

On probing molecular monolayers: a spectroscopic optical waveguide approach of ultra-sensitivity

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Abstract: A broadband, multichannel, single-mode, planar waveguide based spectrometer was developed for probing molecular monolayers. A protein sub-monolayer (thickness $h \cong 3$ nm, imaginary part of refractive index $k_i \leq 0.01$) immobilized on the waveguide surface was characterized by the waveguide attenuated total reflection (ATR) spectrometer. A sensitivity enhancement of 4 orders of magnitude, compared to conventional transmission measurements, has been experimentally achieved in the characterization of ultra-thin films. In addition, polarized spectroscopic measurements at the TE and TM waveguide modes were implemented to determine the average orientation angle of the adsorbed molecules. The work developed here is a new research tool for the investigation of some fundamental aspects of molecular films and a novel platform to develop new technological devices of high sensitivity and selectivity such as biosensors.

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1. Introduction

Due to their potential technological applications, there has been an increase in the interest of molecular monolayers in recent years as reported in the critical review of Swalen *et al.* [1]. Applications in optical and electronic devices, electroanalytical chemistry, and biological interfaces illustrate the intense attention on organic thin films in the monolayer and submonolayer regime [2]. To fully exploit the technological possibilities offered by molecular films, a number of scientific problems need to be addressed. Among them is the relationship between the structure and orientation of molecular adsorbates and their corresponding physical, chemical, and biological properties. For instance, transduction in biosensors, affinity-based separations, and materials biocompatibility are governed by the structural and functional properties of organic molecules accumulated in a thin film at a solid-liquid interface. These properties (*i.e.* conformation, orientation, bioactivity) are in turn known to be dependent on the physical and chemical properties of the interface [3]. However these relationships are not well understood primarily because *in situ* study of molecular films is a technically difficult challenge. Elucidating these relationships is a prerequisite to the projected use of such films in molecular device technologies.

The future for thin molecular films lies in designing organized assemblies to perform new and unique functions, which demands experimental tools able to perform *in situ* non-destructive analysis with the high sensitivity required for monolayer detectability. Existing surface science tools need to be refined and new characterization methods and techniques suitable for monolayer analysis need to be developed.

Presented here is a novel optical technique to spectroscopically characterize films in the molecular monolayer regime. The approach represents not only an analytical tool that allows the investigation of some fundamental problems of molecular assemblies but also a platform to develop new technological devices of high sensitivity and selectivity such as biosensors.

2. Principle of the technique

Multimode optical fibers have been widely employed for evanescent-wave spectroscopy of interfacial films and optically based chemical sensing [4-6]. An alternative configuration that has seen increasing use for research in thin film structure and surface characterization as well as chemical sensing is the planar waveguide. A single-mode, step-index, planar waveguide is an inherently more sensitive geometry for probing ultra-thin films. At visible wavelengths, a single-mode planar waveguide supports up to several thousand reflections per cm of beam propagation using a ray optics model. This reflection density is about 4 orders of magnitude greater than using bulk optical elements in conventional attenuated total reflection (ATR) techniques and yields a concomitantly much higher sensitivity [7,8]. Nonetheless almost all previous waveguide ATR based studies have utilized monochromatic sources. A few works have reported spectroscopic detection either by using several discrete laser lines [9,10] or a monochromator [11,12] to sequentially select frequencies to perform absorbance

measurements. We describe here the design and implementation of a multichannel, broadband, single-mode, and planar optical waveguide based spectrometer. The instrument is schematically described in Figure 1. An achromatic waveguide coupler [13,14] was used to efficiently couple an incoming light beam of broad spectral width into the waveguide. The evanescent wave of the propagating mode was employed to probe the absorption properties of a molecular film immobilized on the waveguide surface. After propagation the guided mode was then out-coupled and spectrally dispersed by a diffraction grating. An optical system composed of a cylindrical lens and a CCD array detector allowed for parallel spectral detection.

