EVANESCENT FIELD ENHANCED FLUORESCENCE ON A PHOTONIC CRYSTAL SURFACE

BY
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DISSERTATION
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ABSTRACT

Photonic crystal (PC) surfaces have been demonstrated to be a compelling platform for improving the sensitivity of surface-based fluorescent assays used in disease diagnostics and life science research. PCs can be engineered to support optical resonances at specific wavelengths at which strong electromagnetic fields are utilized to enhance the intensity of surface-bound fluorophore excitation. Meanwhile, the leaky resonant modes of PCs can be used to direct emitted photons within a narrow range of angles for more efficient collection by a fluorescence detection system. The multiplicative effects of enhanced excitation and enhanced photon extraction combine to provide improved signal-to-noise ratios for detection of fluorescent emitters, which in turn can be used to reduce the limits of detection of low concentration analytes, such as disease biomarker proteins. Fabrication of PCs using inexpensive manufacturing methods and materials that include replica molding on plastic and nano-imprint lithography on quartz substrates result in devices that are practical for single-use disposable applications. In this dissertation I will address design, fabrication and characterization of PCs that employ the guided mode resonance effect to enhance fluorescence detection in the context of molecular diagnosis and gene expression analysis though the use of PC surfaces. A PC that can enhance the emission from multiple fluorescent species on its surface will be demonstrated. This capability is desirable in experiments using multiple fluorophores within a single imaged area like DNA microarrays. I will also demonstrate the design and fabrication of a PC on a low autofluorescence quartz substrate. This new quartz-based PC is shown to further lower the limits of detection of analytes and improve the signal-to-noise ratio. For the first time, a PC coupled to an optical cavity will be demonstrated. A metal layer added to the bottom of a PC forming an optical cavity will be shown to further improve the signal-to-noise ratio of fluorescence detection
by a factor of $6\times$ compared to detection on a PC without an underlying cavity. Finally a new photonic crystal enhanced fluorescence detection technique will be demonstrated, where fluorophores will be imaged on the surface of the PC while it acts as a feedback reflector of an external cavity laser. This new detection scheme will not only ensure optimal on-resonance coupling even in the presence of variable device parameters and variations in the density of surface-adsorbed capture molecules but also give $\sim 10\times$ increase in the electromagnetic enhancement factor compared to ordinary photonic crystal enhanced fluorescence.
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LIST OF ABBREVIATIONS

1D    one-dimensional
2D    two-dimensional
3D    three-dimensional
Cy-5  cyanine-5
Cy-3  cyanine-3
DOE   diffractive optical elements
ECL   external cavity laser
EMCCD electron-multiplying charge-coupled device
FDTD  finite-difference time-domain
FSR   free spectral range
FWHM  full width at half maximum
GMR   guided-mode resonance
LD    laser diode
LOD   limit of detection
MEF   metal enhanced fluorescence
NA    numerical aperture
NIL   nano-imprint lithography
PC    photonic crystal
PCEF  photonic crystal assisted enhanced fluorescence
PCEFM photonic crystal enhanced fluorescence microscope
PDMS  polydimethylsiloxane
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>PET</td>
<td>polyethylene-terephthalate</td>
</tr>
<tr>
<td>PMT</td>
<td>photomultiplier tube</td>
</tr>
<tr>
<td>PPL</td>
<td>poly (Lys, Phe)</td>
</tr>
<tr>
<td>RCWA</td>
<td>rigorous coupled-wave analysis</td>
</tr>
<tr>
<td>RI</td>
<td>refractive index</td>
</tr>
<tr>
<td>RIE</td>
<td>reactive ion etching</td>
</tr>
<tr>
<td>SA</td>
<td>streptavidin</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
</tr>
<tr>
<td>SFIL</td>
<td>step-and-flash imprint lithography</td>
</tr>
<tr>
<td>TE</td>
<td>transverse electric</td>
</tr>
<tr>
<td>TM</td>
<td>transverse magnetic</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor α</td>
</tr>
<tr>
<td>IL-3</td>
<td>Interleukin-3</td>
</tr>
<tr>
<td>QD</td>
<td>quantum dot</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>UVCP</td>
<td>UV curable polymer</td>
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CHAPTER 1

RESONANCE EFFECT IN ONE DIMENSIONAL PHOTONIC CRYSTALS

1.1 Introduction: What are Photonic Crystals?

Photonic crystals (PCs) [1] form a class of novel optical structures that are characterized by the periodic modulation of their refractive index. Depending on the geometry, they can be divided into three broad categories: one-dimensional (1-D), two-dimensional (2-D) and three-dimensional (3-D) structures. Examples are shown in Fig. 1.

![Fig. 1](image)

Fig. 1| Schematic of (a) 1-D (b) 2-D (c) 3-D Photonic crystals showing materials of two refractive indices period in various directions.

In 1-D PCs, the periodic modulation in the refractive index occurs only in one direction, while the structure is uniform in the other direction. An example of this is a bragg reflector which is widely used in vertical cavity surface emitting lasers. 2-D PCs possess a periodic refractive index modulation in two directions while the structure is uniform in the third direction. A periodic array of dielectric cylinders in air is an example of a 2-D PC. In nature, the pattern on the wings
of a butterfly and its rainbow play is caused by the micro 2-D PCs on the wing. 3-D PCs have refractive index modulation in all the three directions. The most well known 3-D PC that occurs in nature is the gemstone opal.

1.2 Optical Resonance in PCs

The 1-D PCs studied in this thesis comprise of a low refractive index periodic surface grating coated with a high refractive index dielectric thin film. They are a subset of diffractive optical elements or DOEs [2] that modify wavefronts by segmenting and redirecting the segments through the use of interference and phase control. Wide range of novel applications such as modulation, filtering, sensing, anti-reflection, beam shaping, splitting, combining and steering functions combined with the ability to design/simulate and fabricate these structures have spurred significant interest and effort towards the development of PCs. An important building block of the DOE is the diffraction grating, a periodic planar or 3-D structure, which depending on the application can be comprised of dielectric, semiconductor or metal materials. Under certain conditions an uneven distribution of light in a restricted parametric space, such as wavelength or incident angle, can occur in these grating structures. These are called anomalies, and they were first reported by R. W. Wood [3] in 1902 and since then they have undergone a thorough theoretical and experimental investigation [4-12]. These abrupt changes in the grating efficiency curve are categorized into two groups: Rayleigh anomalies and resonance anomalies [4, 13, 14]. A Rayleigh anomaly in a given spectrum occurs at the wavelength for which the scattered wave emerges tangentially to the grating surface. The position of the Rayleigh anomaly can be calculated from the grating equation:
Here $\theta$ is the angle of incidence (measured anticlockwise from the normal to the grating), $\theta_n$ is the angle of diffraction (measured clockwise), $\lambda$ is the wavelength in air and $\Lambda$ is the grating period. The above formula allows one to rigorously calculate the diffraction angle of any scattered order $n$ from the grating period, the angle of incidence and the wavelength of light. Thus the Rayleigh anomaly occurs when $\sin(\theta_n) = \pm 1$. From the Eq. (1.1), the spectral position of the anomaly can be calculated for a light incident at angle $\theta$, as [15]:

$$\lambda = \Lambda/n (-\sin(\theta) \pm 1)$$

(1.2)

**Fig. 2** Examples of dielectric structures with periodic index variation (a) dielectric-waveguide grating (b) planar photonic slab with holes etched into a high refractive index slab.

Resonance anomalies were first studied in metallic gratings and are caused by surface excitation effects [15-19] in them. At the interface between a dielectric and a metal, there are specific conditions under which a charge density oscillation (“electron wave”) can be supported, which carry light intensity away from the incident beam and therefore decrease the diffraction efficiency of the grating. The efficiency curve would show a sharp drop in intensity at the corresponding conditions. In dielectric structures with periodic index variations like dielectric-waveguide grating [10, 20] and photonic crystal slabs [21, 22] (as shown in Fig. 2.), these resonant anomalies can give rise to certain guided-mode resonances (GMR), in which 100% of
optical energy can be switched between reflected and transmitted waves over small parameter ranges. Physically, this is due to the presence of certain guided-modes of a dielectric slab that can couple to the radiation modes and possess a finite lifetime. These modes therefore become *guided-mode resonances*. They are called ‘guided’ since they are closely related to the guided-modes in a uniform dielectric slab (i.e. without any surface variation) and should therefore retain significant portions of the electromagnetic power within the dielectric slab. An example of guided-mode resonance phenomenon is shown in Fig. 3 where simulated transmission and reflection spectra of the photonic slab shown in Fig. 2(b) is plotted [21]. Figure 4(a) shows the electric field amplitude at the transmission monitor point as a function of time for the same calculation that gives the spectra in Fig. 3. The time sequence consists of a distinct pulse and a tail of long decay. The presence of these two stages indicates the existence of two pathways in the transmission processes. The first pathway is a direct transmission process, where a portion of the incident energy goes straight through the slab and generates the initial pulse. The Fourier transformation of the initial pulse should account for the background in the transmission spectra. The second pathway is an indirect transmission process, where the remaining portion of the incident energy excites the guided-mode resonances. The power in the resonances then decays slowly out of the structure and produces the long decaying tail. By Fourier transforming the decaying tail, the typical symmetric Lorentzian line shapes, as shown in Fig. 4(b) is obtained. A similar observation can be made for reflected spectra as well. Thus the transmission or the reflection property of these dielectric waveguide structures is determined by the interference between the direct and the indirect pathways.
Fig. 3 | (a) Transmission and (b) reflection spectra. The solid lines are for the photonic slab structure shown in Fig. 2(b). The dashed lines are for a uniform dielectric slab with a frequency-dependent dielectric constant and thickness same as Fig. 2(b). (Reprinted from [21]).
Fig. 4| (a) Field amplitude at the transmission monitor point as a function of time for the same calculation as shown in Fig. 3. (b) Fourier transformation of the amplitude shown in (a). (Reprinted from [21]).
The application of guided-mode resonances in 1-D PCs towards fluorescence enhancement is the main focus of this thesis. Extensive experimental and theoretical work has been done to study guided-mode resonances in 1–D PC structures [8-11, 21, 23-25]. The nature of the interaction of the 1-D PC with an incident plane wave can be understood by using a simple ray picture model. In its simplest form, a 1-D PC is made up of a substrate, a wave-confinement layer and a grating layer (Fig. 5). When this structure is illuminated by a plane wave, a part of the wave gets transmitted out directly through the structure, while a part gets diffracted by the grating and is trapped in the wave-confinement layer. Some of the trapped light in the wave-confinement layer is then re-diffracted out of the wave-confinement. This re-diffracted wave couples out of wave-confinement layer in both forward and backward directions so that it interferes both with the reflected part and the transmitted part of the wave. At a specific wavelength and angle of incidence, the 1-D PC resonates, that is to say a complete destructive interference occurs with the transmitted light and no light is transmitted through the structure, while a complete constructive interference with the reflected light gives the characteristic resonance response of the PC, namely, a sharp reflection peak. For a PC without any defects or material loss, the reflection peak is 100% [10]. Section 1.3 explores the above in some detail. Since the modes in a planar wave-confinement layer are different depending upon the polarization of the incident field, the resonant response of the structure is different depending on the polarization of the incidence wave. However, it is possible to obtain a polarization independent response with 2-D symmetric PCs [26]. The two incident polarization directions used in this thesis are Transverse Electric (TE) and Transverse Magnetic (TM). TE polarization is same as s-polarization; with the polarization pointing along the grating in case of 1-D PCs. TM polarization is p-polarized light with the polarization pointing perpendicular to the grating direction in 1-D PCs.
Fig. 5| Schematic of a 1-D PC.

Fig. 6| Simulated total reflected and transmitted power for the PC shown in Fig. 5 for a TM polarized light shining normally on the surface. At resonance wavelength 100% of the incident light is reflected back resulting in no transmission at that wavelength.
Figure 6 shows simulated total reflected and transmitted power for the PC shown in Fig. 5 for a TM polarized light shining normally on the surface. The structure was simulated using rigorous-coupled-wave-analysis technique (RCWA). Commercial RCWA software (Rsoft Inc.) was employed for the simulations. RCWA is a non-iterative technique for periodic structures that converges to an exact solution without inherent numerical instabilities. The accuracy of the results obtained by this technique depends on the number of harmonics that are included in the analysis, with convergence being obtained for relatively low amount of computation. The parameters used in this simulation are: grating period ($\Lambda$) = 400 nm, grating depth ($d$) = 40 nm, duty cycle or fill factor = 0.5 times the period and the wave-confinement layer thickness ($t_{\text{TiO}_2}$) = 100 nm. The wave-confinement layer is made up of TiO$_2$ with refractive index of $n_{\text{TiO}_2}$ = 2.35. The medium above the PC is air ($n_{\text{air}}$ = 1) and the substrate is glass with index of $n_{\text{Glass}}$ = 1.46. A sharp dip in the transmission or peak in the reflection signifies the PC resonance ($\Delta \lambda = 8$ nm at $\lambda = 633$ nm). A $Q$-factor, signifying the strength of the resonance can be calculated from this spectral resonance for this PC. $Q$-factor is defined as the ratio of the energy stored in a resonator to the energy supplied to it per cycle. It characterizes the leaky nature of the PC. For this PC, it can be calculated as $\lambda/\Delta \lambda = 70$. PCs with $Q$-factor $\sim 200$ have been demonstrated before. Despite the tendency of the light to couple out of the wave-confinement layer through the grating, a PC has a modest $Q$-factor compared to high-$Q$ resonators like whispering gallery resonators, micro-ring resonators, etc., [27, 28].

The above resonance response of the PC can be well controlled by the physical parameters of the PC like grating depth, material properties, period, etc. This makes a PC very useful in applications like tunable-bandwidth reflectance filters [29]. They can be integrated into classical thin film multilayers to produce high-efficiency filter response and arbitrarily low sidebands over
a large spectral range [30]. They have been shown to operate as high-efficiency reflection filters at the Brewster angle at which TM reflection is classically forbidden [31, 32], as high-efficiency narrow band transmission filters [29, 33], as mid-infrared reflectance filters for discrete frequency infrared spectrometry [34] and as narrow band notch filters [35]. PCs have been used with semiconductor materials to modulate light at specific wavelengths [36-38] and with quantum dots to enhance their down conversion efficiency [39]. PCs with high reflection efficiency have been employed as external mirrors in a dye laser system [40, 41] and as miniature planar focusing elements [42]. Besides there application in filters, PCs are now being extensively studied for their use as high throughput biosensors [43-48] and platform for enhanced fluorescence detection [26, 49-57]. They have been demonstrated to enhance second- and third harmonic generation [58]. Planar organic lasers operating on the basis PCs have also been demonstrated [59, 60]. PCs are also being used to trap light in thin-film solar cells to increase optical absorption [61-63]. PCs can be coupled with other optical cavities like a Fabry-Perot cavity to enhance emission from nanoemitters within the cavity [64, 65]. They are being coupled with plasmonic sensors to enhance Raman scattering [66-68].

1.3 Guided-Mode Resonance in 1-D Photonic Crystals: Ray Picture Model

This section builds upon the simple ray model analysis by Rosenblatt et. al [10, 36] to understand guided mode resonances in 1-D PCs. We begin by considering an asymmetric wave-confinement slab with grating etched into it, as shown in Fig. 7(a). The refractive index of the wave-confinement layer is given by \( n_3 \) and the indices of superstrate and substrate are \( n_1 \) and \( n_4 \) respectively. The structure is assumed to be of infinite extent and is illuminated by a TE polarized plane ray.
Fig. 7 | (a) Schematic showing paths of various rays when a TE polarized light is incident at an angle $\theta$ on a 1-D PC. The transmitted rays are labeled as t and s. The rays propagating in the wave-confinement layer are marked in orange. (b) Geometry for computing the optical path difference between rays t and s. The wavefronts at point A and E are marked in dashed blue.

When the TE polarized plane ray is incident at point A, a part of the wave in the forward direction undergoes 0th order diffraction (i.e. it refracts through the wave-confinement layer into the transmitted ray t), while the rest of it gets diffracted into the 1st order light that travels inside the wave-confinement layer according to the phase matching equation:
Here \( k = 2\pi/\lambda \) is the wavevector of light making angle \( \theta \) with the normal, \( m \) is the order of the diffraction, \( K = 2\pi/\Lambda \), \( \Lambda \) is the grating period and \( \psi \) is the internal diffraction angle. For a certain grating period, this diffracted light is coupled into the guided-mode and undergoes total internal reflection at point C. Then, arriving at point D, a part of the light is re-diffracted and emerges out of the wave-confinement layer on the transmitted side (marked s) while the rest is reflected back into the wave-confinement layer and propagates as its resonant mode and the above cycle is repeated. Figure 7(a) shows this ray picture. The rays in red inside the wave-confinement layer get transmitted out while the rays in orange are coupled to the guided modes of the wave-confinement layer.

For such an asymmetric wave-confinement slab, the propagating guided-mode of order \( m \) should satisfy the following phase condition [10, 69]:

\[
2k_3 d + 2\Phi_{13} + 2\Phi_{34} = 2m\pi
\]  

(1.4)

Here \( k_3 = n_3 k \sin(\psi) \) is the wavevector of light in the z direction in the wave-confinement layer. \( \Phi_{13} \) and \( \Phi_{34} \) are the phase shifts related to the two Fresnel coefficients of the medium. \( \Phi_{13} \) is the phase shift due to refractive index difference between layers 1 and 3, while \( \Phi_{34} \) is the phase shift due to refractive index difference between layers 3 and 4 [10, 69]. Thus when condition (1.3) is satisfied, the trapped rays, which bounce back and forth within the wave-confinement layer, form a single wave that propagates as a guided mode in the layer.
On the transmitted side, rays t and s undergo interference with each other. The phase difference, $\Phi$, between these two rays arises from the phase shifts introduced by diffractions, total internal reflection and the difference in the optical paths of these two rays i.e.

$$\Phi = \Phi_{\text{path}} + \Phi_{r} + 2\Phi_{d} \tag{1.5}$$

Each of the terms in the above equation can be calculated as follows:

(i) $\Phi_{\text{path}}$ (Optical path difference). Consider Fig. 7(b), which shows the paths of each ray. To calculate the optical path difference between t and s we will make the following considerations to simplify our calculations: We will draw a wavefront for the incident ray at point A (shown as u in blue). Consider a point F on that wavefront s.t. the incident ray originating from F will hit the grating at a point B, where the distance between A and B is $p\Lambda$, with $p$ being an integer. Now let us draw a wavefront on ray CD that will also intersect the grating at point B. This gives us the same starting points for the two transmitted rays, now marked as t’ and s’. The path difference between t’ and s’ is now calculated as: distance travelled by light in the wave-confinement layer before it hits point B i.e. ACD-DE; distance travelled by incident light in the superstrate before it hits B i.e. BF. Then the optical path difference between t’ and s’ is:

$$\Delta = n_{3}(ACD - DE) - n_{1}BF$$

$$= n_{3}\left(\frac{2d}{\sin(\psi)} - \cos(\psi)\left(\frac{2d}{\tan(\psi)} - p\Lambda\right)\right) - n_{1}p\Lambda \sin(\theta) \tag{1.6}$$

$$= n_{3}(2d \sin \psi + \cos(\psi) p\Lambda) - n_{1}p\Lambda \sin(\theta)$$

Using Eq. (1.3), the last equation above can be reduced to:
\[ \Delta = 2dn_3 \sin(\psi) + \frac{mp2\pi}{k} \]  

(1.7)

The optical phase difference due to paths is then given by:

\[ \Phi_{\text{path}} = k\Delta = 2kd n_3 \sin(\psi) + mp2\pi = 2k_3d + mp2\pi \]  

(1.8)

The factor of \( mp2\pi \) can be ignored as it adds integral multiples of \( 2\pi \) phase to \( 2k_3d \).

(ii) \( \Phi_r \) (Total reflection phase). This phase difference can be derived from Fresnel’s equation for TE polarization as [70]:

\[ \Phi_r = 2\Phi_{34} \]  

(1.9)

(iii) \( 2\Phi_d \) (Phase due to two diffractions, each at point A and D respectively). This phase has two contributions: \( \pi/2 \) coming from classical diffraction [71] and the other coming from Fresnel phase shift due to the refractive index contrast between layers 1 and 3. Thus:

\[ \Phi_d = \Phi_{13} + \frac{\pi}{2} \]  

(1.10)

Finally adding all of the above phases, we get the total phase difference between rays t and s as:

\[ \Phi = \Phi_{\text{path}} + \Phi_r + 2\Phi_d \]

\[ = 2k_3d + 2\Phi_{34} + 2\Phi_{13} + \pi \]  

(1.11)

Using equation (1.3), the above equation is reduced to:

\[ \Phi = 2m\pi + \pi \]  

(1.12)
Thus, we find that at resonance the transmitted and diffracted wavefronts are completely out of phase and interfere destructively to give 0% transmitted power. A similar exercise can be done to show that there is 100% reflection at resonance.

