a stronger enhanced fluorescence, photobleaching may become a more difficult problem. Due to the enhanced excitation not being any stronger than the Si-PC, though, photobleaching may actually be less of an issue as the fluorophores are not as strongly excited. Ultimately, more tests involving the surface chemistry and fluorescence must be performed to finally utilize the mirror-PCs.
REFERENCES