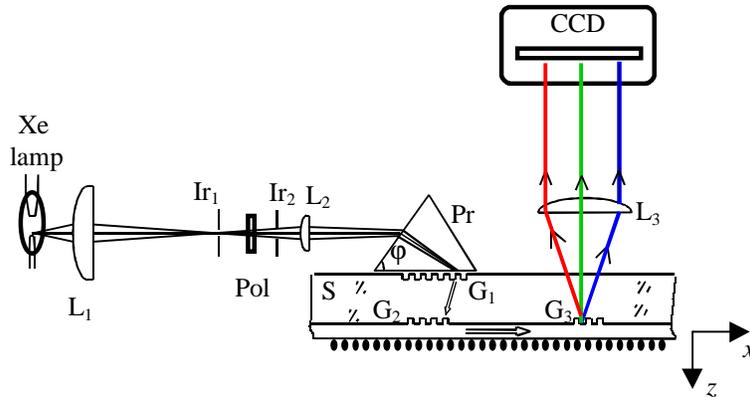


Fig. 1: The single-mode, broadband, multichannel, planar waveguide based spectrometer. Xe lamp of 150 W, $L_1 = 10$ cm focal length lens, Ir_1 , Ir_2 = iris, Pol = linear polarizer, $L_2 = 17$ cm focal length lens, Pr = prism of $LaSF_3$ and $\phi = 52.2^\circ$, G_1 , G_2 , G_3 = diffraction gratings, S = fused silica substrate, CCD = thermo-electrically cooled, charge-coupled array detector, $L_3 = 25.4$ mm focal length cylindrical lens. Grating parameters: G_1 , period = $1.921 \mu\text{m}$, depth = $0.34 \mu\text{m}$; G_2 , period = $2.252 \mu\text{m}$, depth = $0.12 \mu\text{m}$; G_3 , period = $0.3 \mu\text{m}$, depth = $0.12 \mu\text{m}$. The distance between the input and output grating couplers is $L = 8$ mm.

2.1. Theoretical expressions

The attenuation of a guided mode due to an absorbing dichroic layer immobilized on the waveguide surface can be properly described by the ray optics model. The structure and notation is indicated in Figure 2.

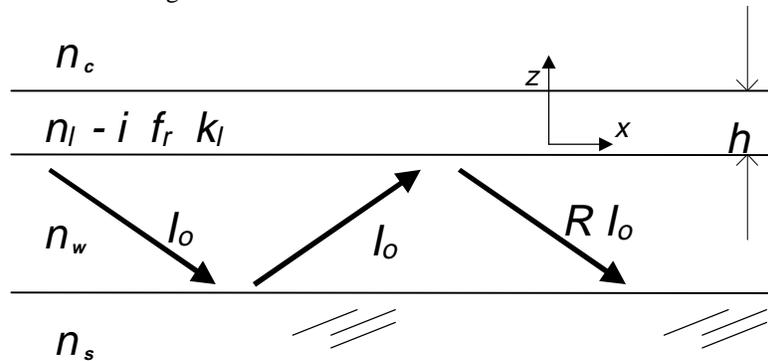


Fig. 2: Schematic representation of the waveguide structure with an absorbing dichroic molecular layer (refractive index $n_l - i f_r k_l$ and thickness h) on the surface of the guiding film.

The total internal reflection of the guided mode is partially attenuated by the presence of an absorbing thin layer of index of refraction $n_l - i f_r k_l$ and thickness h . The terms f_r , with $r = x, y, z$, are geometrical factors accounting for the dichroism of the molecular film. These factors

are calculated [15] from the average angular orientation of the molecular dipoles adsorbed on the waveguide surface. Assuming a weakly absorbing ($k_l / n_l \ll 1$) and very thin ($h / \lambda \ll 1$) layer, conditions that are usually satisfied in typical experiments for probing molecular monolayers, then the ray optics model gives the following expressions for the absorbance, A , in each polarization [15]:

$$A_{TE} = \frac{4\pi k_l h}{\lambda \ln 10} \left\{ \frac{2 n_l f_y (n_w^2 - N_{TE}^2)}{N_{TE} (n_w^2 - n_c^2)} \frac{L}{t_{eff,TE}} \right\} \quad (1)$$

and

$$A_{TM} = \frac{4\pi k_l h}{\lambda \ln 10} \left\{ \frac{2 n_l n_w^2 (n_w^2 - N_{TM}^2) [f_x (N_{TM}^2 - n_c^2) + f_z (n_c / n_l)^4 N_{TM}^2]}{N_{TM} [n_w^4 (N_{TM}^2 - n_c^2) + n_c^4 (n_w^2 - N_{TM}^2)]} \frac{L}{t_{eff,TM}} \right\} \quad (2)$$

where λ is the optical wavelength. The waveguide effective refractive index, N , and the effective thickness, t_{eff} , are calculated from the waveguide dispersion equation without the presence of the absorbing layer as this is assumed to only weakly perturb the waveguide mode profile. The propagation length of the mode inside the waveguide, which is the distance between the input and output couplers, is expressed by L .