1.4 Classification of 1-D Photonic Crystals

The general construction of PCs is basically the same i.e. a wave-confinement layer with a grating coupled to it. However, depending upon their shape and continuity of the wave-confinement layer, they can be broadly classified into three categories: (a) the waveguide-grating, (b) the symmetric slab type grating and (c) the doubly corrugated grating. Although the design approach and the fabrication methodology among these three categories may be different, the underlying principle of operation is the same i.e. excitation of guided-mode resonances. The materials that are suited to make wave-confinement layers in the visible region include titanium dioxide (TiO$_2$), tantalum pentoxide (Ta$_2$O$_5$) and hafnium dioxide (HfO$_2$). The low index material used as substrates or gratings in these structures include silicon dioxide (SiO$_2$), glass, plastics like Polyethylene terephthalate and heat cure or UV cure polymers.

![Waveguide grating type planar PC. (a) The grating is etched into the wave-confinement layer. (b) The grating is etched into a low refractive index material which is deposited onto the wave-confinement layer.](image)

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**Fig. 8** Waveguide grating type planar PC. (a) The grating is etched into the wave-confinement layer. (b) The grating is etched into a low refractive index material which is deposited onto the wave-confinement layer.
The waveguide-grating type PC is shown in Fig. 7. Within this category it can have two types of configurations. Figure 8(a) shows the first type where a high refractive index material forms a wave-confinement layer on a substrate and the grating is etched onto it. Such type of PC can be fabricated by depositing the wave-confinement layer using evaporation or sputtering technique and then using e-beam lithography, nano-imprint lithography, deep-UV lithography or photolithography to pattern the layer using a resist as a mask. Finally the wave-confinement layer can be dry-etched to a required thickness to form the grating on the top. However, patterning the wave-confinement layer to etch it back is best suited for cases where the grating depth to be etched is small i.e within 10 nm – 30 nm. The reason for this limitation is that the dry-etch process which can be very slow for certain metal oxide dielectrics due to the formation of non-volatile etch-byproducts. Thus the selectivity between the common resists used in lithography and the metal oxide dielectric can be of concern, limiting the depth to which they can be etched. A way to get around this problem and to have deeper gratings is to deposit a second layer of lower refractive index material such as silicon dioxide that has higher selectivity with respect to the lithography resist and pattern it with periodic lines. This allows for possibilities of longer etch times, thus giving deeper grating profiles. Figure 8(b) shows this second type of waveguide-grating PC. Using this technique grating depths up to several 100 nm can be realized.

The second type of planar PC structure is a symmetric slab type [72, 73]. Figure 9 shows this structure. The structure of this PC is very similar to the waveguide-grating type PC with the grating region etched into the wave-confinement layer, however unlike waveguide-grating structure symmetric slab structure does not have a substrate and the grating region is completely etched through the wave-confinement layer. Thus these structures are hardest to fabricate, as the substrate needs to be removed after patterning the grating region. However they do possess an
advantage of enhanced sensitivity in applications such as sensing and modulation due to considerably enhanced volume between the PC and its surrounding environment.

Fig. 9 | Side view and top view of a symmetric slap type planar PC. The wave-confinement layer is patterned through the PC and is surrounded by background with refractive index $n_1 < n_2$.

The third type of planar PC is called a doubly corrugated grating and is shown in Fig. 10. In this structure there is no clear demarcation between the high index wave-confinement layer and the grating regions as both of them are structured [74]. Unlike the waveguide grating structure and symmetric slab structure which contain a well differentiated waveguide layer, the doubly corrugated structure does not contain the waveguide in the sense of its usual meaning as there is not any uninterrupted layer of a high refractive index material that is designed to convey a wave from one plane to another. This structure is designed to be lossy and the light does not propagate in the direction perpendicular to the grating by the process of partial back reflections and the development of an electromagnetic standing wave. The advantage of this structure is the ease and cost effectiveness of the fabrication process [74, 75]. This PC structure is fabricated by first
forming the grating region in a low refractive index material. Besides using lithography to pattern the grating, nano-replica molding process can be used to fabricate the grating region on flexible substrates like a plastic sheet [76]. In this process a master wafer is first fabricated with the negative image of the final grating pattern by one of the many lithography techniques. A UV cure polymer is then sandwiched between the wafer and a plastic sheet and cured under UV light. Finally the plastic sheet is peeled away from the master wafer leaving the grating region imprinted onto the hardened polymer. A thin layer of high refractive index material can then be deposited on top of the grating region completing the PC structure. This technique allows the fabrication of PC devices in a high throughput roll-to-roll method.

![Fig. 10](image.png) Doubly corrugated planar PC. The high index layer is deposited onto the replicated grating region.

In this thesis the doubly corrugated PC has been studied for its application towards enhanced fluorescence detection due to its ease and cost-effectiveness of fabrication.
Fig. 11| Layout of the one-dimensional doubly corrugated photonic crystal. The structure is comprised of a low refractive index quartz substrate \((n = 1.456)\) containing one-dimensional periodic structure \((\Lambda = 400 \text{ nm})\) and coated with a high index layer of TiO\(_2\) \((t_{\text{TiO}_2} = 119 \text{ nm}, n = 2.35)\). The physical parameters of the device, refractive indices of the materials and the launch angle \((\theta)\) of the incident beam determine the resonance wavelength.

1.5 Parameters Affecting Resonance Response of a Photonic Crystal

The layout of the one-dimensional PC is shown in Fig. 11. The structure is comprised of a one-dimensional grating of height \(d\), which is fabricated in a low refractive index \((n)\) substrate. The structure is then coated with a layer of high refractive index material of thickness \(t\). The period of the structure is given by \(\Lambda\). As a representative example, we study the response of such a device under external illumination using RCWA. An index resolution of 1.5 nm and 30 harmonics were used in the calculations. The materials were assumed to be lossless and dispersionless at about \(\lambda = 633 \text{ nm}\). One period of the device was simulated with a periodic boundary condition applied in the x direction. The device is exposed to a superstrate i.e. air. The device parameters used in the
The simulation were \( \Lambda = 400 \) nm, \( d = 25 \) nm, \( n_{\text{TiO}_2} = 2.35 \), \( n_{\text{Quartz}} = 1.456 \) and \( t = 119 \) nm. The fill factor or the duty cycle for the above PC is 50%.

Figure 12 (a-b) shows the polarization dependent response of this PC as a function of incidence angle (\( \theta \)) and the wavelength (\( \lambda \)) of the light. The color scale shows the diffraction efficiency (far-field reflectivity). From the Fig. 12 we can see that the resonance response of the PC can be broken down into two parts, namely, the position of resonance defined by the wavelength and angle of incidence and the linewidth of the resonance. It is seen that while the spectral location is mainly governed by the structural parameters of the PC like its period and the thicknesses of various layers, the linewidth is controlled essentially by the losses in the PC – absorption loss and diffraction loss. Considering the PC shown in Fig. 11, it is helpful to understand the relationship of spectral position of the resonance and the linewidth on various physical parameters of the PC.
Fig. 12| Calculated resonant mode band structure for the one-dimensional photonic crystal structure, shown for (a) TE polarized incidence and (b) TM polarized incidence. Y axis shows the spectral location of the resonance for a given incident angle in degrees. The color scale shows the far-field reflectivity of the resonance, and the widths of the bands are inversely related to the resonance \textit{Q-factor}.

1.5.1 Period of the Grating

For simplicity let us consider a wave incident normally onto the PC. In order to couple this beam into the PC modes, the phase matching condition that needs to be satisfied can be written as:

\[
\lambda = n_{\text{eff}} \Lambda
\]  

(1.13)
Here $n_{\text{eff}}$ is the effective index of the resonant mode at the resonant wavelength of $\lambda$ for a PC with grating period $\Lambda$. The above condition is the same as Bragg’s law. Thus for a given set of PC materials, the spectral location of the resonance is set by its period. Figure 13 plots this relationship for the PC, when its period is varied from 300 nm to 500 nm. A continuous increase in the resonance wavelength is seen as the period is increased. On the other hand, the period of the PC doesn’t seem to have much effect on the linewidth of the resonance.

![Figure 13](image)

**Fig. 13**| Variation of the TM-mode resonance with respect to the period of the grating.

### 1.5.2 Thickness of the Waveguide

Figure 14 shows the effect of the TiO$_2$ thickness on the spectral position of the resonance. Since, for the doubly corrugated PC there is no clear demarcation between the waveguide and the grating, it is not straightforward to study the dependence of waveguide thickness on the resonance. However, it is observed that as the thickness of the TiO$_2$ is increased on top of the PC, the spectral location of the resonance shifts towards the red end of the spectrum, although
the shift is not linear with respect to the thickness. Another observation that can be made is that as the TiO$_2$ thickness is increased, higher order resonances are observed (above TiO$_2$ thickness of $\sim 200$ nm). These higher order resonances corresponds to the higher order GMR and appear as the thicker waveguide can now support higher order guided modes. A slight increase in the resonance linewidth is also observed for modes at the higher wavelengths.

![Fig. 14](image)

**Fig. 14** TM-mode resonance with respect to the TiO$_2$ thickness. As the high-index layer becomes thicker, the resonant wavelength shifts towards the red end of the spectrum, due to increased effective index. After a specific TiO$_2$ thickness, higher order GMR modes appear.

1.5.3 Grating Profile and Refractive Index Contrast

The effect of the grating depth on the PC resonance is shown in Fig. 15. The grating in a PC is a diffractive element that couples the light into and out of the PC and thus is a source of the diffraction loss in the PC. The diffraction loss is directly related to its diffraction ‘strength’ which is essentially related to its depth and to the index contrast between the grating and its surrounding. As the depth of grating decreases, the linewidth of its resonance also decreases thus
increasing the $Q$-factor of the structure. Similarly as its modulation decreases its diffractive strength decreases, increasing the $Q$-factor again. Figure 16 plots the reflection spectrum for a rectangular grating profile and a triangular grating profile.

**Fig. 15** TM-mode resonance with respect to grating depth. As the grating depth increases, the diffractive strength of the grating increases, increasing the diffraction loss in the structure. This is depicted by increase in the linewidth of the resonance.

**Fig. 16** Reflection intensity for a rectangular and a triangular grating profile of depth 50 nm. Remaining PC parameters are same for both the structures. The reduction in linewidth for triangular profile is clearly evident from the plot.
Fig. 17| TM mode resonance with respect to the loss introduced in the high index layer. Increasing waveguide loss results in increase of the resonance linewidth and reduction in the reflectivity.

1.5.4 Absorption Loss

Absorption loss is due to the complex value (κ) of the refractive index of the materials used in a PC. Since the resonant modes are localized mainly in the high refractive index layer, the resonance is most sensitive to the absorption loss in this layer. A large absorption loss will cause a drastic reduction in the reflectivity of the PC and will make linewidths very wide. Figure 17 shows the effect of the absorption loss in the TiO$_2$ layer. As the value of absorption is varied from $\kappa = 0$ to 0.005, a dramatic reduction in the reflectivity from 100% to 40 % is observed. The linewidth of resonance also increases. In the experiments outlined in this thesis, the high refractive index material used was sputtered TiO$_2$. The average value of $\kappa$ for TiO$_2$ was measured to be less than $10^{-6}$ at visible wavelengths.
1.6 Modal Profile and Field Enhancement at Resonance

In section 1.3 it was shown how all the trapped waves within the waveguide layer form a single wave that propagates as a guided mode in the layer. This implies that the incident wave that is diffracted into the structure is in phase with the guided mode and continuously re-enforces the guided-modes in the structure in the steady state. Thus, the energy density within the waveguide increases and saturates in the steady state. The magnitude of the energy density in the steady state is set up by the diffraction loss encountered by the quasi-guided modes.

![Diagram of field amplitude build-up at the resonance within the structure for analyzing field enhancement.](image)

Consider the dielectric waveguide grating of section 1.3 again (shown in Fig. 18). Let the incident field amplitude be $E$. Let $c$ be the coefficient defining the coupling efficiency of the incident field into the guided-modes of the waveguide at the point A and $d$ be the coupling efficiency of the wave to couple out of the structure at the point D. The electric field amplitude of the wave entering the waveguide at point A is then given by $Ec$. The field amplitude of this
wave after point D becomes $Ec(1-d)$. On the other hand, the illumination at point D adds another $Ec$ field to the existing amplitude in the waveguide. Thus after point D, the total field amplitude becomes:

$$E_1 = Ec(1 - d) + Ec$$  \hfill (1.14)

Here both $c$ and $d$ are $< 1$. If we consider this wave continuously propagating in the waveguide, the total field amplitude after infinite number of diffractions and re-enforcements becomes:

$$E_{total} = \left[(Ec(1 - d) + Ec)(1 - d) + Ec\right](1 - d) + Ec \ldots (1)$$  \hfill (1.15)

The sum of the above series is given by:

$$E_{total} = \frac{Ec}{d}$$  \hfill (1.16)

Thus the electric field intensity within the waveguide under steady state becomes:

$$I = \frac{E^2 c^2}{d^2}$$  \hfill (1.17)

The coefficients $c$ and $d$ depend on the parameters of the grating and define the $Q$-factor of the structure. Thus the ratio of $c/d$ controls the amplification of the field intensity within the structure. Typically the diffraction loss in the structure decreases as the perturbation provided by grating goes to zero.

Figure 19(a) shows the modal profile of the $E_x$ electric field component for the structure shown in Fig. 11 at normal incidence resonance at the wavelength $\lambda = 633$ nm. The dramatic amplification of fields at the resonance is clearly evident from the figure. The sinusoidal
variation in intensity above the device surface represents the incident and the reflected waves.

Figure 19(b) shows a slice of Fig. 19(a) through the center.

**Fig. 19** Steady state electric field response for TM resonance. (a) cross-sectional field distribution and (b) a vertical cut through x = 0 nm for $E_x$ field amplitude.
Fig. 20| Steady state electric field response for TM resonance. (a) cross-sectional field distribution and (b) a vertical cut through $x = -104$ nm for $E_z$ field amplitude.

Figure 20(a) shows the modal profile of the $E_z$ electric field component at the normal incidence resonance. There are no incident and reflected waves above the structure and $E_z$ is composed of purely resonant modes within the waveguide with evanescent tails extending above and below the high index layer. Figure 20(b) shows a slice through the plot at the center of a
hotspot showing the intensity profile along the z direction. This forms the basis of various sensing applications for PCs. When an optically distinct material is deposited within the extent of the evanescent tail on the surface of the PC, it alters the effective refractive index and thus the path length of the guided modes. This results in a shift of the resonance condition and is manifested as a change in the position of the reflection and the transmission wavelengths or angles. Also, the heightened field intensity on the surface makes it possible to drive the fluorescence process for molecules on the surface of the PC with much greater efficiency.

1.7 Conclusion

This chapter introduces photonic crystals and the phenomenon of guided-mode resonance. Throughout this thesis, the field enhancement property of the 1-D PC is applied to enhance the signal intensity emitted from fluorophores that are bound to the surface of the PC. The following chapters will talk in detail the motivation to enhance fluorescence and various approaches that have been applied with the PC towards this goal.
CHAPTER 2

ENHANCING FLUORESCENCE ON A PHOTONIC CRYSTAL

2.1 Introduction

Biological assays based upon fluorescence detection are the cornerstone of applications that include molecular diagnostics, gene expression microarrays, genome sequencing, protein-protein interaction screening, cell imaging, and many others [77]. The ability to couple a wide variety of fluorescent materials such as organic dyes, fluorescent proteins, and quantum dots with biological molecules and cells has revolutionized our ability to understand complicated biological phenomena, and to selectively detect biological targets within complex media. Typical fluorescence-based assays include determining how much of a specific biomolecule is present in a test sample and visualizing how molecules function within cells. A vast array of fluorescent labeling materials, illumination sources, and detection instruments have been developed for fluorescence-based assays that enable these methods to achieve detection of disease biomarker proteins in serum at concentrations below 1 pg/mL, to perform sub-diffraction limit imaging, and to perform highly multiplexed biomolecular interaction analysis with throughput exceeding millions of individual locations on a single chip [78, 79]. Fundamentally, fluorescence is an electromagnetic and quantum mechanical phenomenon in which electrons within the fluorescent molecule are excited to oscillate as a dipole by an externally applied electromagnetic field. Electrons remain in the excited state for a short time, and subsequently relax to their ground state by emission of a photon, which then propagates away as an electromagnetic field [77]. Seen as a dipole antenna for collection and re-emission of electromagnetic energy, a fluorophore is capable of interacting with its immediate environment.
A fluorophore’s rate of excitation, excitation lifetime, and emission direction can be engineered to achieve much greater detection sensitivity than would be possible for the same emitter attached to a plain glass surface or suspended in solution.

Efficient excitation and recovery of light emitted from fluorescent molecules by employing photonic structures or nano-patterned substrates can result in greatly enhanced signal-to-noise ratio (SNR) fluorescence detection. A high SNR measurement is highly desirable for fluorescence experiments involving fluorophore-tagged analytes at low concentrations. There is now a wealth of literature citing improvements in fluorescence output when metallic surfaces are placed in close contact with a fluorescent system [80-83]. Metal-enhanced fluorescence (MEF) is partly due to a modification of the radiative decay rate of a fluorophore in close proximity to a metallic surface [84, 85]. The result is a reduced spontaneous decay time, an increased probability of photon emission, and consequently a higher quantum yield. Metallic nano-islands and colloids support localized surface plasmon resonance and create local uniform local electromagnetic field “hot spots” with which fluorophores can interact and fluorescence with greater intensity, due to enhanced light absorption rate. However, the lossy metallic material dissipates energy from the fluorophores in a direct non-radiative process, known as the metal quenching effect, which leads to reduced fluorescence output [86]. Because maximum quenching occurs within the same locations close to a metal surface where the electric field enhancement is also maximized, MEF methods have not achieved the same scale of enhancement factor that is available from high quality-factor dielectric-based resonators.

Recent research by the Cunningham Group at the University of Illinois has demonstrated that a nanostructured photonic crystal (PC) optical surface is capable of enhancing the signal of fluorescent dyes by more than two orders of magnitude [87]. In contrast to MEF, the PC
enhanced fluorescence (PCEF) provides a consistent and highly efficient platform for enhancement of fluorescent signal by exploiting its optical resonance. A wide variety of PC structures have been studied and fabricated for an enormous range of applications since the periodic nanostructure was first proposed to control the spontaneous emission of materials embedded within the PC by engineering the photonic density of states [88]. Here, the PCs designed for enhanced fluorescence applications are comprised of a periodically modulated low refractive index dielectric surface structure coated with a high refractive index dielectric thin film. Optical resonance in the PC is excited when evanescently diffracted orders couple to modes existing in the high refractive index layer [10, 36]. PCs can be engineered to interact strongly with any optical wavelengths of interest through selection of their materials and the parameters of their geometry.

PCEF takes advantage of PC resonances associated with two phenomena: *enhanced excitation* and *enhanced extraction*. The simultaneous implementation of both enhancement features on a PC substrate has been shown to boost the emission from dye molecules by greater than three orders of magnitude [87].

PCEF can be applied to any surface-bound fluorescence assay. Being of particular interest for high-throughput analysis, PCEF was implemented for DNA and protein microarrays. The microarray, consisting of a solid substrate populated with spots of immobilized biological capture molecules, has become an important analytical tool for life sciences research [89]. A microarray may be comprised of thousands or even millions of capture probes that bind to target molecules in a complex biological sample. When a test sample is incubated with the microarray, the analyte molecules can bind specifically to their complementary immobilized ligands. A fluorescent label is added either before or after this binding reaction. The fluorescence from
microarray spots is quantified with optical instrumentation to quantify the amount of each specific analyte molecule probed during the assay. Currently, microarrays are performed on glass microscope slides, and are capable of detecting analytes over ~5 orders of magnitude in analyte concentration, with detection limits typically on the order of 5-10 pg/ml, depending upon the affinity of the capture molecule for its target, and the assay conditions [90, 91]. By using photonic crystals as the substrate to perform the microarray assay, we can take advantage of both enhanced excitation and extraction effects to reduce detection limits, to increase SNR for analytes that are near the detection limits, and to enable a detection instrument to operate using lower-cost components. Such a capability will enable disease biomarkers to be detected at lower concentrations for earlier disease diagnosis, and increases the potential for multiplexed biomarker assays to be performed in a clinical setting.

