Polymer thin film structures for ultra-low cost biosensing

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ABSTRACT

Reflectance change due to binding of molecules on thin film structures has been exploited for bio-
molecular sensing by several groups due to its potential in the development of sensitive, low cost, easy
to fabricate, large area sensors with high multiplexing capabilities. However, all of these sensing plat-
forms have been developed using traditional semiconductor materials and processing techniques,
which are expensive. This article presents a method to fabricate disposable thin film reflectance biosensors
using polymers, such as polycarbonate, which are 2–3 orders of magnitude cheaper than conventional
semiconductor and dielectric materials and can be processed by alternate low cost methods, leading to
significant reduction in consumable costs associated with diagnostic biosensing.

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1. Introduction

Highly multiplexed bio-molecular sensors are indispensable for the development of integrative or systems biology where a large
number of genomic or proteomic variables must be quantified to infer their interdependencies. Such sensing technologies also aid
in the diagnosis of diseases through the detection of molecular marker of disease states. Thin film structures whose reflectance changes
upon molecular binding on sensor surface had previously been reported [1–4] with noise floors in the range of 1–10 pm matching the
performance of surface plasmon resonance (SPR) based sensors [5]. The fundamental mechanism of these sensors is based on the
reflectance change produced by specific molecular binding on a thin film structure. The sensors described previously [1–4] are single
film structures using common semiconductor manufacturing materials like SiO2 on Silicon substrates, perhaps due to the easy
availability of these materials and familiarity with associated fabrication processes in academic labs. Single film Si/SiO2 or Si3N4
based structures reported in the literature have attained good sensing performance and these materials and associated fabrication
resources such as clean room, and e-beam thin film deposition systems may be easily accessible for a well equipped academic lab.
However, these materials and the associated fabrication resources require significant capital investments and running costs resulting
in the sensor chips being unaffordable by a large population in developing countries who ironically are probably in the greatest
need for such technology [6,7]. On the other hand polymer thin film materials are significantly cheaper than conventional
semiconductor grade materials (cost of silicon, glass slides and polycarbonate (PC) is $2800/m² [8], $185/m² [9] and $19/m²
[10], respectively, calculated from information on vendor websites) and can be assembled via several low cost manufacturing
processes such as spin coating, electrostatic layer-by-layer self assembly (LbL), and inkjet printing [11], potentially leading to the
construction of thin film reflectance biosensors on low cost substrates such as polycarbonate (PC), which is the substrate used in
the manufacturing of optical compact discs. We advocate the use of polymer based thin film biosensors over conventional semicon-
ductor materials based sensors for the following reasons. Firstly, such thin film biosensors could, for instance, be used as ultra-low
cost label free microarrays among other applications specifically targeted for developing countries, where there is a great need
for such technology. It is true that a polymer thin film biosensor would only lead to a potential cost reduction in the sensing chip while still
requiring relatively expensive optics for detection. However, the sensing instrumentation is a one-time capital expenditure whereas
the sensor chip, which is disposable, is a continuously running cost. Therefore, as one can imagine, it is the cost of the sensor chip
that drives the long-term unit cost of sensing and diagnostics and not the detection instrumentation, unless of course the detection
instrumentation is prohibitively expensive, which is certainly not the case with thin reflectance sensing instrumentation described
previously requiring a rather simple optical detection setup [1,2].

Secondly, it has been argued that even sensors based on silicon and similar expensive materials can be made inexpensive if
made in large volumes. The author is not aware of any commercial endeavor where this argument has been practically realized. Practical
realization of volume scaling cost advantage is difficult to attain because the biosensors market, particularly for label-free biosen-
sors, is still in its early development and therefore highly fragmented

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with several competing technologies such as electrochemical, electrical and acoustic sensing vying for the same user base. In such a scenario, it is unrealistic to talk of volume scaling analogous to that of IC chips for computers or consumer electronics. Moreover, even if, in the long-term it turns out that volume scaling effects lead to cost reduction, a polymer thin film based system with comparable performance to that of a conventional dielectric thin film based system, will in any case be cheaper due to the lower material and fabrication costs described earlier.

