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Intrinsic structure of biological layers: Vertical inhomogeneity profiles characterized by label-free optical waveguide biosensors

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ABSTRACT

When various analytes and functional layers are deposited on the surface of optical waveguide based biochips one faces difficulties in correctly interpreting the experimental data. For example, the deposited layer can be highly ordered (lipid bilayers, oriented receptors etc.) or can have significant inhomogeneity both perpendicular and parallel to the sensor surface (adsorbed polymer films, living cells). The generally applied simple optical model, which treats the deposited analyte layers homogeneous, isotropic and usually relatively thin compared to the wavelength of light, therefore, fails in most of the practical cases. In the present contribution, we systematically investigate the limitations of the widely applied optical models, when the analyte layer on the sensing surface has a vertically inhomogeneous refractive index profile. As examples of more realistic density profiles, the step-index, linear, exponential, power law and Gaussian refractive index distributions on various types of waveguide biochips are investigated using analytical and numerical model calculations. The limitations and the possible errors of the homogeneous thin adlayer model are pointed out. It is shown that for all of the vertically inhomogeneous profiles the refractive index obtained from the homogeneous thin layer modeling underestimates the true averaged refractive index of the layer. The calculated thickness can be over or underestimated, even taking up negative values in some cases. This behavior is similar to what was observed for positively birefringent thin adlayers treated with the homogeneous and isotropic model. It is also shown that the surface mass coverages calculated using the thickness and refractive index obtained from the homogeneous and isotropic modeling underestimate the real coverage values. The above errors are smallest when the reverse waveguide sensor design is applied to investigate vertically inhomogeneous analytes.

1. Introduction

It is increasingly important to in-situ monitor various nanometer scale biochemical and biological analytes at solid surfaces in aqueous solutions [1,2]. For example, the analyte can be a protein, a small ligand or a biopolymer adsorbing on the surface of an implant or on a layer of surface immobilized receptors [3]. In other case, the interaction of living cells with its substrate has to be followed in real time [4,5]. It is especially useful when these interactions are followed without the incorporation of any fluorescent or radioactive labels, for example, by following refractive index variations at a surface caused by the adsorbing analyte itself [6,7].

Various planar optical waveguide [8] based surface sensitive techniques were developed to monitor the above mentioned changes, such as optical waveguide lightmode spectroscopy (OWLS) [8,9], grating coupled interferometry (GCI) [10,11], resonant waveguide grating (RWG) [12]. These techniques measure the shift in refractive index near the sensor-surface, in the range of the penetration depth of the so-called evanescent field (typically up to 100–200 nm above the surface). Usually, the effective refractive index of the waveguided modes are followed with high resolution; and from the effective refractive indices one can calculate the optogeometrical parameters of the surface bound analytes. By in-situ monitoring the effective refractive index changes one can on-line monitor protein adsorption and desorption [13], affinity binding [3], cellular adhesion [12,14] and signaling [15,16]. The most traditional waveguide based sensor, the OWLS, measures the effective refractive indices of the zeroth order TE and TM polarized waveguide modes. The recorded optical data is usually interpreted using the homogeneous and isotropic thin adlayer model [17] and from the measured two effective refractive indices the thickness and refractive index of the analyte layer is calculated.

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But, in many cases the analyte layer is neither isotropic nor homogeneous. For example, a surface supported lipid bilayer is strongly anisotropic having positive birefringence [18]. It was shown that applying the isotropic model for the deposited lipid bilayer the adlayer thickness is strongly overestimated and the refractive index is underestimated [18]. Similarly, unrealistic optogeometrical parameters were observed for glycoprotein films [13], for denaturated BSA layers [19] and for oriented flagellin films [20]. Previously, the effect of optical anisotropy in the adsorbed adlayers was investigated using analytical model calculations [21].

Moreover, the assumption of the thin layer approximation is only valid for nanometer scale adlayers and fails for a layer of adhering living cells or adsorbed polymers with high molecular weight. The adlayer can have vertically inhomogeneous density profile for many adsorbed polymer systems. Linear, exponential, power law and Gaussian density profiles were reported in the literature [22]. Obviously, an adhered layer of living cells are even more complicated. These films are inhomogeneous in all directions [4,5,23].

In the present work we systematically investigate the effect of vertical analyte inhomogeneities on the optical data obtained using planar optical waveguide based label-free sensors. The paper is structured as follows. First, we consider real examples of vertically inhomogeneous refractive index profiles of adsorbed layers assembled from proteins or polymers. After, the analytical and numerical treatments of such refractive index distributions on planar waveguide surfaces are discussed, the relevant equations are summarized. Next, three typical waveguide designs are considered for monitoring refractive index profiles using numerical calculations and we systematically investigate the errors arising when the vertically inhomogeneous refractive index profiles are treated as homogeneous and isotropic thin films. The validity of the obtained refractive index, thickness and the surface adsorbed mass density is discussed. Finally, we summarize our main findings in the conclusions.