Besides microarrays, other fluorescent assays would also benefit from greater signal-to-noise ratios. Improvement in the efficiency of fluorescence excitation and the efficiency of emission photon collection can enable detection instrumentation to utilize miniature, low power laser sources and inexpensive uncooled imaging cameras, which would result in portable, low-cost detection systems that can be used in clinical settings.

In this chapter I will discuss various aspects of PCs that enhances the emission of the fluorophores kept in its proximity. The effects of enhanced excitation and enhanced extraction briefly mentioned in the introduction will be explained in more detail. Conditions that couples light effectively to a PC in order to benefit from enhanced fluorescence will be discussed and the three fluorescence detection systems: the confocal laser scanner, the fluorescence microscope using a collimated laser illumination, and the laser line-scanning instrument, that make use of
them will be described. Finally I will give some examples where PCEF have been utilized in the past to improve the sensitivity of detection of a biological assay.

**Fig. 21** Simulated near-electric field intensity profiles for the resonant modes at $\lambda = 632.8$ nm for (a) TE polarized incidence, showing enhancement of only the $E_y^2$ field component and TM polarized incidence showing enhancement of both (b) $E_x^2$ and (c) $E_z^2$ field components. The color scale associated with each figure represents the intensity of the electric field and is normalized to the unit intensity incident wave.
2.2 Enhanced Excitation

In the context of increased fluorescence output from a dye molecule on the PC surface, the increase in the evanescent electric field intensity is called *enhanced excitation*. Physically, the optical resonances of a PC are excited when an external light source like a laser shines onto the surface of the PC while satisfying all the conditions of a resonance, for example the wavelength, polarization and the incidence angle. In addition, these optical resonances can be engineered to have a very high electromagnetic field density on the surface of a PC during their finite lifetime. The magnitude of this electromagnetic density localized within the PC is directly proportional to the $Q$-factor of the resonance or its lifetime, which in turn is controlled by adjusting the device parameters. The intensity of emission from the fluorophores can be greatly enhanced by placing them in the proximity of the PC surface which has its resonant wavelength overlapping the absorption spectrum of the fluorescent species due to an enhanced absorption rate.

Since the resonant response of the structure is different depending on the polarization of the incidence, with TM resonances being narrower in linewidth, implying a higher $Q$ for the same device structure, they have higher electromagnetic field density on their surfaces and are preferred to TE resonances for the purposes of enhanced excitation. As fluorescence processes mainly arise from electric dipole oscillations, only electric field components are considered in this work. The near electric fields produced by a PC in response to both TE and TM polarized incidences are shown in Fig. 21. Figure 21(a) shows the electric field intensity ($E_y^2$) for TE polarized incidence. Clearly, the maximum near-field intensity occurs where the resonant mode is confined, with the evanescent tails penetrating both substrate and superstrate of the PC. For the purpose of fluorescent enhancement, these enhanced fields represent a greater incident energy density. From the near-field plots in Fig. 21(b) and 21(c), we see the consequence of the higher
$Q$ TM mode on the near field intensity, which is clearly much greater than the TE case. Another point to note is that the TM resonance causes enhancements for both the $E_x$ and $E_z$ near-field components, which might be more beneficial (assuming similar overlap intensities for all the field components) than just the $E_y$ field enhancement produced in the TE case, as the fluorophore dipole moments in ensemble experiments can be assumed to be equally distributed in all three dimensions. For the TM polarized case, the continuity of the displacement field across dielectric boundaries requires that the electric field must be lower in the higher index material, which is evident in Fig. 21(b-c). From the Fig. 21 it can be clearly seen that at the PC resonance, the electromagnetic field intensity that drives the fluorescent output can be as high as 200 times the incident field for a modest $Q$-factor PC ($Q \sim 370$) at normal TM illumination.

Enhanced excitation works selectively in regions within close proximity to the PC surface due to the exponential decay of the electric field intensity from the PC into the superstrate region (i.e. air) above the PC (as seen in Fig. 21). The average decay length of the fluorescence enhancement factor from the surface of the device, for a resonant wavelength $\lambda = 632.8$ nm, has been found $\sim 85$ nm [35]. For a given set of material refractive indices, the decay length is essentially controlled by the resonance wavelength. The relationship between the decay length and the resonance wavelength is approximately linear in the wavelength range where the dispersion of the materials is small. This exponential decay in the strength of the evanescent field allows the PC to share one of the advantages of TIRF microscopy, namely only fluorophores close to the substrate surface are excited [36]. Rather than utilizing a TIRF microscope, a conventional confocal microarray scanner or a modified fluorescence microscope can be used for rapid imaging of large areas without a coupling prism.
The effect of enhanced electromagnetic field intensity on the emission intensity of a surface-bound fluorophore (quantum dots, QDs 405) can be seen in Fig. 22. Quantum dots were dispersed on the PC surface and the un-patterned surface surrounding the PC (acting as the control for comparison) as marked by the circles in the Fig. 22. The quantum dot emission intensity image on

![Fig. 22](image)

_Fig. 22_ Fluorescence (pseudocolor) scan images of the PC with quantum-dots dispensed on the surface. (a) Scan taken when the PC is resonant with respect to the incident beam ($\theta = 11.2^\circ$), showing an enhancement factor of over 108 times and (b) Scan taken when the PC is not resonant with the incident beam ($\theta = 0^\circ$), showing an enhancement factor of over 13 times. The circular regions represent the area over which intensity information was averaged. In both the images, the circle to the left shows the control region where no PC is present. (Reprinted from [92]).
the left (Fig. 22(a)) represents the condition when the PC is resonant (resonant wavelength $\lambda = 405$ nm at $\theta = 11.2^\circ$) with respect to the incident laser beam ($\lambda = 405$ nm), showing an enhancement factor of over 108 times with respect to the control. On the right is the emission intensity image when the PC is not resonant with the incident laser ($\theta = 0^\circ$), i.e. without any pockets of strong localized electromagnetic fields. For this case the PC shows enhancement of 13 times with respect to the control. This off-resonant enhancement can be understood in terms of the enhanced *extraction effect* which will be explained in the following section.

### 2.3 Enhanced Extraction

The presence of rich and highly flexible photonic bands of a PC allows for a possibility to engineer the spatial relationship between its resonant modes. This enables a coupling effect to take place, where the emission from a fluorophore kept in the proximity of a PC can couple to one of the resonant modes of the PC and then emerge out in an angle dependent direction. This provides us with a benefit of a powerful mechanism where the light emitted by a fluorophore can be redirected without loss towards specific directions where it can be detected with much higher sensitivity. Such a scheme is particularly useful in improving the detection efficiency of instruments meant to gather the fluorescence signal and can further help in reducing the detection limits of the fluorescent species.

When a point source like a fluorophore is kept in the proximity of a PC, it emits directly into the superstrate and the substrate. However when there exists a PC resonant mode that overlaps with the fluorescence emission spectrum of relevant polarization both spatially and in wavelengths, the emission gets coupled into the resonant mode and emerges out at angles that can be computed by using the phase matching condition for that PC:
\[ \sin(\theta) = \left( \beta \pm \frac{m2\pi}{\Lambda} \right) k_0 n_s \]  

(2.1)

Here \( k_o \) is the free-space wave-vector, \( n_s \) is the index of the medium from where the external light is incident at an angle \( \theta \) with the surface normal of the PC, \( m \) is the diffraction order and \( \Lambda \) is the period. \( \beta \) is the real part of the propagation constant of the resonant mode of the PC. This implies that for any given wavelength of coupled light, the angle of escape can be arbitrarily chosen by appropriate choice of the photonic structure.

Fig. 23| Leaky mode dispersion and angle-resolved fluorescence. Comparison between the (a) RCWA calculated leaky mode dispersion and (b) the resonant features in the angle-resolved fluorescence measurements for the hexagonal lattice along the directions of high symmetry. (Reprinted from [93]).

In most fluorescence detection instruments the detection optics are kept directly above the emitting species. When fluorescent species bound to the PC surface are imaged with such
instruments, the PC is engineered so as to direct the coupled radiation normally towards the detection optics. This enables the majority of the emitted radiation to be detected efficiently, unlike the radiation emitted by a fluorophore on the glass substrate with its emission almost uniform in all directions. Experiments have been performed to verify this process and, understand its role, in fluorescence enhancement for PCs possessing different symmetries [93]. It has been shown that for a narrow spectral and angular detection instrument, a PC with increased symmetry leads to greater enhancement of emitted radiation in the normal direction. This has been attributed to increase in the number of diffraction planes that can interact with the omnidirectional radiation. Enhancement factor over 200 times has been observed for detected fluorescent radiation for a PC with hexagonal lattice symmetry compared to an unpatterned surface using an objective of \( NA = 0.15 \). Figure 23 shows the effect of enhanced extraction for a PC with hexagonal symmetry. The simulated dispersion diagram of the PC matches well with the angle resolved fluorescence measurement demonstrating that the emitted radiation couples to the resonant mode of the PC and then re-radiates along the angles satisfying the phase matching condition for the PC. The increase in the measured fluorescence intensity along the normal incidence angle suggest that the re-radiation of most of the emission was along the normal incidence and hence the decrease in the measured intensity along other angles.

The effects of enhanced excitation and enhanced extraction act independent of each other. Hence when they act together, their effects get compounded. These two effects can be independently optimized to design a PC that provides very large fluorescence enhancement factors. With the combined effect of the two, enhancement factors as large as 7500 have been demonstrated [87], making PCs very desirable platform for a wide array of fluorescent biological sensing applications.
2.4 Instrumentation for PCEF Detection

This section will discuss the parameters for efficient excitation of PC resonant modes and the design of detection instruments that have been used for PCEF.

2.4.1 Coupling condition for 1-D PC

When a 1-D PC is illuminated by a broadband light source, highly efficient reflections from its surface represent the resonance modes at a specific wavelength and a specific angle combination. Figures 24(b) and 24(c) show such measured resonant modes for the PC shown in Fig. 24(a) when the angle of incidence is scanned from 0° to 20°, both in \( \phi \) and \( \theta \) directions respectively (labeled in Fig 24(a)). Here, \( \theta \) is the angle between the incident beam and the grating’s normal vector in the plane perpendicular to grating direction and \( \phi \) is the angle in the plane along the grating direction. These photonic band diagrams were obtained by illuminating the device with collimated broadband light from a tungsten lamp, and analyzing the reflected light with a spectrometer (USB 2000, Ocean Optics) as a function of incident angle. It is evident from the figures that the resonant wavelength is not very sensitive to the angles along \( \phi \) direction while it changes dramatically when the angle of incidence is varied along the \( \theta \) direction. Using a narrow bandwidth light source, such as a solid-state laser, a misalignment of incidence angle by 0.1° with respect to the resonant angle \( \theta_R \) will reduce the coupling efficiency of the laser to the PC by a factor of 10. On the other hand, the resonant modes exhibit a very small angular dependence (~0.3 nm/°) along the \( \phi \) direction. Therefore, to efficiently couple light into the 1-D PC surface, the excitation laser beam only needs to be collimated and tuned along the \( \theta \) direction.
Fig. 24| (a) Schematic of the PC structure (not to scale). The grating is oriented along the y-axis. (b) Simulated photonic band diagram of the PC surface with $\phi$ varied from $0^\circ$ to $20^\circ$ for a fixed $\theta = 0^\circ$ and (c) simulated photonic band diagram of the PC sensor with $\theta$ varied from $0^\circ$ to $20^\circ$ for a fixed $\phi = 0^\circ$.

2.4.2 Confocal laser scanner with tunable angle of incidence

A confocal microarray laser scanner (LS Reloaded, Tecan Inc.) was the first detection instrument used to study PC enhanced fluorescence. The schematic of the scanner is shown in Fig. 25(a). This system is equipped with a He-Ne laser and a solid state Nd-YAG laser as the excitation sources and a photomultiplier tube (PMT) to detect the emitted fluorescence signal. The angle of incidence of the lasers can be tuned from $0^\circ$ to $25^\circ$. In order to form an image, the substrate is scanned and the fluorescence signal intensity for each pixel is acquired. The instrument uses a lens to focus the laser beam onto the sample and collects the fluorescence signal resulting from this excitation. Due to the focusing effect, the laser beam has a beam divergence $\sim 2.5^\circ$. Since
the beam is not collimated, a substantial portion of the excitation energy does not get coupled into the resonant mode of the PC, thus compromising the enhancement performance of the PC.

![Diagram](image)

**Fig. 25** (a) Schematic diagram of the confocal laser scanner. (b) Schematic drawing of PC enhanced fluorescence microscope. (Reprinted from [94]).

### 2.4.3 PC Enhanced Microscope using collimated laser source

The schematic of a custom-built fluorescent detection system, which is referred to as the PC enhanced fluorescence microscope (PCEFM), is shown in Fig. 25(b). In the PCEFM system, the fluorescent sample is imaged by an electron-multiplying charge-coupled device (EMCCD, Hamamatsu Inc.) via a 4× microscope objective (numerical aperture $NA = 0.1$). Unlike the confocal laser scanner, the PCEFM works in an imaging mode, which significantly improves the measurement throughput. A He-Ne laser is used as an excitation light source, and a bandpass filter is placed in front of the detector to reject excitation laser light.

The PCEFM setup is designed specifically to use a collimated illumination with a tunable angle of incidence. As shown in Fig. 25(b), the output of the He-Ne laser is expanded to produce a beam with diameter of 20 mm and divergence $< 0.037^\circ$ using a beam expander. In order to
accurately control the angle of incidence, the PCEFM system utilizes a high-precision angle-tuning gimbal-mounted mirror that is mounted on a motorized linear stage and moves as the mirror rotates. The movement of this linear stage compensates for the beam shift due to any incidence angle variation and thereby ensures a fixed illumination area. The angle tuning resolution of this configuration is 0.005°, enabling one to test PC devices with angular dependence on resonance as narrow as 0.01°. Using this system a coupling efficiency of 98% has been achieved with a PC surface with angular dependence of 0.3° [95].

2.4.4 Objective Coupled Line Scanner for PCEF

It has been shown that the resonant modes of a 1-D PC are not very sensitive to the changes in angle along $\phi$ direction. Although the PCEFM does an excellent job at coupling the incident light to the modes of a PC, there is considerable loss in the excitation power in the process of collimating it. In order to solve this problem of loss in power density, a newer detection modality, which employs excitation source in form of a line, has been demonstrated recently [96]. The goal of this line scanner is to focus light in the $\phi$ direction while maintaining the collimation in the $\theta$ direction, thus increasing the power density supplied to the fluorescent dye molecules and consequently improving the fluorescent signal strength.

A schematic diagram of this new configuration is illustrated in the Fig. 26. The illumination setup of this system comprises of a 70 mW solid-state laser (AlGaAs) at $\lambda = 637$ nm coupled to a polarization maintaining fiber, a half wave plate, a cylindrical lens, a long pass dichroic mirror, and a $10\times$ objective (Olympus Plan N) of focal length 18 mm. The fiber tip is coupled to a fiber collimator giving a highly collimated output beam of 3.4 mm in diameter. The output beam then passes through a half-wave plate, which is used to rotate its polarization to match with the PC-
resonant mode to be excited. The laser beam is then focused in the $\phi$ direction to a line by a cylindrical lens ($f' = 100$ mm). The focused laser line is then directed onto the back focal plane of the microscope objective via a dichroic mirror. The output of the objective is thus a laser beam focused to a line.

![Diagram of the objective-coupled line-scanning microscope.](image)

**Fig. 26** Schematic of the objective-coupled line-scanning microscope. The incident beam path is clearly demarcated in red and the collection beam path is demarcated after the dichroic mirror in burgundy. The collection and illumination beam paths overlap in the region between the dichroic mirror and the photonic crystal. (Reprinted from [97]).

The PC is placed on a motorized sample stage (MS2000, Applied Scientific Instruments) that is translated perpendicular to the laser line for a fast scan (750 lines/second). The fluorescence image is constructed by sequential scanning across the sensor in fixed increments. The PC, placed at the focal plane of the infinity corrected $10\times$ objective ($f_0 = 18$ mm), interacts with a beam that appears collimated in one plane but focused in the other. The theoretically expected line width of a focused beam is given by:

$$w_0 = \left(\frac{4\lambda}{\pi}\right)\left(\frac{f}{D}\right)$$

(2.2)

Here $\lambda$ is the wavelength of the laser beam, $f$ is the focal length of the focusing lens and $D$ is the diameter of the incident beam. The theoretical linewidth of the beam focused on the back focal
plane of the objective is calculated to be 23.85 µm. This linewidth is important when characterizing the angle of divergence for the incident beam. The theoretical linewidth in the front focal plane of the objective is calculated to be 4.29 µm. These calculations are subject to the assumptions of a perfectly Gaussian laser beam and perfectly aspherical lenses.

The assembly of the cylindrical lens, half-wave plate and fiber collimator are mounted on a two-dimensional adjustable stage. In order to achieve angle tuning, the line-focused beam is translated on the back focal plane of the objective by tuning the position of the cylindrical lens-wave plate-fiber collimator assembly. This fine stepping is achieved by utilizing a motorized linear stepping stage (Zaber LSM-25). The result is a change in the incident angle in the $\theta$ direction. The emitted fluorescence signal is collected by the objective and projected onto a CCD camera (Hamamatsu 9100C) by a tube lens ($f = 150$ mm).

The ultimate goal of the line scanner is to provide illumination that efficiently couples excitation photons to the PC, while providing spatial resolution sufficient for visualization of microarray spots that may be 5-200 µm in diameter.

**Fig. 27** Fluorescent images of microspots from a sandwich immunoassay (TNF-α and IL-3) for two different concentrations obtained using the objective coupled line scanning system. (Reprinted from [98]).
2.5 Photobleaching on Photonic Crystal Enhanced Fluorescence Surfaces

As PCEF works by enhancing the localized fields near the fluorescent species, the rate of photobleaching of fluorophores is an obvious question to ask. A study was performed by Chaudhery et al. to compare the rate of photobleaching of the fluorophores on a PC surface to their rate on unpatterned substrates [99]. Figure 28 shows the averaged fluorescence intensities collected for over a period of 159 sec for 16 streptavidin conjugated Cy-5 spots both on a PC substrate and a glass substrate. The spots on the two substrates were excited by He-Ne laser for various incidence angles, one of which was the resonance angle of the PC. In the figure the highest fluorescence intensity value corresponds to the resonance angle of the PC (i.e. $\theta = 2.03^\circ$) when the localized electric fields are the strongest. As expected, when we move away from the resonance angle, the strength of the localized fields decreases and the fluorescence intensity gets lower till we reach an angle of $\theta = 20^\circ$ where the laser is not at all coupled to the PC resonance modes. Note that even when the PC is illuminated at an angle that is far from the resonance condition ($\theta = 20^\circ$), we still obtain greater signal output compared to the glass surface. This high signal output is due to the enhanced extraction effect. Thus from Fig. 28 we see that for higher degrees of enhancement there is a higher rate of photobleaching. It is important to note here that even though the rate of photobleaching is higher for the PC on resonance when compared to the unpatterened glass, after hundreds of individual exposures, an enhancement factor $> 100\times$ is still observed relative to measuring the same fluorophore on a glass surface after the final scan. In fact, it can be extrapolated from the figure that both the surfaces would have to be subjected to a constant exposure of the laser incident at the resonance angle for over 2000 sec for the fluorescence intensity on the PC to equal that on the glass. Thus, for all practical experimental
time frames, the fluorescence signal for the PC on-resonance will be higher than the fluorescence
signal from an unpatterened glass substrate.

![Figure 28](image)

**Fig. 28** Plot of the measured fluorescence intensity versus time for continuous exposure at different
angles of incidence for excitation light. (Reprinted from [99]).

### 2.6 Utilizing PCEF for Improving the Sensitivity of Microarray Applications

Since their first introduction in the scientific literature in 1982, microarrays have revolutionized
the study of gene expression. Microarray analysis has extended to the high throughput study of a
host of other biological analytes such as proteins, peptides, tissue, cells, antibodies, and chemical
compounds. In our work, we have demonstrated significant gains in detection sensitivity by
applying the PCEF technology to the microarray platform in the context of DNA and protein
microarrays. Below are the two examples where a PC fabricated on a plastic substrate was
applied to improve detection sensitivity of microarrays.