Finally, compared to the single film structures described previously [1–4], polymer multi-layer structures described here can be designed to provide optimal performance for any noise distribution of the detection instrumentation. As we show in this article, the optimal thin film structure for bio-sensing depends on the relative distribution of the system noise between intensity correlated noise (laser intensity noise, shot noise, etc.) or intensity un-correlated noise (electronic noise, dark noise, etc.). We present a design method to fabricate polymer thin film stacks that are optimal for any given noise distribution. Additionally, polymer based thin films permit the incorporation of nanoparticles, dye molecules and so on for additional functionalities.

The rest of the article is organized as follows. Section 2, provides an analysis of the signal to noise ratio (SNR) of detection using thin film reflectance based biosensors and shows that a thin film stack with a purely imaginary reflectance coefficient maximizes the SNR of molecular sensing, irrespective of the system noise being dominated by intensity correlated or intensity uncorrelated noise. The magnitude of this optimal reflectance coefficient depends on the relative distribution of noise between intensity correlated and intensity uncorrelated terms. Section 3 presents a method to fabricate thin film stacks with arbitrary purely imaginary reflectance coefficients using polymers that commonly have purely real refractive indices. The last section provides a discussion of the utility of the design method presented here.

2. Performance analysis of thin film biosensors

Fig. 1 shows a generic thin film reflectance based biosensor. A biological recognition layer, which could be antibodies, single stranded DNA, etc., is immobilized on the sensor surface. When specific target molecules, such as antigens complementary to the immobilized antibody, bind to the recognition layer, local optical phase change leads to a change in the reflected light intensity. Using the transfer matrix method [12], any general thin film stack can be represented by a net reflectance coefficient \( r_s' \) which gets modified to \( r_s \) upon the addition of a bio-molecular layer. As the beam scans the surface there is a net reflectance coefficient at point A without the bio-layer and a different reflectance coefficient at point B where the bio-layer is present. Throughout the paper, we assume that the ambient medium is air \((n = 1)\). Let \( n_f, d_f \) and \( r_f \) be the refractive index, thickness and reflectance, respectively, of the bio-layer. The net reflectance at points A and B is then,

\[
r_A = r_s \quad \text{and} \quad r_B = \frac{r_f + r_s' e^{-j2\delta_f}}{1 + r_f r_s' e^{-j2\delta_f}}; \quad \delta_f = 2\pi \frac{n_f d_f}{\lambda}
\]  

(1)

The bio-layer, which could be DNA or proteins, has a purely real refractive index \( n_f \sim 1.3–1.5 \) [2] and consequently a purely real Fresnel reflection coefficient \( r_f \) when the ambient medium also has a real refractive index. At point B, one can calculate \( r_s' \) by modeling the stack as a product of individual thin film matrices as follows [12]:

\[
M = D_a^{-1} \prod_{i=1}^{N} D_i P_i D_i^{-1} D_s
\]

(2)

Here the subscripts ‘a’ and ‘s’ refer to the ambient medium and the substrate, respectively. And the matrix \( D_i \) for any i is written as,

\[
D_1 = \begin{pmatrix} 1 & 1 \\ n_i \cos \theta_i & -n_i \cos \theta_i \end{pmatrix} \quad \text{for TE polarization},
\]

(3a)

and

\[
D_2 = \begin{pmatrix} \cos \theta_i & \cos \theta_i \\ n_i & -n_i \end{pmatrix} \quad \text{for TM polarization}
\]

(3b)

\[
P_i = \begin{pmatrix} e^{j\phi_i} & 0 \\ 0 & e^{-j\phi_i} \end{pmatrix}; \quad \phi_i = 4\pi n_i d_i / \lambda
\]

(3c)

Using Eq. (3), it is easy to show that when the ambient medium changes from air to the bio-layer, as in points A and B, respectively, in Fig. 1, the reflectance of the stack for any polarization, gets modified to,

\[
r_s' = \frac{r_s - r_f}{1 - r_s r_f}
\]

(4)

Substituting Eq. (4) in (1), we get,

\[
r_A = r_s
\]

(5a)

\[
r_B = \frac{(e^{j\phi_f} - e^{-j\phi_f}) r_f - (r_f^2 e^{j\phi_f} - e^{-j\phi_f}) r_s}{(e^{j\phi_f} - r_f^2 e^{-j\phi_f}) - (e^{-j\phi_f} - r_f^2) r_s}
\]

(5b)