2. Vertically inhomogeneous refractive index profiles of surface deposited analyte layers

The refractive index distributions of adsorbed layers found in the literature are shortly summarized in Table 1. Note, in the literature usually the density distributions of the various layers are treated and numerically investigated. Throughout the present work we assume that the refractive index of the layer is proportional to its density. This assumption is often used in previous works [24].

The simplest profile is the step-index distribution characterized by a homogeneous refractive index n_A (usually larger than the refractive index of the aqueous cover solution n_C) and a thickness d_A. In the waveguide sensor literature thin films adsorbed on the sensor surface are mostly treated with this model and it is also assumed that $d_A \ll \lambda$. Where, λ is the wavelength of the light [17]. Clearly, this homogeneous and isotropic thin layer model is only realistic in limited cases; for example for dense layers formed from nanometer scale homogeneous objects like compact proteins. Other profiles are simply not treated in the OWLS literature, but would have relevances.

For example, layers grafted on a non-attractive surface and formed from rod-like, rigid polymer chains can be well-characterized with a linearly decaying refractive index distribution [28]. At the surface the layer refractive index having a maximum value n_A^{max}, decaying to the refractive index of the cover solution n_C at a distance d_A. But, when the surface is attractive the layer can be characterized by a power law profile [28–31,35]. Where again, n_A^{max} is the refractive index at the surface and d_A is the thickness

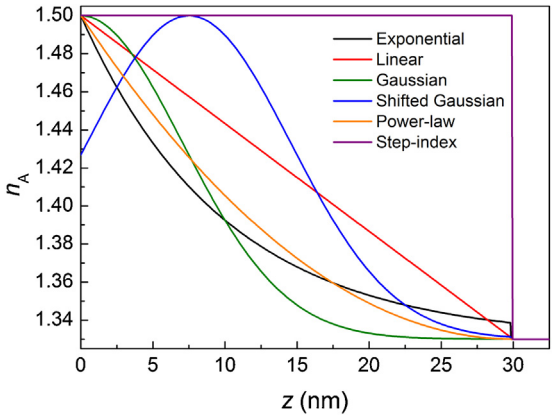

Fig. 1. Examples of vertically inhomogeneous refractive index profiles treated in the present work ($n_A^{max} = 1.5$, $d_A = 30$ nm. For the relevant equations see Table 1).

of the whole profile. When flexible polymer chains are assembled on an attractive surface the deposited film can be described by an exponentially decaying refractive index distribution [28–31]. But, when the flexible chains are grafted on a non-attractive surface the refractive index distribution is Gaussian [29–38]. Another profile found in the literature is the shifted Gaussian. This is the case for self-assembled layers of most polymers [28–38]. Note, the adlayer profile and its stability strongly depend on the environmental conditions such as temperature, pH, ionic strength [39–44]. For simplicity, in the calculations we always assume that the total layer thickness is limited, above a thickness d_A the refractive index is assumed to be equal to the refractive index of the cover solution n_C. Fig. 1 visually overviews the above mentioned various refractive index profiles with n_A^{max}=1.5, n_C = 1.33 and d_A=30 nm.

3. Modeling of vertically inhomogeneous profiles

3.1. Analytical formulas for the averaged thickness and refractive index

Coffey et al. defined the following weighted optical averages [22] providing a basis to compare the averaged effect of various refractive index profiles.

$$\overline{n} = \frac{\int_0^\infty n_A(z)[n_A(z) - n_C]dz}{\int_0^\infty (n_A(z) - n_C)dz}, \quad (1)$$

$$\overline{d} = \frac{\int_0^\infty (n_A(z) - n_C)dz}{\overline{n} - n_C}. \quad (2)$$

In the following, we calculate the above defined optical averages for the different refractive index profiles shown in Fig. 1. For the step-index refractive index distribution, as expected, we obtain the following formulas:

$$\overline{n}_{step} = n_A^{max}, \qquad \overline{d}_{step} = d_A.$$

While the calculation result in the following formulas for the linear, exponential and Gaussian profiles summarized in Table 1.

$$\overline{n}_{lin} = \frac{2(n_A^{max} - n_C)}{3} + n_C, \qquad \overline{d}_{lin} = \frac{3d_A}{4} \quad (3)$$

$$\overline{n}_{exp} = \frac{e^3(n_A^{max} + n_C) + n_A^{max} - n_C}{2e^3}, \qquad \overline{d}_{exp} = \frac{(e^3 - 1)2d_A}{3(1 + e^3)} \quad (4)$$

$$\overline{n}_{Gauss} = 0.707n_A^{max} + 0.293n_C, \qquad \overline{d}_{Gauss} = 0.418d_A \quad (5)$$

$$\overline{n}_{s.Gauss} = 0.772n_A^{max} + 0.228n_C, \qquad \overline{d}_{s.Gauss} = 0.654d_A \quad (6)$$