#### 2.6.1 PCEF for Differential Gene Expression Analysis

The DNA microarray gained prominence as a fluorescence-based tool for the high throughput
quantification of gene expression, allowing a large number of candidate genes to be examined
for differential expression simultaneously without extensive prior knowledge of gene functions.
Eukaryotic gene expression is typically characterized by a large number of genes expressed at very low levels and a decreasing number of genes expressed at high levels [100, 101]. Usually the fluorescence intensity of only high expression genes - a small fraction of all genes in a cell population - can be detected above the noise in the experiment. However, the profiling of low expression genes, many of which have important housekeeping functions, is also of keen biological interest. To better quantify these low abundance genes, we began by pursuing the engineering and fabrication of surface PCs on plastic substrates. The nanoreplica fabrication process produces PCs with uniformity over large areas necessary for high density microarrays comprised of thousands of capture probes. The PCs were fabricated in the format of standard microscope slides, allowing them to be seamlessly incorporated into the standard assay workflow alongside control glass slides, and imaged using a commercially available fluorescence microarray scanner.

To facilitate the binding of DNA molecules to the PC, the slides are functionalized with a vapor-based epoxysilane-based surface chemistry [102] and each PC was paired with a silanized glass microscope slide that acted as the control sample. A set of 192 oligonucleotides representative of soybean genes [103] were printed in replicates of 40, for a total of 7680 spots per slide; the spots were incubated and then UV crosslinked. Total RNA was extracted from freeze dried soybeans seeds (Glycine max cultivar Williams), purified and labeled with Cy5 by reverse transcription. Printed slides were blocked with bovine serum albumin and then hybridized overnight at 42°C with the labeled samples. Approximately 40 µg of total RNA was used per slide. All slides received identical treatments throughout the assay. All slides were scanned using a confocal microarray scanner (LS Reloaded, Tecan) using a TM polarized laser (λ = 632.8 nm) and an emission filter with a range of 670-710 nm. All slides were scanned at
identical PMT gain settings and at a pixel resolution of 10 μm. Glass slides were scanned at normal incidence (0 degrees) while the PCs were scanned at their respective resonant angles ($\theta_{\text{excitation}}$). Spot SNR was calculated as the local background subtracted spot intensity divided by the standard deviation of the local background.

The overall effect of the PC enhanced fluorescence phenomena is to amplify the fluorescent signal from molecules within approximately 100 nm of the PC surface. This PC was engineered to enhance the common microarray dye Cyanine-5 (Cy5) by more than one order of magnitude when scanned in a commercial microarray scanner [104]. The application of this PC design to a 1-color microarray experiment was pursued where the differential expression was assessed between *Glycine max* cotyledons and trifoliates, which represent tissues from two distinct developmental stages in the soybean plant [26].

The signal enhancement factor is defined as spot intensity minus the local background observed on the enhancement substrate divided by the same value observed on the glass slide or control substrate. The signal enhancement factor observed from Cy-5 spots with high expression genes in this microarray experiment was approximately $60\times$ (see Fig. 29).

However, SNR enhancement is a more relevant measure over signal enhancement because microarray data analysis programs use SNR values to classify spots as detected or not detected. It is possible to achieve good signal enhancement without achieving similar SNR enhancement if a substrate enhances fluorescence but also has a large noise value, thus avoiding any advantages of fluorescence enhancement. We observe that the PC not only attains a large signal enhancement but it also achieves an SNR enhancement of approximately $10\times$ (measured over all spots in the experiment), suggesting that the array can detect hybridization at concentrations $10\times$
lower than can be detected on glass substrates. Since the SNR is enhanced by more than one order of magnitude on PC substrates, genes with expression levels that were lower than the noise floor on glass substrates can now be measured on PCs. This allows researchers to preserve the advantageous throughput of microarrays while increasing the sensitivity of their measurements. The practical effect of the PC is to improve the dynamic range of the expression measurements and allow for quantification of low expression genes. The direction of differential expression in these low expression genes is confirmed by sequencing data, which agreed with the microarray analysis for 39 of the 41 genes identified as differentially expressed only on PC microarrays. By expanding the dynamic range of the microarray experiment, the number of genes for which statistically significant changes in expression could be observed improved from 26 to 66 genes, or from 13\% to 34\% of the genes probed in the experiment. This suggests that the detection
capabilities of current microarray protocols can be greatly expanded by just the substitution of conventional substrates with an enhanced fluorescence substrate like the PC. By enhancing fluorescence, more than double the number of genes were identified on the PC as differentially expressed between the trifoliate and cotyledon tissues, demonstrating that enhanced fluorescence offers practical benefits to a DNA microarray experiment (Fig. 30).

Fig. 30] Replicate-averaged SNR values for 192 genes probes are presented in a logarithmic plot. Genes are ordered by their average SNR value and a cutoff line at SNR = 3 is used in each graph to determine the number of genes detected. Cotyledon expression profiles are plotted in (a) for a glass slide and (c) for a PC slide, respectively. Trifoliate expression profiles are presented in (b) for a glass slide and (d) for a PC respectively. (Reprinted from [26]).

The increased SNRs provided by the PCs may allow researchers to perform experiments that are currently problematic on glass slides. Because lower amounts of bound sample can be detected with the PC, sample sizes may be reduced to volumes that would be difficult to probe using normal glass substrates. This may be particularly helpful for profiling gene expression in limited tissue samples or small populations of rare cells. This approach is not limited to
conventional DNA microarray experiments. Any surface-bound biomolecular assay can be performed on these PCs for improved performance, as we will next illustrate with protein microarrays.

2.6.2 PCEF for Multiplexed Protein Biomarker Detection

Antibody-based protein microarrays are a valuable tool for studying cellular protein production with potential applications as a clinical tool in disease diagnosis and drug discovery [105-108]. Protein microarrays are a favorable platform for the detection of circulating biomarkers because they combine multiplexed detection, minimal reagent usage, and high sensitivity. By running calibration standards alongside patient samples, protein microarrays provide quantitative measurements of analyte concentration. Furthermore, protocols have been developed that demonstrate multiplexed detection of biomarkers in serum through fluorophore-tagged secondary antibodies [109, 110]. In many clinically relevant applications, such as for detection of biomarker proteins that are expressed by cancer cells at a tumor site and subsequently diluted by the total blood volume of a person, a target protein may only be present at concentrations in the 1-100 pg/mL range [111-114]. There is substantial interest in extending the limits of detection (LOD) and generally increasing the SNR in order to diagnose disease at the earliest possible stage and to quantify biomarker levels at concentrations that were below previous limits of detection. Fluorescent-based detection of chemically tagged analytes has been demonstrated as a robust, highly specific, and easily multiplexed method for achieving high sensitivity [115-120].

We have successfully utilized PCEF to develop a high-sensitivity platform for the detection of a panel of >20 breast cancer biomarkers in a protein microarray format [51, 52]. Our results show that the resonant excitation effect increases the signal-to-noise ratio by 3.8- to 6.6-fold, resulting
in a decrease in detection limits of 6-89%, with the exact enhancement dependent upon the antibody-antigen interaction. Dose-response characterization of the photonic crystal antibody microarrays demonstrates the capability to detect common cancer biomarkers in the <2 pg/mL concentration range within a mixed sample.

The first step in the assay is to print capture antibodies on the slide; replicate arrays are printed to assess the experimental variability in each slide. The slide is blocked to limit the non-specific binding of analytes in subsequent steps. The slide is then incubated with a test sample consisting of a mixture of biomarkers. Next, the slide is washed to remove all unbound biomarkers and then incubated with a mixture of biotinylated secondary detection antibodies. Finally, the secondary detection antibodies are labeled by incubation with streptavidin-Cy5 (Fig. 31). In this platform, alongside the test samples, it is routine to generate a standard curve by assaying a concentration series that covers a 10000-fold range of protein concentrations.

**Fig. 31** (a) Schematic diagram of the protein microarray assay format. Each microscope slide is divided into 16 wells and each well consists of 20 capture antibodies printed in quadruplicates. (b) A fluorescence image of a well obtained at the completion of the assay; spot color is representative of biomarker concentration.
Fig. 32| (a) Fluorescence images of Cy5 labeled protein microarray spots from a block exposed to a mixture of 20 biomarkers. Images on the left and right indicate the block on the PC scanned on resonance and off resonance, respectively. Images were obtained with a confocal microarray laser scanner and at identical gain settings. (b) Comparison of the replicate-averaged fluorescence intensity at PC resonance and off PC resonance for all functional assays in the array. Error bars indicate +/- one standard deviation of 8-replicate spots. (Reprinted from [51]).

The effect of PC enhanced fluorescence was determined by comparing the fluorescence output when the PC is illuminated with the excitation laser at an incident angle matched to the PC resonant angle ("on resonance", in this case $\theta = 0^\circ$) to the fluorescence output when the illumination angle is not at the PC resonance ("off resonance", in this case $\theta = 20^\circ$). The fluorescent image of one block of an array exposed to the second highest concentration in the dilution series depicts the typical signal enhancement observed when the PC is imaged on-resonance. The fluorescence signal intensity was enhanced by a factor of 11 to 20-fold by illuminating the PC at its resonant condition (Fig. 32).
The PC enhances the output of any fluorophore within the evanescent field region, regardless of whether the source of the fluorescence is a Cy5 molecule within the capture spot area, a Cy5 molecule nonspecifically bound outside the capture spot area, autofluorescent material within the device structure, or autofluorescence from the chemical functionalization layer. It is observed that when the PC is on-resonance, the background intensity is 4 to 5-times higher than in the off-resonance condition. Even so, an overall SNR enhancement of 3.8- to 6.6-fold was observed for the assays because the magnitude of the PC enhancement is greater within the capture spots than in the regions between the spots. Observed enhancements in SNR is particularly important for detecting antigens at low concentrations – for example, two antigens EGFR and uPAR were detected at concentrations as low as 3.6 and 7.1 ng/mL, respectively. At the PC off resonance, the spot signals for EGFR and uPAR at these same concentrations were noise-limited (SNR < 3) and could not be differentiated from the local background fluorescence. However, at the PC resonance, these same spots were detectable (SNR > 8) over the background. The ability to detect reduced concentrations of such antigens is extremely important to the early detection of disease biomarkers, which in general are present at very low concentrations in serum. The signal intensities from each dilution in the concentration series were used to generate standard curves using the Protein Microarray Analysis Tool (ProMAT) software, developed by Pacific Northwest National Laboratory. A representative standard curves for TNFα when the PC is on-resonance and off-resonance is presented in Fig. 33. We found that when on-resonance, the PC demonstrated better precision as indicated by the steeper slope in the linear region of the standard curves, and ~10-fold reduced limit of detection.
Fig. 33 Standard curves for TNF-a when the PC is imaged at its resonance condition (solid curve) and off resonance (dashed curve). The PC demonstrates higher detection sensitivity at its resonance condition as indicated by the steeper slope in the linear region of the solid curve. Sensitivity here is defined as the change in fluorescence signal per unit change in its concentration. (Reprinted from [51]).

2.7 Conclusion

So far we have seen how PCs can be utilized for enhancing fluorescence in the context of multi-spot microarrays. In the next few chapters, I will present the work I have done over the course of my PhD to advance this technology. So far I have only discussed enhancing fluorophores bound to PC surfaces excited by one specific wavelength, exciting the PC resonance at only that wavelength. In general there are many applications that require more than one fluorophore that are excited at different wavelengths to be imaged simultaneously. These applications require PC substrates that can enhance multi-color fluorophores simultaneously. Chapter 3 will discuss the work done in this area. Utilizing PCs as substrates amplifies the fluorophore intensity relative to the substrate fluorescence intensity; however the impact of substrate fluorescence on the measurements is of major concern. Substrate auto-fluorescence is an important contributor to the experimental noise in a microarray, making the choice of this material critical. To address this concern, Chapter 4 will discuss new developments in the area of PC fabrication utilizing ultralow
autofluorescence substrates. Chapters 5 and 6 will introduce a new enhanced fluorescence detection modality that combines the PC assisted enhanced fluorescence discussed so far with optical cavities to obtain enhanced fluorescence assisted by PC coupled to a cavity. This new technique has interesting features and benefits compared to traditional PCEF.
CHAPTER 3
MULTI-COLOR FLUORESCENCE ENHANCEMENT FROM A PHOTONIC CRYSTAL SURFACE

3.1 Motivation

Many biological fluorescence assays, such as gene expression microarrays [121, 122], have been developed using multiple fluorescent dyes within a single imaged area. In order to enhance the emission from multiple fluorescent dyes, the PC surface must be designed with resonant wavelengths coinciding with the wavelength of multiple lasers that are used to excite the target fluorophores. The PC structure intrinsically supports resonant modes in a wavelength range as wide as 200 nm [123], because each wavelength couples resonantly with the structure for a distinct angle of incidence. Therefore, by designing a PC that can resonantly couple light from multiple excitation lasers – each at a distinct incident angle – it is possible to provide enhanced fluorescence for multiple dyes. The fluorescence images of each dye can then be taken using a multi-laser confocal scanning detection instrument which has the ability to excite the PC surface with a selected angle of incidence, and the wavelength/angle combination that yields PCEF. In this work, I describe the design and characterization of a PC surface that is used to enhance the fluorescence from fluorophores Cyanine-5 (excited by a $\lambda = 632.8$ nm HeNe laser) and Cyanine-3 (excited by a $\lambda = 532$ nm diode pumped solid state laser).
Fig. 34 | Cross-sectional diagram (not to scale) of the PC. The dimensions are as follows: period: $\Lambda = 360$ nm, grating depth: $d = 25$ nm, SiO$_2$ thickness: $t_{SiO_2} = 80$ nm and TiO$_2$ thickness: $t_{TiO_2} = 120$ nm. The grating width is 35% of the period.

Fig. 35 | RCWA simulated dispersion diagram for the PC used in this study. Resonance for enhanced excitation for the TM mode is $\theta \sim 9.2^\circ$ for Cy-5 and $\theta \sim 13.8^\circ$ for Cy-3 excitation.
Fig. 36| RCWA Simulated near field distribution for one period of the PC used in this study at $\lambda = 532$ nm and $\lambda = 633$ nm.

3.2 Sensor Design and Fabrication

A cross-sectional diagram (not to scale) of the PC surface is shown in Fig. 34. The one-dimensional (1-D) grating structure was formed in ultraviolet curable polymer (UVCP) on a polyethylene-terephthalate (PET) substrate by the nanoreplica molding technique [124]. The polymer grating surface was coated with a high refractive index dielectric layer of TiO$_2$ which functions as a wave confinement layer. Under broadband illumination, a highly efficient reflection represents resonance at a specific wavelength and a specific angle. A simulation tool (DiffractMOD, RSoft Design Group) based on the rigorous coupled-wave analysis (RCWA) technique was used to design the 1-D surface PC structure. In order to support resonances at $\lambda = 632.8$ nm and $\lambda = 532$ nm concurrently, RCWA simulation results stipulated the use of a grating with a period of $\Lambda = 360$ nm. Other device parameters include the grating depth of $d = 25$ nm, TiO$_2$ thickness of $t_{\text{TiO}_2} = 120$ nm, UVCP refractive index of $n_{\text{UVCP}} = 1.47$, and TiO$_2$
refractive index of $n_{\text{TiO}_2} = 2.35$. The transmission spectra were calculated by RCWA and the minimum transmissions (or maximum reflections) in the spectra were used to identify the resonant mode supported by the designed structure [92]. As shown in Fig. 35, a photonic band diagram for the structure shown in Fig. 34 was calculated for TM modes in the $510 \text{ nm} < \lambda < 660 \text{ nm}$ wavelength interval where the incident angle varied from $0^\circ < \theta < 20^\circ$. From the photonic band diagram, a resonant angle of $\theta = 13.8^\circ$ corresponds to a resonant wavelength of $\lambda = 532 \text{ nm}$, while a resonant angle of $\theta = 9.2^\circ$ corresponds to a resonant wavelength of $\lambda = 632.8 \text{ nm}$. Figure 36 shows the spatial distribution of the simulated near-field electric field intensity (normalized to the intensity of incident field) for one period of the PC for the excitation of the resonant mode at $\lambda = 532 \text{ nm}$ and $\lambda = 632.8 \text{ nm}$. The influence of the resonance phenomenon on the resulting near-fields is clearly visible in the electric field intensity.

**Fig. 37** Schematic diagram of the fabrication procedure for replica molding process. (a) The process begins with a silicon master wafer fabricated by deep UV lithography and reactive-ion etching. Liquid UVCP is poured onto the silicon master wafer. (b) A plastic sheet is pressed against the UVCP so as to form a uniform layer of the polymer between the sheet and the master wafer. (c) Polymer is cured under UV light. (d) After the polymer is cured, the plastic sheet is peeled away. (e) To complete the PC fabrication TiO$_2$ is sputtered over the structure.
Fabrication of the device was carried out using a plastic-based nanoreplica molding process. The steps of fabrication are shown in Fig. 37. Briefly, a silicon wafer with a negative surface volume image of the desired grating pattern was fabricated using deep-UV lithography and reactive ion etching. A liquid UVCP was sandwiched between a PET sheet and the silicon master wafer, and was subsequently cured using a high intensity UV lamp (Xenon, Inc). The hardened polymer grating adhered to the PET and was peeled away from the master, and the replica was cut and attached to a 1×3 inch² microscope slide (Fig. 38). A thin SiO₂ intermediate layer ($t_{SiO_2} = 80$ nm) on the grating surface helps to reduce autofluorescence background from the underlying polymer material. Following SiO₂ deposition, 120 nm of TiO₂ was sputtered by an RF sputtering system (PVD 75, Kurt Lesker). The photonic band diagram of the device was obtained by illuminating the device with collimated white light and measuring the transmitted spectrum as a function of incidence angle with a spectrometer (USB 2000, Ocean Optics). The schematic of the transmission setup is shown in Fig. 39 and the consequent band diagram obtained is shown in Fig. 40, which agrees well with the simulated band diagram shown in Fig.
As highlighted in Fig. 40, resonances for $\lambda = 532$ nm and $\lambda = 632.8$ nm modes lie at $\theta = 15^\circ$ and $\theta = 9^\circ$, respectively.

**Fig. 39** Schematic of the transmission measurement setup used to study the dispersion diagram of the fabricated PC.

**Fig. 40** Measured dispersion diagram for the PC used in this study by RCWA. Resonance of the TM mode is at $\theta \sim 9^\circ$ for Cy-5 excitation and $\theta \sim 15^\circ$ for Cy-3 excitation.
3.3 Fluorescence Enhancement

In order to demonstrate the fluorescence enhancement performance of the fabricated sensor, a detection experiment using a dye-labeled protein was carried out on the PC surface and a reference glass slide. Both the PC surface and the glass slide were pre-cleaned with O$_2$ plasma (TI Plasma) for 3 min. and functionalized by overnight incubation in an enclosed glass container with 5% 3-glycidoxypropyltrimethoxysilane in dry toluene at 100 °C. After incubation the silanized devices were cleaned by sonication in toluene, methanol and deionized (DI) water and then dried under a nitrogen stream. Cy-5 and Cy-3 conjugated streptavidin (GE Healthcare) at 50 µg/ml was spotted onto the slides as two separate arrays by a piezo dispenser (Piezorray, PerkinElmer). After overnight incubation, the devices were washed by gently dipping them in a protein blocking buffer (Phosphate buffered saline at pH 7.4 with Kathon antimicrobial agent) solution for 60 sec. followed by DI water rinse. Fluorescent images of the spots were obtained using a commercially available confocal laser scanner (LS-Reloaded, Tecan) equipped with a
λ = 632.8 nm He-Ne laser and a λ = 532 nm diode pumped solid state laser. The angle of incidence of both lasers can be tuned from θ = 0° to 25°. The schematic of this confocal scanner is shown in Fig. 41. The Cy-5 conjugated streptavidin and Cy-3 conjugated streptavidin spots were sequentially illuminated by TM-polarized λ = 632.8 nm and λ = 532 nm lasers with Cy-5 and Cy-3 emission filters (λ = 692 ± 20 nm; λ = 575 ± 25 nm). The measured images were analyzed by image processing software (ImageJ). The net fluorescence intensity was calculated by averaging spot intensities over the 16 replicate spots minus the local background intensity.