Eq. (5b) can be simplified by noting that typical values of \( n_f \) and \( d_f \) are 1.3–1.5 and 2–3 nm, respectively [2], implying \( \phi_f \ll 1 \) and \( r_f \approx 0.15 \). Therefore, collecting only linear terms in \( \phi_f \) and \( r_f \), which are the most significant terms in the expansion, we can rewrite Eq. (5b) as,

\[
r_B = r_s + 2j\phi_f (r_f^2 r_s - (r_f^2 - r_s))
\]

(6)

Note that \( r_f \) is purely real whereas, \( r_s \) is in general complex valued. The reflectance difference \( \Delta R \) with and without the bio-layer is then,

\[
\Delta R = |r_B|^2 - |r_A|^2 = 4\phi_f r_f |\text{Im}(r_s)| (1 - |r_s|^2)
\]

(7)

Eq. (7) provides the fundamental design guideline for constructing thin film reflectance sensors for detection of biological molecules. Two important aspects of this equation must be noted here. Firstly we see that thin film structures that have a purely real reflectance coefficient or are perfectly reflecting do not produce any reflectance contrast to the first order in \( \phi_f \) and \( r_f \). In other words, the most significant contribution to the reflectance change due to molecular adsorption, which is a real valued perturbation of the original reflectance, comes from imaginary component of the original reflectance, which has a 90° phase offset (quadrature) with the perturbation. It is interesting to note that the quadrature concept is a common theme tying various self-referencing interferometric
schemes [2]. Secondly, this equation has been derived considering a spot with biolayers and one without bio-layer. However, in a real biosensor the difference in reflectance comes from binding of specific molecules on a spot already containing a biological layer of recognition molecules. But one can easily see that Eq. (7) can still be used in the performance analysis even in this situation because the effect of the substrate (terms with subscript ‘s’) is separable from the effect of biolayers (terms with subscript ‘f’) and only appears as a common multiplicative factor.

In thin film reflectance biosensing one is interested in designing a thin film stack with \( r_s \) which results in optimal performance. In order to find the optimal \( r_s \), one cannot simply maximize the expression in Eq. (7), but instead should maximize the signal to noise ratio (SNR) as noise in optical systems has intensity dependent terms. Generally one can write the total noise per bandwidth as [13],

\[
\sigma_{\text{tot}}^2 = \sigma_{\text{RIN}}^2 + \sigma_s I + N_0^2 \tag{8}
\]

Here \( \sigma_{\text{RIN}} \) is the relative intensity noise coefficient, \( \sigma_s \) is the shot noise coefficient, \( N_0 \) is back ground noise, for e.g. from detector electronics, and \( I \) is the mean intensity of the detected optical beam. As pointed out in Ref. [13], practical laser diodes are not shot noise limited and the dominant intensity correlated noise is the laser’s relative intensity noise (RIN) with \( \sigma_{\text{RIN}} \approx 10^{-3} \sim 10^{-2} \) [14,15].

The signal to noise ratio can then be written as,

\[
\text{SNR} = \frac{P_0 \Delta R}{N_0 (1 + (\sigma_{\text{RIN}} P_0 R_s)^2 + \sigma_s P_0 R_s)^{1/2}} \tag{9}
\]

where \( \sigma_{\text{RIN}} = \sigma_{\text{RIN}}/N_0, \sigma_s = \sigma_s/\sigma_s, P_0 \) is the incident laser power, \( R_s \) is the amplitude reflectance of the thin film structure and \( \Delta R \) is calculated from Eq. (7).

It can be shown that SNR given by Eq. (9) is maximized by \( r_s = \pm j \beta \), for any \( \sigma_{\text{RIN}} \) or \( \sigma_s \), value of \( \beta \) dependent on the operating noise regime (value of \( \sigma_{\text{RIN}} \) and \( \sigma_s \)). As a specific case, when \( \sigma_{\text{RIN}} = \sigma_s = 0 \), SNR given by Eq. (9) is maximized when \( r_s = \pm (1/\sqrt{3}) \).

Fig. 2 shows normalized SNR as a function of the real and imaginary parts of the substrate reflectance for \( \sigma_{\text{RIN}} = \sigma_s = 0 \), and \( \sigma_{\text{RIN}} = 100 \) and \( \sigma_s = 10 \).