The sensitivity factor, defined as the ratio between the waveguide absorbance and the direct transmission absorbance, is given by the term in the bracket of Eqs. 1 and 2. Figure 3 shows the dependence of the sensitivity factor on the thickness of the guiding film and allows for the determination of the optimum thickness to maximize the device response in each polarization. From this analysis we can observe that higher sensitivity is obtained for tightly confined waveguides, which explains the much higher sensitivity of step-index waveguides compared to graded-index (e.g. ion-exchange) waveguides.

Using direct transmission measurements it is extremely difficult and often impossible to reliably obtain absorbance data of molecular films that are only a few nanometers thick. An enhancement of four orders of magnitude in sensitivity illustrates the attractiveness of measuring ATR of ultra-thin films in the waveguide configuration.

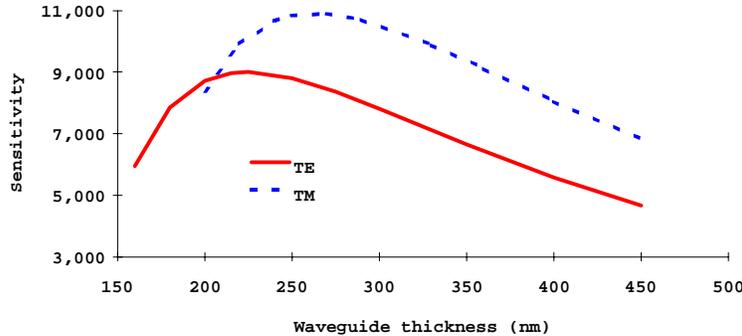


Fig. 3: Sensitivity vs. waveguide thickness. For the calculations, it was assumed: propagation length $L = 1$ cm, wavelength $\lambda = 550$ nm, waveguide index of refraction $n_w = 1.56$, substrate index of refraction $n_s = 1.46$, real part of the adsorbed layer index of refraction $n_l = 1.33$, cladding index of refraction $n_c = 1.33$. The molecular film is assumed isotropic ($f_x = f_y = f_z = 1$). The results are for the lowest order waveguide mode and they are independent of the adlayer thickness, h , and absorption coefficient, k_l .

2.2. Broadband waveguide coupler

To efficiently couple a broad spectral width of an incoming beam into a planar, single-mode waveguide, we developed an achromatic coupler [13,14]. A combination of dispersive components was designed and inserted in the path of the incoming beam to direct each spectral component into the proper incident angle at the grating coupler. The effects of chromatic dispersion on an incident beam are evaluated in Figure 4, where the mismatch between the incoming effective index and the waveguide effective index is plotted for several waveguide couplers. The conventional couplers ((a) grating and (b) prism) show a sharp increase in the mismatch function even for small detunings from the central wavelength (defined as $\Delta\lambda$ in Fig. 4). A first order chromatic dispersion compensation (c) was achieved by Li and Brazas [16]. A correction up to the second order (d) was implemented in our work [13] to provide for a broader spectral bandwidth.

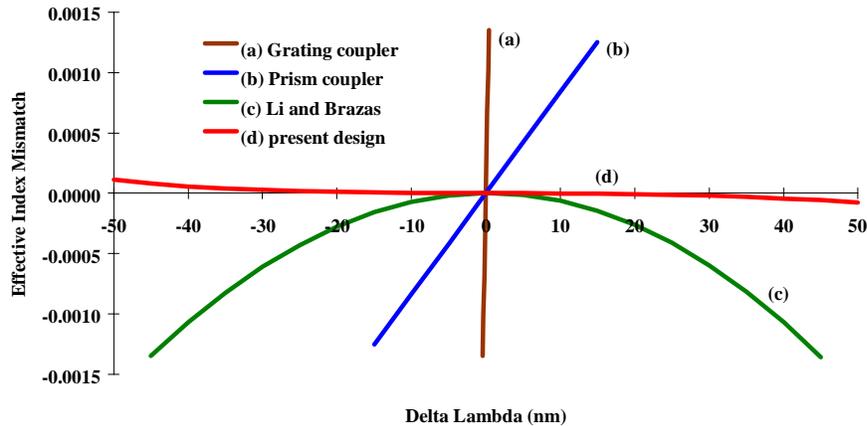


Fig. 4: Mismatch in the effective index of couplers and waveguides as described in [13].