He-Ne laser at θ = 15° was used to excite the Cy-5 conjugated streptavidin spots, which demonstrated an amplification of Cy-5 emission on the PC surface by a factor of 32 compared to the glass slides measured under same condition. Similarly, Cy-3 conjugated streptavidin spots were excited by the green laser at θ = 9°, which showed an enhancement by a factor of 25, compared to the glass surface. The scanned images from the PC and glass substrates are compared in the insets of Fig. 42 (images were plotted using the same color scale). Line profiles generated by extracting fluorescence intensities from a single line of pixels through eight spots on the PC and glass images for both fluorophores are also shown in Fig. 42. This profile clearly illustrates amplification of the emission signal from both fluorophores on the PC surface. The slight increase in background fluorescence in these images can be attributed to simultaneous enhancement of the auto-fluorescence originating from the polymer material used for nanoreplica molding the grating structure. The fluorescence images of the spots were taken at the small pinhole setting in the confocal laser scanner (LS-Reloaded, Tecan) with 10×10 μm² pixel resolution. A photomultiplier tube (PMT) gain of 100 was used to scan SA-conjugated Cy-3 spots and a PMT gain of 130 was used to scan SA-conjugated Cy-5 spots.
Fig. 42] Intensity profile as a function of distance for a single line of eight spots of (a) Cy-5 conjugated streptavidin and (b) Cy-3 conjugates streptavidin on the glass slide and the PC. The scanned images are shown in insets.

3.4 Discussion

In summary, a single PC surface was used to enhance fluorescence emission from both Cy-5 and Cy-3 dyes. The PC was fabricated by a low cost replica molding method that can be performed uniformly over a large surface area. To excite resonant modes at a desired wavelength, the
excitation light needs to illuminate the PC at a specific resonant angle by tuning the angle of incidence of the detection instrument. For the device demonstrated here, the resonance angle for $\lambda = 632.8$ nm is $\theta = 9^\circ$ while for $\lambda = 532$ nm, $\theta = 15^\circ$. Compared to a glass slide, the PC sensor exhibited an enhancement of $32\times$ for Cy-5 and $25\times$ for Cy-3. This particular PC is capable of enhancing emission from fluorophores with the excitation wavelengths lying within a range of $532$ nm $< \lambda < 660$ nm, thus providing compatibility with a large variety of commonly used dyes, such as Rhodamine, Texas Red, and Alexa fluor 532 to 647, which are widely used in life science research, diagnostic testing, and environmental detection [125].
CHAPTER 4

PHOTONIC CRYSTAL ENHANCED FLUORESCENCE USING A QUARTZ SUBSTRATE TO REDUCE LIMITS OF DETECTION

4.1 Introduction

Our previous results with PCEF have been obtained using polymer grating structure fabricated by the nanoreplica molding technique [124] on flexible plastic substrates. Fluorescence detection limits of PCs incorporating polymer materials have been limited by the background fluorescence (autofluorescence) signal produced by the plastic substrate and polymer grating. It is well known that plastic materials show significant autofluorescence when excited by near-UV or even visible radiation [126-128], with autofluorescence increasing as the illumination source photon energy is increased. This phenomena was demonstrated clearly in a the previous chapter where the PCEF surface designed for multiple excitation wavelengths ($\lambda = 532 – 660$ nm) showed best signal-to-noise sensitivity performance for longer excitation wavelengths, however the detection limit for short excitation wavelengths was limited by substrate autofluorescence [129]. Meanwhile, a great deal of research activity is directed towards developing new plastic substrates with lower autofluorescence [130-132]. PMMA, PDMS, Topas and Zeonex have been shown to have lower autofluorescence compared to other plastic materials and have been identified for potential applications in high throughput screening devices that rely on fluorescence detection. Despite these efforts, no polymer material provides autofluorescence comparable to quartz.

The use of quartz surfaces for the PCEF applications have been limited by the requirement to produce surface structures with subwavelength dimensions over surface areas large enough to
encompass entire DNA microarrays or protein microarrays, which are usually performed on substrates as large as standard microscope slides (1×3 in²). Conventional lithography methods, such as e-beam lithography and DUV lithography, are either too expensive or low throughput to produce subwavelength structures over such large surface areas. To address these issues, “step-and-flash” nanoimprint lithography (NIL) tools (Molecular Imprints, Inc.) can be employed [133-136]. Step-and-flash imprint lithography (SFIL) has been successfully demonstrated for hard drive disks, LEDs, and CMOS manufacturing. SFIL is capable of producing patterns with feature sizes less than 20 nm. In the SFIL process, a template with a pre-defined pattern is pressed into UV-curable liquid dispensed over a substrate. Once exposed to UV light, the liquid is cured, and after separation, a replica of the pattern on the template is imprinted into the solidified polymer surface. A step and repeat procedure is used to replicate the pattern over the entire substrate surface as shown in Fig. 43. After SFIL patterning, reactive ion etching (RIE) is used to transfer the imprinted pattern into the substrate. Using this approach, 1-D grating structures with a period of 400 nm have been fabricated upon wafers as large as 8 inches in diameter.

**Fig. 43** | Schematic representation of S-FIL process on 8 inch silicon wafer. Reprinted from [http://www.molecularimprints.com](http://www.molecularimprints.com).
This chapter will describe the fabrication and the characterization process of a quartz-based PC produced using SFIL nano-imprint lithography. The device design is described in Section 4.2, and the fabrication process is detailed in Section 4.3. Section 4.4 characterizes the performance of the PC, demonstrating low auto-fluorescence and a strong fluorescence signal enhancement when a laser at $\lambda = 632.8$ nm illuminates the device at the resonance angle. The detection of a dye labeled peptide on a PC is shown in Section 4.5.

### 4.2 PCEF surface design

A cross-sectional diagram (not to scale) of the PC that was fabricated is shown in Fig. 44. The subwavelength grating was formed on the surface of the quartz, which has a refractive index of $n_{\text{sub}} = 1.456$. A layer of a high refractive index dielectric material with a specific thickness ($t_{TiO2}$) and refractive index ($n_{TiO2} = 2.35$), was subsequently deposited over the grating structure. The wavelength, angle of incidence, bandwidth, and efficiency of the PC surface are determined by the geometry of the structure as described in chapter 1. By adjusting the physical parameters of the PC that includes the grating period ($\Lambda$), grating depth ($d$), duty cycle ($f$), thickness and refractive index of the dielectric coating, a PC surface can be designed to efficiently interact with the absorption and emission spectra of a specific fluorophore.
In this study, a PC was designed to enhance the emission from fluorophores which have an absorption band near $\lambda_{ex} = 632.8$ nm and an emission band near $\lambda_{em} = 690$ nm. The detection instrument was equipped with a HeNe laser ($\lambda = 632.8$ nm) as the excitation source and an emission filter with a center wavelength of 690 nm and bandwidth of 40 nm. A PC structure usually supports two orthogonal modes: TE polarized and TM polarized. Compared to TE modes, the resonant modes associated with TM polarization have higher $Q$-factor, resulting in stronger field intensity near the device surface. Therefore, the PC was designed to have one TM mode at $\lambda_{ex} = 632.8$ nm, which spectrally matches with the excitation laser wavelength for near field enhancement, resulting in enhanced excitation. In order to direct the emitted photons toward the collection optics, a TM mode at $\lambda_{em} = 690$ nm, which spectrally overlaps with the pass band of the emission filter, was designed with a resonance angle $\theta_{em} = 0^\circ$.

RCWA simulation package was used to aid the design of the PC with multiple TM modes and with precisely defined wavelength/angle coupling conditions. For the PC structure, transmission...
efficiency minima (or reflection efficiency maxima) in the calculated spectra were used to identify the resonance modes. RCWA simulations stipulated a use of a structure with a period of \( A = 400 \) nm, grating depth of \( d = 40 \) nm, duty cycle \( f = 50\% \), and TiO\(_2\) thickness of \( t_{TiO2} = 130 \) nm. For this design, the transmission efficiency of the TM resonance mode was calculated in the wavelength range of \( 600< \lambda < 800 \) nm as the incidence angle was varied from \( 0^\circ < \theta < 20^\circ \). Figure 45(a) plots the photonic band diagram of the PC shown in Fig. 44. From the photonic band diagram, the resonance angle of \( \theta_{ex} = 10.8^\circ \) corresponds to the resonance wavelength of \( \lambda_{ex} = 632.8 \) nm for enhanced excitation. Figure 45(b) shows the spatial distribution of the simulated near-field electric field intensity (normalized to the intensity of incident field) within one period of the PC structure at the resonance wavelength of \( \lambda_{ex} = 632.8 \) nm and the resonance angle of \( \theta_{ex} = 10.8^\circ \). At \( \theta_{em} = 0^\circ \), the upper band edge with \( \lambda_{em} = 690 \) nm is used for enhanced extraction (shown in Fig. 45(a)). The enhanced extraction effect offers the possibility of efficiently collecting fluorescent emission using a low \( NA \) lens. The fluorescence detection instrument used in this study is installed with a microscope objective with \( NA = 0.1 \). This low \( NA \) objective can only accept light within an angular acceptance cone of \( 5.7^\circ \). Since the designed excitation angle of \( \theta_{ex} = 10.8^\circ \) is much larger than \( 5.7^\circ \), the excitation beam will not be visible to the detection system. This feature of the detection instrument is extremely important for obtaining low background intensity measurements as even a small fraction of excitation light leaking into detection optics can pass through the emission filter increasing the noise in the detected signal and thus compromising the signal-to-noise ratio of the measured signal.
Fig. 45 (a) RCWA simulated dispersion diagram for the PC used in this study. Resonance for the enhanced excitation for the TM mode is at $\theta \sim 10.8^\circ$ (b) Simulated near field distribution at $\lambda = 632.8$ nm (normalized to the intensity of the incident field).

4.3 PCEF surface fabrication

The subwavelength grating surface (1$\times$3 in$^2$) was prepared by nano-imprint lithography using the SFIL process, performed on a commercially available tool (Molecular Imprint Inc. Imprio-55) [137-139]. The imprint template consists of two primary regions, the active field area ($9 \times 9$ mm$^2$) and the 15 $\mu$m recessed non active area as shown in Fig. 46(a) and is patterned by e-beam lithography and RIE [140, 141]. The active field area contains the PC features to be printed on the wafer. Figure 47 outlines the steps for the template fabrication. Briefly a thin layer of chrome $\sim 15$ nm was evaporated on the quartz substrate (65 mm $\times$ 65mm $\times$ 12.5 mm) and then a layer of e-beam resist (ZEP) was spin coated upon chrome. E-beam was then used to pattern the active
area. After resist development, chrome etch was performed to expose the quartz beneath it. Finally the quartz was etched to a depth of 120 nm. The depth of the grating in the template does not define the depth of the grating in the PC fabricated using imprint lithography; however, a deeper grating facilitates the imprint process. Finally to define the 15 \( \mu \text{m} \) pedestal, the template was recoated with a photoresist (AZ 4620) and UV light was used to generate the pedestal pattern. The chrome was etched and the template was wet etched in 10:1 buffered etch oxide (BOE) to generate the pedestal. The template is then cleaned by sonication in acetone to remove wax-like contaminations. Then, it was treated in a standard piranha solution at 120\(^\circ\)C for 30-60 minutes, rinsed in deionized water and baked at 100\(^\circ\)C for 10 minutes. The above cleaning process produces a clean hydroxylated surface. Prior to using the template to imprint, its surface was treated with an anti-adhesion layer (RelMat) by dispensing few drops over the active area and then blown dry using clean filtered nitrogen.

![Imprint template schematic showing the top view, cross-section view and the active area patterned with 1-D grating.](image)

**Fig. 46** Imprint template schematic showing the top view, cross-section view and the active area patterned with 1-D grating.
Figure 47 outlines the fabrication of the imprint template.

Figure 48 outlines the steps of nano-imprint lithography. Quartz wafers (4 inch diameter) with very-low autofluorescence were chosen as the imprint substrate. Before imprinting, wafer was cleaned in Piranha solution for 1 hr. and then washed in Spin-Rinse-Dry (SRD 19A Verteq Process Systems Development) for 1 hr. to remove particles. For the purpose of planarization, the wafer was pre-coated with Transpin (Molecular Imprints Inc.) and hard baked for 60 sec. on a hotplate at 160 °C to produce a 60 nm thick film. The wafer was then loaded into the tool and the imprint resist (MonoMat, Molecular Imprint Inc.) was dispensed onto the substrate. The dispense pattern and volume of the resist was precisely controlled so as to uniformly fill the active field area in the template with minimum resist protruding out of it and to have a uniform
base layer thickness. The template was then slowly pressed against the dispense pattern and a force of 3 N was applied for 70 sec. followed by 20 sec. UV exposure of the resist. After UV cure, the template was pulled away from the hardened imprint resist with the grating pattern in it with a base layer thickness of ~ 60 nm. The above steps were repeated within an x-y grid to cover the entire quartz wafer surface.

After the imprint, RIE was used to transfer the imprinted pattern into the quartz substrate. In order to improve fidelity of the etch, the imprinted wafer was coated with a silicon- or silixane-containing polymer layer (SilSpin, Molecular Imprint Inc.) at 3,000 rpm and baked on a hotplate at 200 °C for 90 sec. (Fig. 48(d)). As shown in Fig. 48(e), during oxygen RIE, the Silspin surface forms a SiO₂ layer which functions as a high selectivity mask for the etching of MonoMat and TranSpin. To etch back SilSpin, RIE was performed with 30 sccm of CHF₃ and 2.7 sccm of O₂ at a chamber pressure of 35 mTorr. This etching step is precisely timed so as to expose imprint resist at the end of the SilSpin etch. Using Silspin as an etching mask, the imprint resist and TranSpin layers were etched by an anisotropic RIE with 12 sccm of Ar and 2.7 sccm of O₂ to expose the quartz substrate. Finally, a RIE with CF₄ was used to transfer the grating pattern into the quartz wafer. After this, the wafer was cleaned by Piranha to remove all residues of the imprint polymers. The substrate was then cut into two standard microscope slides (1×3 in²) by a dicing saw (600 Series, Disco Corporation). The RF sputter (PVD 75, Kurt J. Lesker) with a ceramic TiO₂ target was used to coat ~130 nm of TiO₂ on top of the imprinted grating structure.
**Fig. 48** Schematic diagram of the fabrication procedure: (a) The process begins with a dispense pattern of MonoMat on a planarized quartz wafer; (b) The template is pressed against the dispense pattern and then UV cured; (c) The template is pulled away from the solidified grating pattern; (d) A layer of Silspin is spin coated onto the patterned surface; (e) RIE of the imprint resist is performed to expose the quartz; (f) RIE of quartz is performed to transfer the pattern; (g) Piranha clean of the wafer; (h) TiO₂ deposition onto the grating.

**Fig. 49** (a) SEM image of the top view of the TiO₂ coated grating structure on quartz substrate; (b) AFM image of the PCEF surface showing the grating depth of 40 nm, (c) Photograph of the PCEF surface on 1×3 in² substrate.
Figure 49(a) shows an SEM image of the top view of the fabricated quartz PC. It is apparent from the SEM image that the sidewalls of grating are free from debris and essentially vertical. An atomic force microscope (AFM) surface profile of the imprinted structure after the TiO$_2$ deposition is shown in Fig. 49(b). The geometric dimensions (grating period of $\Lambda = 402$ nm and grating depth of $d = 44$ nm) match closely with the design dimensions. Fig. 49(c) shows a photograph of the PC in a standard 1×3 in$^2$ microscope slide format with 21 imprint replicas. The PC surface shows excellent uniformity across the entire area. Capable of accurately reproducing subwavelength feature sizes over a large range, the nano-imprint process reported here is well suited for mass production of PCs.

4.4 Device characterization

Several experiments were conducted to characterize the transmission spectrum, autofluorescence intensity, enhanced excitation, and directional emission properties of the fabricated PC.

4.4.1 Transmission spectrum of PC sensor

In order to measure the resonant wavelength and angle of the fabricated device, the wavelength transmission spectra were collected by illuminating the PC with collimated broadband light from a halogen lamp and analyzing the transmitted spectrum, using a spectrometer (HR2000, OceanOptics) coupled to an optical fiber. The blue curve in Fig. 50(a) represents the transmission spectrum at the incidence angle of $\theta = 0^\circ$, with full width at half maximum (FWHM) of ~1 nm. Designed as a mode for enhanced extraction, this mode has a resonance wavelength of $\lambda_{em} = 689.7$ nm that overlaps with the pass band of the emission filter of the detection instrument. In Fig. 50(a), the red curve shows the transmission spectrum at the incidence angle of $\theta_r = 11.4^\circ$. At this angle, the resonance wavelength matches the excitation
laser wavelength of 632.8 nm with a FWHM of ~4 nm. The resonance angle ($\theta_r$) was also identified by illuminating the device with a TM polarized HeNe laser (35 mW), tuning the angle of incidence, and recording the transmitted light intensity. The illumination spot size was ~ 3 mm in diameter. The measured angle spectrum is shown in Fig. 50(b) with FWHM$_\theta$ = 0.3°. To fully take advantage of the enhanced excitation effect, the angle of incidence needs to be well tuned to efficiently couple the excitation light into the resonance mode at $\theta_r = 11.4^\circ$. A PC enhanced fluorescence microscope (PCEFM) developed by Cunningham research lab and described fully in a chapter 2 [142], was used to perform the angle tuning. When the illumination angle was tuned to $\theta_r$, the fluorescence images were taken.

![Fig. 50](image)

**Fig. 50** | (a) Wavelength transmission spectrum (b) Angle transmission spectrum at the excitation wavelength of $\lambda = 632.8$ nm.

### 4.4.2 Autofluorescence characterization of the quartz PCEF surface

Reducing the background fluorescence from the sensor substrate is critical for the detection of fluorescent tags present at low concentrations. The flame-fused quartz substrate (University Wafer) used in this experiment exhibits very-low autofluorescence when excited by a red laser source. The autofluorescence level of the quartz substrate was compared to a commercial glass
slide and a PC fabricated on a plastic substrate [17]. Glass slides and the plastic-based PCs are currently the most commonly used substrates for surface-based fluorescent assays. Both the plastic-based PC and the quartz-based PC have a resonance mode at $\lambda_{ex} = 632.8$ nm with the resonance angle at $13^\circ$ and $11.4^\circ$, respectively.

![Graph](image)

**Fig. 51** | Autofluorescence intensity from a normal glass slide, a plastic-based PC, and a quartz-based PC measured using the PCEF microscope under identical measurement settings.

Before the measurement, all devices were cleaned in oxygen plasma for 5 min. to remove possible organic contaminations. All devices were measured using the PCEF microscope. A 35 mW HeNe laser was used as the excitation source; a 20× objective ($N.A. = 0.4$) was used to collect the autofluorescence signal and an emission filter ($\lambda = 690 \pm 20$ nm) was used to block the laser light from reaching the CCD camera in the microscope. All the images were taken at 1.2 sec integration time. Figure 51 shows the autofluorescence of the glass slide, plastic-based PC and the quartz-based PC for illumination angles between $0^\circ$ and $16^\circ$. The glass slide sample showed a nearly constant autofluorescence signal of $\sim 50$ counts. The plastic-based PC exhibited strong background fluorescence near the resonance angle of $13^\circ$ due to the enhancement of the
fluorescence from the polymer material lying within the strong evanescent field region. The quartz-based PC showed background fluorescence as low as 15 counts, which is 15 times lower than the plastic-based PC at resonance and 5 times lower than the glass slide.

4.4.3 Enhanced excitation and extraction

A simple testing scheme was developed to characterize the signal enhancement capability of the quartz-based PC. A fluorescent dye (LD-700, Exciton, Inc) with a peak absorption wavelength of 647 nm and a peak emission wavelength of 673 nm was doped in SU-8 \( (n_{SU8} = 1.58) \) and spin-coated onto the PC surface [143]. The SU-8 host medium was prepared by mixing SU-8 2000.5 with SU-8 thinner (Microchem Corporation) at a volume ratio of 1:12. The LD-700 dye molecules were first dissolved in methanol at \( 10^{-6} \text{ M} \) (538 ng/ml) and then mixed with diluted SU-8 solution at a volume ratio of 1:2. The LD-700 doped SU-8 solution was then spin coated onto the PC surface at 5000 rpm for 30 sec and then air dried. The coated film thickness was measured as \( \sim 50 \text{ nm} \) by an ellipsometer. The deposition of dye-doped SU-8 film causes a shift in the resonance angle by 3.5°, as the film slightly increases the effective refractive index of the resonance mode.