3. All polymer thin film biosensors

We now demonstrate a design to achieve any arbitrary reflectance of the type \( r_s = \pm j \beta \) using materials which have purely real refractive indices (most polymers).

For the structure in Fig. 3, let \( r_{f1} \) and \( r_{f2} \) be the reflectance of the system comprising the substrate and the stack, calculated with air and the top layer as the ambient medium, respectively. The thin film stack consists of \( N \) bi-layers, denoted as stack length (alternate layers of high and low refractive indices with \( \lambda/4 \) optical thickness). Let \( n_i, d_i \) and \( r_s \) be the refractive index, thickness and reflectance, respectively, of the top layer. Then from Eq. (4),

\[
r' = \frac{r_s - r_f}{1 - r_f r_s} \tag{10}
\]

Following Eq. (1), the net reflectance of the structure (substrate + stack + top layer) is,

\[
r_{\text{net}} = \frac{r_f + r'_s e^{-2k d_s}}{1 + r_f r'_s e^{-2k d_s}} = \pm j \beta \tag{11}
\]

Solving Eq. (11) one gets two relationships,

\[
\frac{[r'_s(n + r'_s^2) - n(n + r'_s^2)]^2}{(1 - r'_s^2)^2} = \beta^2 \tag{12a}
\]

\[
\cos \left( 4\pi \frac{n_i d_i}{\lambda} \right) = \frac{r_f}{r_s} \left( \frac{1 + r'_s^2}{1 + r_f^2} \right) \tag{12b}
\]

Eq. (12) serves as the design criteria for the thin film structure. The recipe would be to choose any available polymer material (for e.g. \( n_i = 1.5 \)) as the top layer, thus fixing \( r_f \). Then Eqs. (12a) and (10) gives the appropriate value of \( r_s \). The reflectance of an alternating high and low index quarter-wave thin film stack depends on the stack length, asymptotically reaching unity with increasing \( N \). Therefore, for a given \( r_s \), one can always find the required stack length for a given combination of high and low index polymers. Once \( r_s \) is fixed Eq. (12b) gives the appropriate thickness of the top layer. By interchanging the order of the stack, sign of net reflectance can be reversed.

Fig. 4(a) shows the real and imaginary reflectance coefficient of a multilayer stack structure using a polycarbonate substrate (\( n = 1.6 \)), top layer of \( n = 1.5 \) (e.g. poly-methyl methacrylate), stack layers with \( n = 1.5 \) and 1.6, and design parameters obtained from Eq. (12). We see that proposed stack structure is able to create any arbitrary (limited by the discreteness of stack length) reflectance of the type \( r_s = \pm j \beta \) for normal incidence. Unlike the Si/SiO\(_2\) structures (Fig. 4(b)) reported earlier [4,5], these stacks can be used to create optimal conditions for bio-molecular sensing under any operating noise regime and can achieve similar or better sensing performance as the Si/SiO\(_2\) ones limited only by issues such as scattering or chemical stability of polymers. Our group is pursuing the
fabrication of such stacks on polystyrene substrates using a variety of techniques such as electrostatic layer by layer assembly and spin coating in the fabrication of low cost, all polymer reflectance sensors for bio-molecular sensing.

4. Conclusions

We showed that a purely imaginary substrate reflectance coefficient with magnitude dependent on the noise regime of operation maximizes the SNR of reflectance based bio-molecular sensors. Additionally, a thin film stack structure capable of creating any arbitrary purely imaginary reflectance coefficient was presented, thus demonstrating that there is no fundamental theoretical limitation in the fabrication of low cost, all polymer reflectance sensors for biological applications suitable to meet the demands of developing countries where low sensor cost is of paramount importance for widespread adoption of diagnostic chip technology.

While it is true that in a practical sensing system there are several other issues affecting performance such as surface roughness, long range uniformity and chemical stability of the polymer layers, non-specific binding on sensor surface and so on, it is important to demonstrate that there is no fundamental theoretical limitation in developing polymer based thin film stacks for bio-molecular sensing instead of conventional dielectrics (SiO₂, TiO₂, Si₃N₄) on Si or quartz. Our work demonstrates that all polymer thin film biosensors based on reflectance change are possible and can theoretically attain similar performance of that of sensors bases on conventional dielectrics. We also provide the design method for fabrication of such sensors.

References