The dye/polymer coated PC was tested using the PCEF microscope, which illuminates the PC from beneath the structure with a collimated light that can be swept rapidly through a range of incidence angles [142, 144]. A 35 mW HeNe laser was used to excite the dye, and the emission was collected by a 0.1 \( NA \) objective (positioned above the device) with a 690 ± 20 nm emission filter. An electron multiplied CCD (EMCCD) camera measured the fluorescence signal. By varying the angle of incidence from 10° to 20° in increments of 0.02°, the emission intensity was recorded and is shown in Fig. 52. The images were taken with an integration time of 30 msec.
At the resonance angle ($\theta_r = 14.88^\circ$), the measured fluorescence intensity was $\sim 45,000$ counts. At off-resonance illumination ($\theta = 20^\circ$) the fluorescence signal was only $\sim 45$ counts. The enhancement factor of the PC was calculated by subtracting the substrate background intensity from the signals and then dividing the net on-resonance signal by the net off-resonance signal. Using the above method an enhancement factor of $1500\times$ was obtained, representing the effects of enhanced excitation only.

**Fig. 52** Fluorescence output as a function of angle of incidence for a $\sim 50$ nm film of dye-doped polymer spin-coated onto the PC surface.

Using the same sample, the enhanced extraction effect was also studied with the PCEF microscope. An LD-700 coated PC was illuminated at an off-resonance angle ($\theta = 20^\circ$) with the TM polarized HeNe laser. An integration time of 1.2 sec was used for the EMCCD camera because the fluorescence intensity from the glass control was too low to be detected when integration times lower than 100 msec were used. A background subtracted off-resonance fluorescence intensity of 10940 counts was collected by the $NA = 0.1$ objective for the PC. A glass control with LD-700 coated at the same concentration was also studied using the PCEF
microscope under the same detection conditions and a background subtracted fluorescence intensity of 2050 counts was obtained for it. The ratio of background subtracted off-resonance signal and the background subtracted glass control signal gives the extraction enhancement factor of the device. From the above data, the PCEF surface gave a factor of $5\times$ for enhanced extraction.

A combined enhancement factor, (defined as the product of the enhanced excitation and the enhanced extraction factors) of $7500\times$ was calculated for the device for the given concentration of LD-700. This enhancement factor represents the maximum achievable enhancement for the fabricated design that can be obtained when the fluorescent material is allowed to fill a $\sim$50 nm thick volume that conforms to the corrugated PC surface, with no spacer materials between the fluorophores and the PC surface. A point to note here is that $\sim$50 nm SU8 layer also improves the Q-factor of the PC by $\sim$10% and thus has some contribution to this enhancement factor. As we will show, detection of biomolecules tagged with fluorophores results in lower total enhancement factor, as fractional monolayers of proteins with $\sim$1 dye molecule each do not fill the evanescent field volume as efficiently as the SU8-doped layer.

Another short study was done to look at the angle-dependence of the enhanced extraction effect of the PC by measuring the angle and the wavelength-resolved plot of a fluorophore’s emission. The measurement setup designed to collect and analyze the fluorescence intensity as a function of the angle of collection is shown is Fig. 53. In order to demonstrate that enhanced extraction can be used with any combination of dye and laser, a diode pumped laser ($\lambda = 532$ nm; 300 mW) was used as an excitation source on LDS-720 ($\lambda_{abs} = 529$ nm, $\lambda_{emi} = 699$ nm) doped SU8 layer ($\sim$50 nm) coated onto the surface of the PC. The green laser beam was used to illuminate the sample surface at a fixed angle of incidence ($\theta = 0^\circ$) and the dye emission was
collected by an optical fiber mounted with a collimator. The acceptance angle of the collimator coupled optical fiber lies between 0° and 0.3°. The fluorescence intensity from the dye was collected by rotating the detection setup about the center of the sample for angles between 0° to 9° in increments of 0.25°.

**Fig. 53** Schematic of the optical setup to study extraction effect on the PC surface.

**Fig. 54** Angle-resolved fluorescence measurement on the quartz PCEF surface.
Figure 54 shows the measured angle and wavelength-resolved emission plot of the fluorescence. The detection window of the PCEF microscope lies within the wavelength range of $680\text{ nm} < \lambda < 720\text{ nm}$ and an angle range of $0^\circ$ to $5.7^\circ$, corresponding to the collection window of $NA = 0.1$ objective (as marked in Fig. 54). Within this detection window, 77% of the fluorescence was gathered, which is $\sim 9 \times$ higher than the fluorescence intensity that was gathered in the same experiment performed upon a conventional glass substrate. This factor was calculated by assuming that the dye emission on the glass is spherically symmetric. This enhanced collection of dye emission is marked by a strong peak at $\lambda = 690\text{ nm}$ for an exit angle of $0^\circ$ in Fig. 54. As mentioned previously, this TM peak was used to couple the emission of the dye molecules and direct it towards the collection optics.

4.5 Detection of dye labeled polypeptide

In order to demonstrate the enhancement in the SNR and lowering of the limit of detection of the analytes on the PC in the context of a multispot microarray assay, a detection experiment using a dye-labeled protein was performed. Dye-labeled polypeptide for a range of dye concentrations was spotted directly to the PC and a glass surface, and the fluorescence of the spots on each surface was compared in a dose-dependent manner. Before spotting both the PC and the glass slide were cleaned with O$_2$ plasma for 3 min., sonicated in acetone, isopropanol and DI water and then dried under a nitrogen stream. Poly (Lys, Phe) conjugated with Alexa-647 (Invitrogen) at a range of concentrations was spotted onto the slides by a piezoelectric dispenser (Piezorray, Perkin Elmer) with a center-to-center separation of $500\mu m$ and a spot radius of $\sim 200\mu m$. After an incubation period of 50 min, the devices were washed by gently dipping them in DI water for 60 sec. Fluorescence images of the spots were then taken using the PCEF microscope. The Alexa-647 labeled polypeptide (PPL-Alexa 647) spots were excited with the TM polarized HeNe
laser at the angle of incidence $\theta = 11.4^\circ$ and the emission was collected with a $Na = 0.1$ objective, as described previously. The measured images were analyzed by image processing software (ImageJ). The net fluorescence intensity was calculated by averaging intensities over the 9 replicate spots minus the local background intensity.

Fig. 55| Gain and exposure-optimized images of PPL-Alexa 647 fluorescence on glass compared the PC surface.

The PPL-Alexa 647 spots demonstrated an amplification factor of $690 \times$ for the emission on the PC surface compared to the glass slide for a concentration of 3.3 $\mu g/ml$ (i.e. the lowest concentration that was visible on the glass slide) measured under the same conditions. This was the highest amplification factor of all the concentrations spotted on the PC surface. The fluorescence images for both the PC and glass slide for four consecutive concentrations are shown side-by-side in Fig. 55 (the images were plotted using the same grey scale). An
integration time of 30 msec was used for all the images taken by the PCEF microscope. Line profiles generated by extracting fluorescence intensities from a single line of pixels through the spots on the PC and glass images are also shown in Fig. 55. For the concentration of 30µg/ml, the spots on the PC saturate at 65535 counts (the saturation limit of the CCD), while for the glass slide the signal is 3150 counts. For the concentration of 9.9µg/ml, the signal on the glass slide has already decreased to 80 counts compared to the PC at 60000 counts. This profile clearly illustrates amplification of the emission signal from the fluorophore on the PC surface. Table I summarizes the measured fluorescence enhancement for the four consecutive concentrations of PPL-Alexa 647 spotted on the quartz-based PC and separates the individual effects of enhanced excitation (measured as the ratio of fluorescence intensities for the PC illuminated on- versus off-resonance) and enhanced extraction (measured as the ratio of fluorescence intensities for the PC off-resonance versus the glass slide control). The trend of decreasing enhanced excitation for higher spot concentration arises because the resonance angle for the 3.3 µg/ml spots was used as the incidence angle to scan the PC surface. Due to the narrow angular linewidth of the resonance used here (FWHM₀ = 0.3°) and because the resonance angle shifts slightly higher with increasing spot density, the high-density spots were illuminated with a small angle offset from their true on-resonant state.

As a more appropriate performance metric for fluorescence-based assays (as opposed to raw intensity enhancement numbers), SNRs are calculated from the optimized images for each PPL-Alexa 647 concentration spotted onto the PC and glass slide and are shown in Fig. 56. The signal is background subtracted and the noise is defined as the standard deviation of the nine local backgrounds taken in each quadrant around the perimeter of each spot. The Limit of Detection (LOD) is defined as the concentration at which a SNR = 3 is obtained. The LOD for the PC was
found to be 35 ng/ml, which is 140× times lower than that of the glass slide. The SNR enhancement for the PC was found to be 330× for the concentration (5 µg/ml) corresponding to the LOD on the glass slide.

**Table I. Measured Photonic Crystal Fluorescence Enhancement**

<table>
<thead>
<tr>
<th><a href="%C2%B5g/ml">PPL-Alexa 647</a></th>
<th>Enhanced Excitation*</th>
<th>Enhanced Extraction†</th>
<th>Total Enhancement‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 (On Res. PC saturates)</td>
<td>35</td>
<td>4</td>
<td>140</td>
</tr>
<tr>
<td>9.9</td>
<td>96</td>
<td>3</td>
<td>288</td>
</tr>
<tr>
<td>3.3</td>
<td>116</td>
<td>6</td>
<td>696</td>
</tr>
<tr>
<td>0.3 (Glass spots not visible)</td>
<td>131</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

* Net spot intensity for PC on resonance divided by net spot intensity for PC off resonance.
† Net spot intensity for PC off resonance divided by net spot intensity for glass control.
‡ Net spot intensity for PC on resonance divided by net spot intensity for glass control.

The difference in the measured maximum enhancement factors between the detection of a dye-tagged polypeptide (696×) and a conformal thin film of dye-doped polymer (7500×) can be explained by several factors. Most importantly, the SU-8 film with a higher refractive index relative to the air, behaves as a wave confinement layer that can hold a greater proportion of the resonance mode within its volume compared to the biomolecule monolayers that are only surrounded by a low refractive index air medium. Also, the SU-8 film is able to apply a conformal volume that is uniformly filled with dye, and that can partially fill the grating grooves, thus resulting in efficient overlap between the physical space occupied by the dye molecules and the volume occupied by the resonance electric field. The peptide layer, in contrast, is confined to a two-dimensional monolayer film that can conform to the PC surface structure, but does not occupy a substantial volume. Characterization of the enhancement factor is presented here using
both the methods to facilitate comparison with approaches that primarily report results using only dye-doped polymer films.

![Signal-to-noise ratio plotted for different concentrations of PPL-Alexa 647 showing an improvement in the limit of detection (LOD) on the PC surface by a factor of 140×.](image)

**Fig. 56** Signal-to-noise ratio plotted for different concentrations of PPL-Alexa 647 showing an improvement in the limit of detection (LOD) on the PC surface by a factor of 140×.

### 4.6 DNA microarrays

*(i) Single Color Microarray*

A single color DNA microarray experiment was performed on a quartz PC and a control glass slide to study the expression profile of a *Glycine max* cotyledon sample [50]. The PC slides were functionalized with an epoxysilane-based surface chemistry described previously [6] and commercially silanized glass slides (Corning GAPS II) were used as control slides. A previously reported [7] set of 192 oligonucleotides consisting of soybean genes were printed in replicates of 40, for a total of 7680 spots per slide. The 70-mer oligonucleotides were printed (QArray2, Genetix) on three PCs along with their glass control slides. Printed slides were incubated
overnight and then UV crosslinked. Cotyledon RNA was extracted from freeze dried soybeans seeds (Glycine max cultivar Williams). The total RNA, extracted from the separated cotyledons, was purified and labeled with Cy5 by reverse transcription. Printed slides were blocked with bovine serum albumin and then hybridized overnight at 42°C. ~ 40 µg of total RNA was used per slide. All slides received identical treatments throughout the assay. All slides were scanned using a confocal microarray scanner (LS Reloaded, Tecan) using a TM polarized laser (λ = 632.8 nm) and an emission filter with a range of 670-710 nm. All slides were scanned at identical PMT gain settings and at a pixel resolution of 10 µm. Glass slides were scanned at normal incidence while the PCs were scanned at their respective resonant angles. Spot segmentation and intensity calculations of the fluorescence scans were performed using Genepix Pro 6.1 (Molecular Devices). Spot SNR was calculated as the local background subtracted spot intensity divided by the standard deviation of the local background.

Figure 57 plots post-hybridization images of an identical sub-array on the PC and glass slide. Not only are signals from high expressing genes enhanced on the PC (Fig. 57 (a)), but also several low expressers that could not be differentiated from the background noise of the glass slide are detectable on the PC (Fig. 15(d)). Figure 58 plots the SNR of all the genes spotted for one PC-glass pair. In this analysis, a gene was defined detectable if the replicate-averaged gene SNR was greater than 3. For the PC-glass pair plotted in Fig. 58, 13.6% of all genes were detectable on the glass slide while, 26.6% were detectable on the PC. On comparing all the three PCs against their paired control glass slides, an average of twice as many genes was detectable on the PC.
Fig. 57] Fluorescence images on (a) a PC and (b) a glass control of the same sub-array at identical laser scan settings. Contrast and brightness of the images has been optimized to maximize feature visibility. Line profiles showing signal intensity of (c) high and (d) low expression genes. (Reprinted from [50]).

Many of the highly expressed genes, detected both on the glass and PC, encode storage proteins which are abundant during this stage of seed development. The additional genes detected only on the PC represent enzymes and important regulatory transcription factors that are expressed at lower levels. Transcription factors provide genetic control over development and can also be important markers of disease state. The quartz based PCs offer some unique advantages for DNA microarray applications over previously reported work using PCs based on plastic substrates with polymer gratings. The quartz PCs have a substrate fluorescence that is 15 times lower than plastic PCs. This is an important consideration when designing a PC to enhance fluorescence emission from Cy-3 ($\lambda_{abs} = 536$ nm), another fluorophore that is routinely used in DNA microarrays.
Fig. 58 Logarithm (base 3) values of replicate-averaged SNR for all 192 genes on (a) the PC and (b) glass. Genes with SNR > 3 or correspondingly log 3(SNR) > 1 are classified as detected. 51 genes were detected on the PC while only 26 were detected on the control glass slide. (Reprinted from [50]).

(ii) Two-Color Microarray

A two-color fluorescence enhancement experiment was performed on a quartz PC for improved microarray sensitivity. The PC slide was functionalized with epoxysilane-based surface chemistry. A commercially silanized glass slide (Corning GAPS II) was used as a control slide in the microarray experiment. A previously reported [103] set of 192 oligonucleotides consisting of soybean genes were printed in replicates of 40, for a total of 7680 spots per slide. The 70-mer oligonucleotides were printed (QArray², Genetix) on PCs matched with glass control slides. Printed slides were incubated overnight and then UV crosslinked. Cotyledon RNA was extracted from freeze-dried soybeans seeds (Glycine max cultivar Williams). The identical aliquots of the purified RNA were separately labeled with Cy5 or Cy3 by reverse transcription. Slides were then hybridized overnight with a solution made from equal parts of Cy3 and Cy5 labeled transcripts. Approximately 40 µg of total RNA was used per slide. All slides received identical treatments throughout the assay.
**Fig. 59** Fluorescence images of a subarray on a PC designed for Cy3 and Cy5 fluorescence enhancement. (a) PC resonance centered at 532.4 nm has been excited (b) PC resonance centered at 632.8 nm has been excited. Images obtained using a microarray scanner. Line profiles (c) and (d) show examples of signal enhancement on the two channels when compared to the paired glass slide. (Reprinted from [145]).

The PC and the glass slide were scanned using a confocal microarray scanner (LS Reloaded, Tecan) equipped with TM polarized lasers ($\lambda = 632.8$ nm and $\lambda = 532.4$ nm) and emission filters for Cy-5 and Cy-3 excitation. All slides were scanned at identical PMT gain settings and at a pixel resolution of 10 $\mu$m. The PC was scanned at its resonant angles ($\theta_{\text{excitation}}$) while the glass slide was scanned at normal incidence (0°).

Fluorescence images of a sub-array from a 2-color DNA microarray experiment on a PC device engineered to enhance the excitation of Cy3 and Cy5 fluorophores are presented in Fig. 59(a, b). Line profiles through a row of spots on the Cy3 and Cy5 channels are presented in Fig. 59(c, d) for both the PC at resonance and the corresponding control glass slide in this pair. For the Cy3 channel, a replicated-averaged spot intensity enhancement of $14.9 \times$ across the set of 192
genes was observed on the PC as compared to the glass slide. On the Cy5 channel, an average spot signal enhancement of $18.3\times$ was observed. Average SNR enhancements of $3.4\times$ and $3.7\times$ were observed on the Cy3 and Cy5 channels, respectively. 23 genes (11.9%) were detectable on the glass slide on the Cy 3 channel while 56 genes (29.2%) were detectable when the PC resonance centered at 532nm was excited. On the Cy5 channel, 28 genes (14.6%) were detected on the glass slide while 59 genes (30.7%) were detected on the PC at resonance. On comparing the PC against its control glass slide, an average of twice as many genes were detectable on the PC for both Cy-3 and Cy-5 excitation.

4.7 Conclusion

This paper reports the design and fabrication of a PCEF surface that was fabricated upon a quartz substrate to lower its auto-fluorescence. The fabricated PC surface demonstrated a maximum enhancement factor of $7500\times$ for a ~50 nm thick layer of LD-700 (concentration = 538 ng/ml) doped SU-8. The LOD of PPL- Alexa 647 on the PC was lowered by $140\times$ compared to its glass control. Using a dose-response characterization of deposited PPL-Alexa 647 spots of variable tagged molecule concentration, a SNR improvement of $330\times$ was obtained for the concentration of PPL-Alexa 647 that correspond to its LOD on the control glass surface. Both single color and two color microarray experiments were performed to demonstrate the fluorescence enhancement in the context of multispot microarray. On comparing the PCs against their control glass slides, an average of twice as many genes were detectable on the PC for both Cy-3 and Cy-5 excitation.

This PCEF surface can be used to provide lower detection limits for broad classes of surface-based fluorescent assays for applications that include DNA microarrays for quantification of
gene expression, protein microarrays for detection of disease biomarkers in blood, and next-generation DNA sequencing applications that utilize fluorescent tags.
CHAPTER 5
ENHANCED FLUORESCENCE EMISSION USING A PHOTONIC CRYSTAL COUPLED TO AN OPTICAL CAVITY

5.1 Introduction

Incorporating PCs on silicon (Si) substrates has the inherent benefits of standard semiconductor processes and hence better control of device properties. Furthermore, it opens up the possibility of combining optical measurements with electronic measurements that may be performed in an integrated circuit. Hence the next generation of the PCEF substrates were designed on silicon substrates. PCs on silicon provide substantial advantages compared to previously reported PCEF surfaces prepared on plastic or quartz substrates. First, these devices can be fabricated on a wafer scale with semiconductor process technology and are thus amenable to inexpensive, high volume manufacturing. Second, the SiO$_2$ and TiO$_2$ materials of the PC have negligibly low levels of autofluorescence, thus enabling weak fluorescence signals generated by low concentration analytes to be more easily observed. Figure 1(a), shows the design of a PC on a silicon substrate. The design utilizes a thick layer of thermally grown SiO$_2$ (~ 800 nm) on top of the silicon substrate. This ensures that silicon reflectivity has no effect on the resonance modes of the PC and that the modes are only determined by the refractive indices of SiO$_2$ and TiO$_2$ and the dimensions of the grating. The first prototype Si-based PC devices were fabricated using the nano-imprint lithography process [146]. Briefly, a thermal oxide with a thickness of ~ 800nm was first grown on a silicon wafer (4” diameter, double side polished) and a step and flash imprint lithography (SFIL) process was carried out using the Imprio-55 nano-imprint tool (Molecular Imprints, Inc.). Finally, for large scale fabrication of these devices on 8” wafers, a
commercial vendor (SVTC technologies) was employed. A proprietary process using deep UV (DUV) lithography and reactive-ion-etching (RIE) was used to transfer the grating pattern from the template to the full area of an 8” wafer. An SEM image showing the surface characteristics of a Si-based PC is shown in Fig. 60(b).

![Figure 60](image)

**Fig. 60** | (a) Schematic of a PC on a silicon substrate. (b) An SEM of the PC.

![Figure 61](image)

**Fig. 61** | Reflection setup schematic. A white light is made incident on the sample placed on a tilted stage to deflect the reflected light onto the detector. The angle of incidence is changed by using the sample rotation stage. The detector is then rotated by twice the angle of incidence to collect the reflected light.
Fig. 62 | Measured reflection spectra of a Si-based PC measured at two different incidence angles. The peaks in the spectra represent the PC resonance while the background curves represent the optical-modes of SiO₂-Si cavity.

The reflection spectrum of the Si-based PC was measured using the reflection setup shown in Fig. 61. The setup consisted of a tungsten halogen lamp (white light), coupled to an optical fiber (Ocean Optics Inc.) with a 50 µm core. The output of the fiber was collimated using an achromatic lens and polarized using a linear polarizer (Thorlabs Inc.). The light was then projected onto the PC that was held in a customized holder. The PC holder was then put on a rotation stage. The entire assembly was then placed on another rotation stage which has a detector mounted on it. The setup design takes advantage of the 1-D PC’s insensitivity to an angle change in the $\phi$-direction. The PC holder was tilted at a fixed angle of $\phi = 5^\circ$ and the detector was placed at a height lower than the height of the incident beam with a tilt of $2\phi = 10^\circ$. This orientation allows for the detection of the reflected spectra at normal incidence. The angle of incidence of the light was changed by rotating the sample stage. In order to measure the reflected wavelength spectrum at an angle of incidence $\theta$, the sample rotation stage was rotated
by an angle $\theta$ in the clockwise direction and the detector rotation stage was rotated by an angle $2\theta$ in the counterclockwise direction. This allows the detector to collect the reflected wavelength spectra at all the angles. Each spectrum was normalized using a gold reflection mirror. Figure 62 plots the reflection spectra of a Si-based PC for a TM-polarized incident light for two angles of incidences. Recently, Si-based PCs were used to demonstrate enhancement in the detection of cancer biomarkers [98].

The optical response of a PC at resonance changes when it is coupled to a Fabry-Perot type optical cavity by adding a layer of gold underneath it. Coupled resonators have been studied in the past and can lead to interesting optical properties like an increase in Q, changes in electric fields, or modification of the far-field reflection properties, that can improve detection in sensing applications [125, 147, 148]. In this work I demonstrate such a coupled-cavity photonic crystal structure. The structure operates by coupling one-dimensional (1D) PC modes to the modes of an underlying Fabry-Perot type optical cavity. This coupling of the two modes results in even higher evanescent fields on the surface of the PC when compared to the fields when the light is resonantly coupled to a PC without an underlying cavity coupled to it. The experimental results are supported by a quantitative theoretical investigation of the cavity-coupled PC structure using RCWA electromagnetic modeling.
Figure 63(a-b) compares the structure of the cavity-coupled PC biosensor to the solitary PC. The cavity-coupled PC structure is formed by adding a layer of gold under the PC at a specific distance. The PC structure is comprised of a periodic linear grating structure ($\Lambda = 360$ nm, depth, $d = 60$ nm) that is patterned in SU8 resist by solvent-assisted soft imprint lithography. A blanket deposition of a TiO$_2$ film (thickness, $t = 120$ nm) is applied by sputtering on top of the imprinted structure. In this work, the PC was designed to have a resonance for a normally incident ($\theta = 0^\circ$) TM polarized light (He-Ne laser) for enhanced excitation and a resonance for a normally incident TE polarized light for enhanced extraction. The geometry of the structure and the indices of the surrounding dielectric media determine the resonance wavelength of the PC and it was shown that the optical cavity strongly modifies these resonances. Here a 1D-PC, formed by a linear
grating structure in low refractive index (RI) polymer with high RI TiO₂ on top, optically couples constructively or destructively to the modes of the underlying optical cavity formed between the PC and the gold layer that acts as a mirror underneath the PC. Figure 63(c) shows a large area optical image of the cavity-coupled PC fabricated on a 2 inch silicon wafer. Figure 63(d) shows a cross-sectional SEM image of the fabricated device with the underlying cavity.

**Fig. 64** | RCWA simulated data for one period of the PC (a) Far-field reflection of the cavity-coupled PC for various cavity lengths. The incidence angle is θ = 0°, which corresponds to the resonance angle of the PC (b) Far-field reflection spectrum for one of the cavity lengths (740 nm) showing the coupling compared to that of the solitary PC (c-d) Near-field electric field distribution for the cavity-coupled PC and the solitary PC at the resonance wavelength showing enhanced fields for the case of the coupled modes
Fig. 65 | Fabrication of a 1-D PC and a coupled-cavity PC using solvent assisted soft imprint lithography comprises of two steps: Bilayer PDMS stamp fabrication: (a) Hard-PDMS is spin coated on a master wafer. (b) Soft-PDMS is poured onto the hard-PDMS and subsequently heat cured. (c) The stamp is peeled away from the master wafer. Solvent assisted imprint lithography: (d) A substrate is spin coated with the imprint resist SU8. (e) Ethanol is dropped so as to cover the area to be imprinted. (f) PDMS stamp is pressed onto the substrate. (g) After ethanol evaporates the stamp is peeled away leaving the nano-imprint on the resist surface that is UV cured and post-baked.

RCWA simulations were performed to model the coupling between the optical cavity and the PC. Figure 64(a) shows far-field reflection spectra computed by RCWA for a cavity-coupled PC for different lengths of the cavity and the incidence angle of $\theta = 0^\circ$ that corresponds to the resonance coupling angle of the PC. The coupling region is represented in dark blue in the plot where the reflection from the device becomes minimum. This represents the region where the incident photon resonates within the cavity before it is either scattered or absorbed by the structure. From the figure, we can see that the coupled mode repeats itself for every $\sim 220$ nm of added cavity length. This length corresponds to $\sim \lambda_{\text{eff}}/2$ that satisfies the condition for constructive interference within the cavity. Here $\lambda_{\text{eff}} = \lambda/n_{\text{eff}}$ is the effective PC resonance wavelength in the cavity. Thus, the coupled-cavity resonance repeats itself with the changes in
the cavity length as the PC resonance comes into and out of overlap with the cavity modes. Figure 64(b) shows one such reflection spectrum for a cavity-length of 740 nm in comparison to a solitary PC reflection spectrum. With the resonant photon now trapped inside the cavity resulting in a strong sharp dip in the reflection, the cavity not only inverts the reflection response of the PC but also enhances the resonance as seen by the increase in the evanescent electric fields on the surface of the PC (Fig. 64(c-d)).

The subwavelength grating was fabricated by solvent-assisted soft imprint lithography [149]. The steps of fabrication are shown in Fig. 65. A soft stamp with a negative volume image of the desired grating structure was used for imprinting. The stamp was prepared by spin coating Hard-PDMS (a mixture of poly(7-8%vinylmethylsiloxane)-(dimethylsiloxane), 1,3,5,7-tetravinyl-1,3,5,7-tetramethylcyclotetrasiloxane, Xylene and poly(25-30% methylhydrosiloxane)-(dimethylsiloxane)) onto an 8-inch silicon “master” wafer that had been previously prepared with a positive surface volume image of the desired grating dimensions by deep UV lithography. After spin-coating the Hard-PDMS on the silicon wafer, a thick layer of Soft-PDMS (10:1 Sylgard 184) was poured over it to provide mechanical strength for handling the stamp. The master wafer with both layers of PDMS on top of it was heat cured overnight in an oven at 65 °C. After curing, the stamp was peeled away from the master wafer and cut into small squares. The cavity-coupled PC and the solitary PC were fabricated on 2-inch Si wafers. SU8 2000.5 was chosen as the polymer that forms the low refractive index layer for the PC and also the cavity layer for the cavity-coupled PC. The solvent assisted imprinting process is performed by first spin coating SU8 onto a clean substrate. A 200 nm gold-coated Si wafer served as the substrate for the cavity-coupled PC while a bare Si wafer was the substrate for the solitary PC. For the case of the solitary PC, ~ 1 um thick SU8 was applied by spin coating in two layers. This ensures
that Si reflectivity has no effect on the resonance modes of the PC and the modes are only determined by the refractive index of SU8 ($n = 1.46$), the refractive index of TiO$_2$ ($n = 2.43$) and the dimensions of the grating. For the cavity-coupled PC, an SU8 thickness of ~750 nm was spin-coated onto the wafer. After spin coating, the SU8 was prebaked at 75 °C for 2 min. A small amount of ethanol was then used to wet the SU8 surface before pressing the substrate against the stamp. The stamp was pressed upon the SU8 coated wafer until the ethanol evaporated (~45 min.), after which it was peeled away and the wafer was UV cured for 2 min. and then hard baked at 90 °C for 2 min. Finally, TiO$_2$ (thickness, $t = 120$ nm) was sputtered onto the grating.

Figure 66(a) plots the measured far-field reflection for the cavity-coupled PC for the cavity length of 750 nm and the solitary PC with the incidence angle of the white light at $\theta = 0^\circ$. The measured spectra indicate that the presence of the cavity beneath the PC results in an inversion of the resonance characteristic from a reflective maxima to a reflective minima, as predicted by the RCWA model.

5.3 Coupled-Cavity Enhance Fluorescence

In order to demonstrate the enhancement in the SNR detection of surface attached fluorophores on the cavity-coupled PC surface, a detection experiment using a dye-labeled protein was performed. 0.7 µl volume of the dye-labeled polypeptide, Alexa 647-Poly-Phe-Lysine (PPL-Alexa 647) was applied using a pipette at a concentration of 30 µM on the cavity-coupled PC with a resonance cavity length of 750 nm, the cavity-coupled PC with a non-resonant cavity length of 650 nm, and the solitary PC surface. After an overnight incubation, the devices were washed by gently dipping them in DI water for 60 sec. Each spot of the fluorophore-tagged protein had a diameter of approximately 4 mm. Fluorescent images of the labeled protein spots
were obtained using a commercially available confocal laser scanner (Tecan, LS-Reloaded) equipped with a TM polarized $\lambda = 632.8$ nm He-Ne laser. The angle of incidence of the laser can be tuned from $\theta = 0^\circ$ to $\theta = 25^\circ$ and using a numerical aperture of .04 to focus the light, only a portion of the illumination is applied at the resonant coupling condition, as discussed in Chaudhery et. al. [95]. The measured images were analyzed by image processing software (ImageJ). Figure 66(b) plots the intensity cross section through the dye-labeled protein spot on each device structure. The angle of incidence for the laser was $\theta = 0^\circ$ to correspond with the resonance coupling angle of the PC. The fluorescence intensity on the cavity-coupled PC is higher than both the solitary PC and the PC with underlying off-resonance cavity length, showing that the increase in the evanescent fields due to the coupling of the two modes gives rise to the enhancement. From the intensity plot, the increase in the SNR for the dye labeled polypeptide on the cavity-coupled PC was calculated as $6 \times$ when compared to the solitary PC, and $10 \times$ when compared to the off-resonant cavity PC. The noise here is defined as the standard deviation in the background intensity around the spot.

5.4 Conclusion

In summary, the work here has demonstrated a novel approach in which, for the first time, a 1-D PC coupled to an underlying Fabry-Perot type cavity was used to further amplify the evanescent electric fields for PC enhanced fluorescence. It was shown that the underlying optical cavity, when coupled to the PC resonance, resulted in an increase of the evanescent field intensity on the PC surface as shown by the improvement in the detected signal intensity of a dye labeled protein. An attractive aspect of this type of cavity-coupled photonic component is that its properties can be tuned dynamically, by changing either the dimensions or the refractive index of the cavity. For example, we can go from using a PC as a narrow wavelength reflective filter to a narrow
wavelength absorptive filter by coupling it to the cavity. This approach shows promise as a method for further increasing the signal-to-noise ratios obtainable for a wide variety of surface-based fluorescence assays used in molecular diagnostics, genomics, and proteomics.

Fig. 66 | (a) Experimental far-field reflection spectrum for the cavity-coupled PC at the cavity length of 750 nm and the solitary PC. The incidence angle for the laser is $\theta = 0^\circ$ which corresponds to the resonance angle of the PC (b) Intensity profile for PPL-Alexa 647 dye for the 3 devices (inset shown in same color scale): Cavity-coupled PC with the cavity length of 750 nm, uncoupled cavity and the PC for the cavity length of 650 nm and the solitary PC.
CHAPTER 6
COUPLED EXTERNAL CAVITY PHOTONIC CRYSTAL ENHANCED FLUORESCENCE

6.1 Introduction

Previous work on fluorescence enhancement has operated through simple illumination of the nanostructured surface with a laser, which often requires illumination with a specific combination of wavelength and incident angle to generate strong surface coupling. In this chapter, a fundamentally new approach to fluorescence enhancement is demonstrated that is capable of generating an even greater enhanced excitation effect, in which the PC itself serves as a tunable mirror of an external cavity laser (ECL). ECLs are generally comprised of a source of optical gain, such as a diode or semiconductor optical amplifier, that establishes a resonant optical cavity with a reflective surface that is spatially separated from the gain source. While PCs have been used previously as a mirror of an ECL [150], it has not been recognized that such a configuration leads to even higher evanescent fields on the surface of the PC that can be used for purposes of coupling energy to surface-adsorbed light emitters. Such an approach will not only benefit fluorescence detection, as demonstrated here, but can also be applied to enhancement of surface-enhanced Raman spectroscopy when it is performed upon a PC surface [151].

This new approach utilizes an antireflection-coated semiconductor laser diode (LD) with a gain spectrum in the 639<\(\lambda\)<645 nm wavelength region as the optical pump source of an ECL with a PC surface as the external mirror. As shown schematically in Fig. 1A, the PC is designed to perform as a narrow bandwidth resonant reflectance filter with a peak reflectance wavelength that falls within the LD gain spectrum. The lasing wavelength of the ECL cavity is selected by
the peak reflectance wavelength of the PC, and is tuned by the adsorption of biomolecular layers upon the PC surface. Chemical fluorescent dyes and semiconductor quantum dots are selected with strong absorption at the lasing wavelength, and are excited by surface-confined electric field standing waves that occur on the PC surface. The experimental demonstrations here were supported by a theoretical investigation on the working principle of this cavity-coupled PC using finite-difference time domain (FDTD) electromagnetic modeling. The model was used to study the electric field distribution that develops on the PC surface with the optical feedback. Compared to the PC without feedback from the laser, the PC in the ECL configuration showed increased surface electromagnetic fields, which in turn provide enhanced illumination intensity to surface-bound fluorophores.

Using PCs fabricated upon quartz substrates by nanoimprint lithography, a 10× stronger evanescent field is experimentally demonstrated upon the surface of the PC in this cavity-coupled PC configuration compared to the field on the PC substrate without a feedback cavity. Upon comparison of the net signal intensity from fluorescence molecules within the PC-ECL configuration to the signal obtained on an unpatterned glass surface, an enhancement factor of ~360× is observed. Direct application of dye-labeled protein to the surfaces of PCs over a range of protein concentrations is used to demonstrate that use of the PC-ECL system provides a 10^5× advantage in the LOD compared to detection upon a glass substrate. It will be demonstrated that the PC-ECL configuration tunes itself to the resonant wavelength of the PC, thus eliminating the need to adjust the incidence angle of the detection instrument when the PC is altered by surface chemistry layers or by capture molecules. The results obtained here show that integration of PCEF with excitation via an external cavity provides efficient coupling from the illumination
source and greater enhanced excitation than an equivalent system that operates without the benefit of an optical feedback.

**Fig. 67** Working principle of the external cavity coupled photonic crystal enhanced fluorescence. (A) The resonantly reflected laser wavelength from the PC provides feedback to the diode. The cavity then lases at the resonant wavelength of the PC. Addition of biomolecules to the surface of the PC shifts the resonant reflected wavelength, which in turn changes the lasing wavelength of the PC. (B) Overlap of resonantly reflected laser by the PC and the gain of the diode with the modes of the cavity determine the lasing wavelength of the cavity.
6.2 Cavity-Coupled Photonic Crystal for Fluorescence Enhancement

Figure 67(A) shows the working principle of the cavity-coupled PC-ECL system, which is comprised of a laser diode (LD), the PC, and a system for detecting fluorescence output as either an intensity output or an image. One facet of the LD has high reflectance (95%) while the other
facet is coated with an anti-reflection layer (reflectance R<10^{-4}). The PC surface is placed directly in front of the AR-coated facet, with a lens between them to collimate the light onto the PC, and to focus the reflection back into the diode. The PC is designed to have a reflection resonance wavelength within a 639<\lambda<645 nm range, to overlap with the gain spectrum of the LD. The PC couples to the modes of this external cavity and provides feedback to the LD. When the PC resonance matches the optical cavity modes lying within the gain spectrum of the diode, the entire cavity lases with single mode output spectra as shown in Fig. 67(B).

As described in previously reported detection instruments that take advantage of PCEF, variability in the PC will lead to variability in the precise incidence angle required to produce resonant coupling for a fixed wavelength, which in turn necessitates precise tuning of the incidence angle in order for the surface to be maintained on-resonance [95]. The PC-ECL configuration eliminates this constraint. As the cavity only lases with the feedback from the PC, the lasing wavelength always corresponds to the PC resonance wavelength. The self-tuning action is shown in Fig. 68(A), for several discrete positions on the PC surface, where a slight gradient in the TiO\textsubscript{2} film thickness results in a gradient in resonance wavelength across the device. It can be seen that with feedback the lasing wavelength corresponds to the peak in the reflection spectrum of the PC. This self-tuning ability of the PC-ECL configuration is useful for efficient excitation of surface-bound fluorophores, as adsorption of capture molecules and other surface chemistry layers also shift the resonance coupling condition of the PC. The adsorption of biomolecules to the surface of the PC increases the effective refractive index of the resonance mode, which in turn shifts the resonance wavelength of the PC to a longer wavelength. A positive shift in the resonance reflection wavelength of the PC caused by a biomolecule adsorption in turn causes a positive shift of the lasing wavelength of the ECL. As a result, a
surface-bound fluorophore is always excited with the PC in the optimal on-resonance condition. While the diode is capable of lasing without feedback from the PC, its interaction with the PC through the formation of an external cavity effectively reduces the lasing threshold. This effect is clearly observed in Fig. 68(B), which shows the emitted power with feedback and without feedback for equal diode injection current. The self-tuning behavior is only observed when feedback from the PC is present.

Fig. 69 | Device design. (A) Cross-sectional SEM of the PC fabricated by nanoimprint lithography. (B) Image of the device after fabrication.

As shown in Fig. 69, the PC was fabricated on a quartz substrate selected for its low autofluorescence (See Methods) [87]. The PC structure was comprised of a periodic ($\Lambda = 400$ nm) linear grating structure that was patterned by nanoimprint lithography and etched into the quartz by reactive ion etching (depth, $d = 40$ nm). After removal of the imprint resist, the quartz grating was coated with a film of TiO$_2$ by sputtering to complete the structure (thickness, $t = 125$ nm). The PC period, grating depth, and TiO$_2$ thickness are selected to produce a surface that resonantly reflects a narrow band of wavelengths that falls within the gain spectrum of the diode when the media above the PC is comprised of air. The width of the lasing peak and the suppression of the sidebands in the lasing profile depend largely upon the reflectivity and the
linewidth of the reflection peak of the PC [152]. In previous work, we have shown that an optimal design for PC enhanced fluorescence for normal incidence illumination utilizes a TM polarized light for excitation and a TE polarized light for extraction of the emitted fluorescence signal [104]. Therefore, the PC was designed for normal-incidence excitation using TM polarized light to have a resonance in the $639 < \lambda < 645$ nm wavelength range, and a TE resonance mode at 690 nm and $\theta = 0^\circ$ for enhanced extraction.

**Fig. 70| Theoretical formulation.** (A) Schematic illustration of a unit cell simulated using FDTD, showing one period of the PC and a plane-wave source sandwiched between the PC and a gold mirror for a cavity of length $L$. (B) FDTD computed far-field transmission intensity for PC-coupled cavity showing cavity modes enveloped by a PC resonance mode. The peak in the transmission spectrum represents lasing action in the cavity. (C) Simulated electric field intensity on the PC surface when coupled to the cavity. (D) Simulated electric field intensity on the PC surface when incident light is resonantly coupled to the PC. On averaging the field in a 5 nm area above the PC surface (for both c-d), an increase of $330\times$ in the average fields is observed for the cavity-coupled PC biosensor.
FDTD simulations were performed to model the coupling between the optical cavity and the PC, using a simplified approach that approximates the behavior of the actual external cavity. Figure 70(A) shows a schematic of the simulated FDTD model. The simulation space must be partitioned into small volume elements with a size scale that can accurately capture the spatial variability of electromagnetic fields near the PC surface. If a spatial dimension of 5 nm for such a cell were used, the entire length of the cavity (30 cm) would be too large to be easily simulated. Because the vast majority of the cavity was comprised of empty space, a shorter cavity (1.85 µm) that produces the same physical effect was simulated, with the only difference being the wavelength spacing between allowed external cavity modes, as described by the free spectral range (FSR):

\[
\text{FSR} = \frac{\lambda_0^2}{2nl\cos(\theta) + \lambda_0}
\]

(6.1)

where \(n\) is the refractive index of the cavity, \(l\) is the length of the cavity, \(\theta\) is the angle of incidence and \(\lambda_0\) is the central wavelength of the nearest transmission peak.

For the 30 cm long experimental external cavity, the spacing between the optical modes is FSR~0.7 pm, ensuring that the PC resonance peak always overlaps with an available cavity mode that provides feedback to the diode. Within the simulation, the laser diode was replaced by a 200 nm thick gold layer that acts as the high reflectivity end of the cavity. A TM polarized plane wave source (overlapping a wavelength range of 639 < \(\lambda\) < 645 nm) was inserted into the cavity. When the PC is not coupled to the cavity, its simulated resonant reflection displays a FWHM of ~ 2 nm, as shown in Fig. 70(B), which is also observed experimentally, as shown in Fig. 70(B). Insertion of the PC into the cavity results in narrowing of the resonant transmission spectrum to FWHM = 0.1 nm (Fig. 70(B)). This phenomenon is also experimentally observed.
(Fig. 67(B)) as the stimulated emission in the diode, and combined with feedback, results in lasing of the external cavity system.

Importantly, the interaction of the PC with the external cavity results in an increased evanescent electric field magnitude on the surface of the PC, thus providing an additional mechanism for PCEF gain. Figures 70(C-D) compare the simulated power density ($|E|^2$) associated with the electric field near the surface of the PC for the PC-ECL configuration, compared to a PC that is resonantly excited by the illumination source without the benefit of an external cavity. Averaging $|E|^2$ within the volume of media that is within 5 nm of the sensor surface (within the air media), where surface-bound fluorophores would be located, an enhancement factor of 330$\times$ is obtained. The simulation here supports the hypothesis that evanescent fields with enhanced magnitude will form upon the PC surface within the cavity, and lead to efficient enhanced excitation of surface-adsorbed light emitters, such as fluorophores and quantum dots. The simulation utilizes uniform perfect plane waves and a cavity that does not suffer from various sources of optical loss; therefore experimental enhancements are not expected to be as high as in the simulation.

### 6.3 Enhanced Excitation of Quantum Dots and Dye-Labeled Streptavidin

To demonstrate enhanced excitation of fluorophores by the cavity-coupled PC, detection experiments were performed using streptavidin labeled with a fluorescent dye (Alexa633), and semiconductor quantum dots (QD) coated with streptavidin. A schematic diagram of the detection instrument is shown in Fig. 71, which enables us to gather fluorescent images of the PC surface from below the substrate, while the cavity is formed above the substrate. An excitation filter was added to the cavity to attenuate laser diode emission at wavelengths greater than $\lambda =$
650 nm, while a quartz window was added to monitor the power inside the cavity at one end, while enabling measurement of the lasing spectrum at the other end. For the 1-dimensional PC, the resonance coupling condition only depends upon the angle between the incident beam and the grating’s normal vector in the plane perpendicular to grating direction (labeled as $\theta$ in Fig. 71). The resonance condition is insensitive to the angle in the plane along the grating direction ($\phi$). The diode illumination was incident on the PC at an angle of $\theta = 0^0$ and $\phi = 17^0$. This configuration ensures that no excitation light leaks into the objective ($NA = 0.28$, acceptance angle $\sim 16^0$) of the imaging setup, thus reducing background noise in the collected signal while the resonance condition for the PC is maintained. The resonantly reflected light from the PC retraces its path back to the diode by reflection against a mirror, thus forming the cavity.

![Detection instrument schematic](image)

**Fig. 71** | Detection instrument schematic. Cavity-coupled PC with the imaging setup used in the experiment. A resonance angle of $\theta = 0^0$ is used as the incidence angle for the laser on the PC. $\phi = 17^0$ is chosen to prevent the transmitted laser light from entering the objective.
To compare the evanescent field magnitude on the surface of the PC in the cavity to its magnitude without the cavity, while avoiding the effects of photobleaching, streptavidin-coated QDs selected for their absorption at $\lambda = 640$ nm were used (QD 705, Life Technologies, emission wavelength = 705 nm). The labeled streptavidin-coated QDs were applied using a pipette at a concentration of 20 $\mu$M on a PC surface that had been functionalized with dimethylethoxysilane. After an overnight incubation and a wash with deionized water, fluorescence intensity was measured with the PC on-resonance and with the cavity-coupled PC at the same incident laser power. This was achieved by blocking and unblocking the resonantly reflected laser beam from reaching the second mirror and adjusting the injection current to the diode. The net fluorescence intensity (emission-background fluorescence) on the cavity-coupled PC was increased by a factor of 10×, compared to the solitary PC, as shown in Fig. 72(A). No measureable increase in the background and noise were detected in the cavity-coupled PC compared to PCEF giving an increase in signal-to-noise ratio ($S/N$) of 10×. This factor represents the gain achieved over “ordinary” PCEF.

To demonstrate the overall gain for this approach compared to detection of the same analyte on an ordinary glass surface, a series of concentrations (1 $\mu$M–1 pM range) of Alexa-633 labeled streptavidin (Alexa-SA) were tested. The net fluorescence signal intensity of Alexa-SA on the PC surface substantially increased for all concentrations tested, compared to the signals from the glass surface (Fig. 72(B)). These measurements were taken using identical incident laser power, camera gain, and integration time. The highest concentration of Alexa-SA on the cavity-coupled PC resulted in saturation of the photon count available from the CCD. On the glass surface, only the two highest Alexa-SA concentrations were visible, while all lower concentrations resulted in fluorescence counts lower than the background noise level. The concentration of 1 pM was
Fig. 72] **Fluorescence enhancement.** (A) (Inset) Fluorescence images of quantum dot labeled streptavidin on a solitary PC and a PC-coupled cavity. Line profile of the images showing an enhancement of 10×. (B) Plot of net fluorescence signal intensity of Alexa-streptavidin versus its concentration to compare the signal intensity from the PC-coupled cavity biosensor and glass. The limit of detection on the PC was ~1 pM compared to an unpatterned glass surface, where no spots were seen below 0.1 µM. 

observed on the cavity-coupled PC biosensor with a signal-to-noise ratio of ~6.1, while no fluorescence was detected on the glass surface for concentrations below 0.1 µM. As the concentration was increased, fluorescence intensity on the cavity-coupled PC increased while the
signal intensity on the glass surface only started to show a detectable signal starting above 0.1 µM (an intensity equivalent to the 1 pM concentration on the cavity-coupled PC). For the concentrations of Alexa-SA that were measurable upon both the PC and the glass surface, comparison of the net signal intensity demonstrated an enhancement factor of ~ 360×.

6.4 Discussion

In this work, I demonstrated for the first time that a PC surface excited within an optical cavity results in enhanced fluorescence with even greater evanescent field magnitude than is available from an equivalent surface that is illuminated by ordinary laser exposure. The higher magnitude evanescent electric fields generated by this method have been shown theoretically and experimentally to reduce achievable limits of detection and to increase signal-to-noise ratio for two varieties of optically pumped light emitters, namely chemical fluorophores and semiconductor quantum dots.

I envision PC-ECL excitation to be applicable to any surface-based fluorescence bioassay that is currently performed upon a glass surface, particularly those that would benefit from reduction of detection limits, or development of a miniature detection instrument for clinical point-of-care applications. For example, “sandwich” assays for detection of disease biomarkers in serum utilize fluorophore-tagged secondary antibodies to specifically label captured analytes [153], but achieve detection limits that are typically two orders of magnitude lower than the dissociation constant for the interaction of the capture antibody and the analyte. PCEF has demonstrated the ability to enhance weak fluorescence signals for low concentration biomarkers, thereby reducing detection limits [154] and potentially achieving the ability to perform diagnosis at an earlier
stage. Further increase in the electromagnetic enhancement factor will enable the detection limits achievable via PCEF to be pushed further.

An important aspect of the detection system is its throughput, as measured by its ability to integrate a large number of biological assays into a small PC surface area. The most basic implementation of a PC-ECL instrument, illuminates a ~2200 µm² region of the PC with a collimated (but not focused) laser beam and measures fluorescence intensity integrated from that entire region, essentially performing one assay with a single illuminated spot. Such an approach will be appropriate to assays performed within standard format microplate wells, in which the entire bottom surface of each well would be covered with PC, and coated with a uniform layer of capture molecules. I also envision, as partially demonstrated here, the ability to capture fluorescent images of the PC surface while it is being excited by the external cavity, which would enable a PC surface to be partitioned into a microarray of discrete capture spots. I have observed that an important factor that must be addressed for the PC-ECL approach to become effective for imaging is to achieve a high level of illumination uniformity. In the implementation of the system presented here, the diode illuminates a ~700 µm diameter region with a Gaussian beam, resulting in only a ~2200 µm² region in the center of the beam experiencing strong excitation. To overcome this limitation, I envision an illumination approach in which the beam will be focused by a cylindrical lens with its cone of incident angles spread in the φ direction, while the orthogonal θ direction couples to the PC in the on-resonance condition [96]. Such an approach would allow a fluorescent image to be gathered for a tightly focused line, which would then be scanned across the PC to gather an image of an entire surface populated with small capture spots. Such an imaging approach could be extended to detection of other surface-bound fluorescent emitters, such as those used to label the surface of cells or tissue. I further expect this
approach to translate to PC structures operating within a microfluidic channel, enabling enhanced detection of analytes (such as virus particles or DNA molecular beacons) flowing through the channel and entering the evanescent field region of the PC [155].

Besides enhancing fluorescence, the cavity-coupled PC may also be employed to further magnify surface enhanced Raman spectroscopy (SERS). As SERS signal scales with $|E|^4$, any increase in $|E|$ would lead to considerable increase in the signal. Thus SERS signal of a molecule linked to metal nanoparticles dispersed on the surface of the PC can be enhanced by overlapping the extinction spectra of the particle with the resonance of the cavity-coupled PC. The optical gain provided by the diode in this case can help overcome sources of optical loss/absorption from metal nanoparticles while enhancing the coupling of the laser to the metal nanoparticle via PC. I expect this to be a topic of future work.

To conclude, I have presented a novel method, supported by experimental demonstrations and theoretical modeling, for sensitive detection of dye-labeled proteins in which a PC surface serves as a tunable mirror of an external cavity laser, where the cavity results in an enhanced evanescent field magnitude above that generated by ordinary PCEF. In addition to greater sensitivity, the approach provides a self-tuning capability that ensures on-resonance excitation of the cavity, even as the PC resonant condition is altered by attached biomolecules. Using both chemical fluorophores and semiconductor quantum dots, I demonstrate detection limits over $\sim 10^5$-fold lower than traditional techniques. Coupled with the simplicity afforded by physical signal enhancement and compatibility with existing biodetection tools, the cavity-coupled PC is expected to find broad use in disease diagnosis and other fluorescence-based sensing applications.
6.5 Methods

6.5.1 PC Fabrication

The PC was fabricated using the step and flash nanoimprint lithography technique, using a Molecular Imprints Imprio50 tool. Briefly, a quartz template was fabricated (Dai Nippon Printing Co., Ltd.) with a linear grating structure (period = 400 nm, depth = 100 nm, 50% duty cycle) by e-beam lithography with a grating area of 9x9 mm. A 4-inch quartz wafer was used as a substrate for the PC. A planarization layer (Transpin, Molecular Imprints) was spin-coated onto the wafer prior to dispensing droplets of the imprint resist (MonoMat, Molecular Imprints) so as to fill the template pattern without extrusions. The template was pretreated with a release layer (RelMat, Molecular Imprints) and was pressed onto the imprint resist creating a replica of the mold. The imprint resist was cured into a solid phase by exposure to ultraviolet light. The template imprint/cure/release process was stepped and repeated across the wafer to create a 4x7 array of gratings. A resist (Silspin, Molecular Imprints) was then spin coated over the imprint resist, to serve as a hard mask for the etching process. Reactive-ion etching was then used to transfer the pattern onto the wafer surface with a depth of 40 nm. After removal of the resist films and planarizing films (using Piranha clean), TiO₂ (~125 nm, refractive index ~ 2.35) was sputtered onto the wafer. Finally, the wafer was diced to produce 2, 1×3 in² slides.
6.5.2 FDTD Model

FDTD simulations (Lumerical FDTD, Vancouver, Canada) were used to model the coupling between the optical cavity and the PC, using a simplified approach that approximates the behavior of the actual external cavity. The detailed FDTD model of the simulated structure appears in Fig. 73 where the cavity lengths, gold mirror, source position, grating period, grating depth and TiO$_2$ thickness are $L$, $M$, $h$, $\Lambda$, $d$ and $t$, respectively. The refractive indices of gold and Quartz were taken from the material database of Lumerical; CRC for gold and SiO$_2$ (Palik) for Quartz. The refractive index of TiO$_2$ was taken as 2.35 for all the wavelengths used in the simulation. The structure was excited by a linearly polarized (TM), plane-wave with the...
wavelength range overlapping $639 < \lambda < 645$ nm. The PC was designed to have a reflection resonance within $639 < \lambda < 645$ nm, by tuning the thickness of TiO$_2$ deposited on the grating surface. A 200 nm thick gold mirror replaced the LD. Periodic boundary conditions were chosen along the grating and PML on the top and bottom surfaces of the simulation region. An accuracy of at least $\lambda/22$ was used within the simulation region with mesh size of 5 nm in the region of the PC. The shorter cavity simulated here produces the same effect as a longer cavity except for the spacing between the optical cavity modes. In order to ensure that at least one optical mode of the external cavity couples to the PC, a simulation was performed for various cavity lengths. Figure 74 plots the transmitted intensity from the bottom of the PC for different cavity lengths for a range of wavelengths. From this data, a cavity length of 1.85 $\mu$m was chosen, for which one of the optical modes coupled to the PC mode. A cross-section of the transmission efficiency at the cavity length of 1.85$\mu$m is shown in Fig. 70(B). The evanescent field profile on the PC surface was then generated at this transmission peak wavelength and is shown in Fig. 70(C). The cavity-coupled PC was then compared to PC without a cavity. For this comparison, the PC in Fig. 73 was simulated without the gold mirror, while the remaining simulation parameters were kept constant. Figure 70(B) compared this PC reflection peak to the cavity-coupled PC. The evanescent field profile on the PC surface generated at this reflection peak wavelength is shown in Figure 70(D).

6.5.3 Far-Field Transmission Measurements

Transmission spectra were collected by a fiber-coupled collimating lens with its distal end connected to a spectrometer with a wavelength resolution of 0.06 nm (Ocean Optics HR 4000).
6.5.4 Dye-Labeled Detection Experiments

The Alexa-SA (streptavidin, Alexa fluro 633 conjugate, Invitrogen) and quantum dot-SA (Qdot 705 streptavidin conjugate, Invitrogen) solutions were prepared in 1× Phosphate-buffered saline (PBS) solution, pH = 7.4. The surfaces used in the experiment were cleaned and functionalized using dimethylethoxysilane by an overnight incubation in a vacuum chamber heated to a temperature of 80°C. The slides were then washed in toluene, methanol, and de-ionized (DI) water to rinse off any excess silane. The Alexa-SA and quantum dot-SA solutions were
deposited in the form of a ~1 mm diameter droplet on the samples using a precision pipette (±5% accuracy at 1µL). The samples were then incubated overnight and then washed and dried in PBS-tween and DI before taking fluorescence measurements.

6.5.5 Fluorescence Measurements

The LD (Ridge Waveguide Laser Diode, SAL-640-100, Sacher Lasertechnik) illuminates a ~700 µm diameter spot on the PC surface. The incident laser power in the cavity was monitored using a power meter and controlled using a diode injection current controller (E3620A, Agilent). All the images were taken using a laser power of 2 mW incident on the substrate both with and without feedback. A 10× objective (NA = 0.28) was used to collect the fluorescence signal, which was then imaged by an EMCCD (Cascade 512B) with a field of view of 800×800 µm². An integration time of 30ms was used for all the images along with a multiplication gain of 3000. A long pass emission filter (λ > 664 nm, SEMROCK-BLP01-664R-25) was used to block any excitation light leaking into the objective. The net intensity for all the fluorophores was calculated by subtracting the background substrate fluorescence from the fluorophore emission.
CHAPTER 7
CONCLUSIONS

This thesis makes a number of contributions to advancing the state of the art in PCEF. It addresses the theoretical analysis, design and fabrication of PCs for enhanced excitation of surface absorbed fluorescing molecules, in particular for fluorescence-based molecular diagnostics applications like gene expression analysis, disease diagnostics and life sciences. The following is a summary of the main contributions.

1. A PC substrate exhibiting resonant enhancement of multiple fluorophores was demonstrated. The device, fabricated uniformly from plastic materials over a ~3×5 in² surface area by nanoreplica molding, utilized two distinct resonant modes to enhance electric field stimulation of a dye excited by a $\lambda = 632.8$ nm laser (Cy-5) and a dye excited by a $\lambda = 532$ nm laser (Cy-3). Resonant coupling of the laser excitation to the photonic crystal surface was obtained for each wavelength at a distinct incidence angle. Compared to the detection of a dye-labeled protein on an ordinary glass surface, the photonic crystal surface exhibited a 32× increase in fluorescence signal intensity for Cy-5 conjugated streptavidin labeling, while a 25× increase was obtained for Cy-3 conjugated streptavidin labeling. The fabricated PC is capable of amplifying the output of any fluorescence dye with an excitation wavelength in the range of $532 \text{ nm} < \lambda < 633$ nm by selection of an appropriate incidence angle. The device was designed for biological assays that utilize multiple fluorescence dyes within a single imaged area, such as gene expression microarrays.

2. A PC surface fabricated upon a quartz substrate using nanoimprint lithography was demonstrated. Quartz was selected for its low autofluorescence characteristics...
compared to plastic-based PCs, improving the detection sensitivity and SNR of PCEF. Nanoimprint lithography enabled economical fabrication of the subwavelength PC structure over a 1x3 in² quartz slide. The demonstrated PC supported a TM resonance mode at a wavelength of $\lambda = 632.8$ nm and an incidence angle of $\theta = 11^\circ$. Meanwhile, another TM mode at a wavelength of $\lambda = 690$ nm and incidence angle of $\theta = 0^\circ$ efficiently directed the fluorescence emission toward the detection optics. An enhancement factor as high as $7500 \times$ was achieved for the detection of LDS-700 dye spin-coated upon the PC, compared to detecting the same material on an unpatterned quartz surface. The detection of spotted Alexa-647 labeled polypeptide on the PC exhibited a $330 \times$ SNR improvement. Using dose-response characterization of deposited fluorophore-tagged protein spots, the PC demonstrated a $140 \times$ lower limit of detection compared to a conventional glass substrate.

3. Enhanced excitation of surface bound fluorophores was demonstrated where the molecules were excited on a PC surface that was coupled to an underlying Fabry-Perot type cavity through a gold mirror reflector beneath the PC. This approach lead to a $6 \times$ increase in the SNR of a dye labeled polypeptide compared to ordinary PCEF.

4. A fundamentally new approach to enhance fluorescence was developed in which surface adsorbed fluorophore-tagged biomolecules were excited on a PC surface that functions as a narrow bandwidth and tunable mirror of an external cavity laser. This scheme lead to a $\sim10 \times$ increase in the electromagnetic enhancement factor compared to ordinary PCEF. In the experiments, the cavity automatically tuned its lasing wavelength to the resonance wavelength of the PC, ensuring the optimal on-resonance coupling even in the presence of variable device parameters and variations in the
density of surface-adsorbed capture molecules. Over $10^5 \times$ improvement in the limit of detection of a fluorophore-tagged protein compared to its detection on an unpatterned glass substrate was achieved. The enhanced fluorescence signal and easy optical alignment makes this cavity-coupled photonic crystal a viable approach for further reducing detection limits of optically excited light emitters that are used in biological assays.
REFERENCES