2.3. Spectral resolution and detection range

A surface relief grating integrated into the laminar waveguide structure, G3 in Figure 1, was employed to outcouple the guided mode and spectrally disperse the light beam. A cylindrical lens was used to image each spectral component onto the CCD, which was placed at the back focal plane of the lens (Fourier plane). The spectral resolution $\Delta\lambda_{res}$ for such a system is determined by:

$$\frac{\Delta\lambda_{res}}{\lambda} = \frac{\Lambda\alpha}{\pi}, \quad (3)$$

where Λ is the period and α is the leakage rate of the outcoupling grating. The spectral resolution was also experimentally determined by measuring the imaged spot size of monochromatic light and using two laser lines to calibrate the CCD. As shown in Figure 5 we measured a spectral resolution of 0.7 nm with the current system.

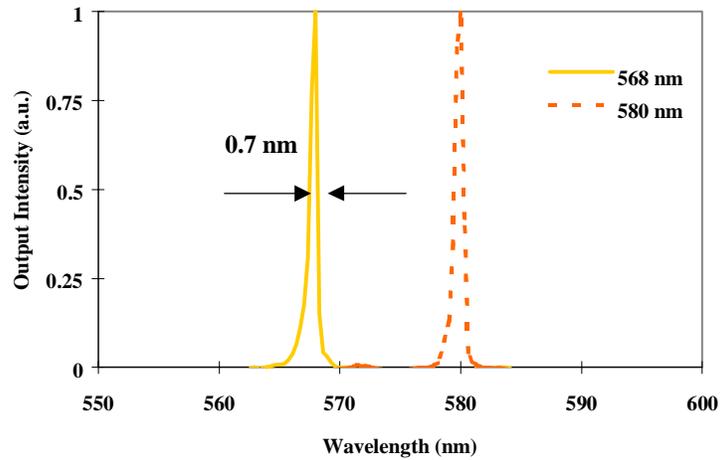


Fig. 5: Output intensity of two laser lines for spectral resolution characterization.

The total spectral content that can be detected by a CCD array of linear length l and with a cylindrical lens of focal length f is given by:

$$\Delta\lambda_{det} \approx \frac{l\Lambda}{f}. \quad (4)$$

The detection range with the system described in Figure 1 is approximately 160 nm. As a performance test of the developed device we measured the transmittance of narrow-band pass filters placed in front of the incident beam. These results were compared to the measurements using a conventional spectrophotometer. Typical curves showing good agreement are plotted in Figure 6.

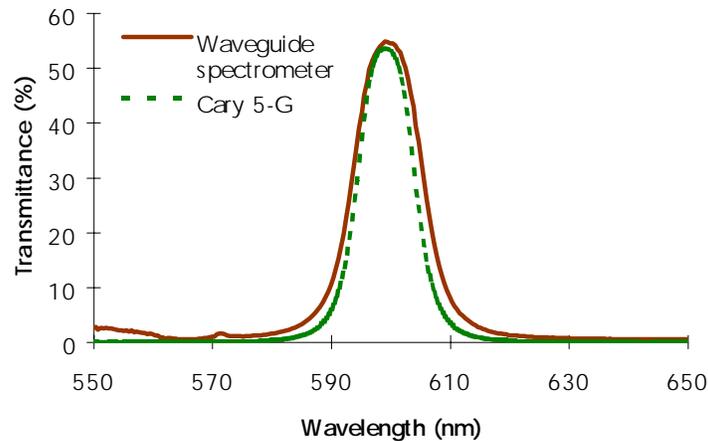


Fig. 6: Performance test with a 600-nm bandpass filter measured in a transmission mode by the waveguide based spectrometer shown in Fig. 1 and a conventional transmission spectrophotometer (Cary 5-G).

3. Applications of Waveguide Absorption Spectroscopy

As an application of the developed system, a molecular monolayer of the protein cytochrome c was adsorbed on the waveguide surface and spectrally characterized [17]. The waveguide film was a 400-nm thick Corning 7059 glass deposited on a fused silica substrate; such structure supports one TE and one TM guided mode in the visible spectral range. A liquid flowcell was clamped to the waveguide surface. Initially the flowcell was filled with phosphate buffer solution and a reference spectrum was collected. Next a solution of ferricytochrome c (oxidized cytochrome c) dissolved in sodium phosphate buffer was injected into the flowcell and allowed to incubate for 30 min. The cell was then flushed with phosphate buffer to remove non-adsorbed species and a spectrum of the adsorbed protein monolayer in the oxidized state was acquired. A solution of sodium dithionite in buffer was then injected to reduce the protein to ferrocytochrome c and the spectrum of the reduced cytochrome c was acquired. The spectra of the oxidized and reduced states of the cytochrome c film are shown in Figure 7. Attempts to characterize these films by conventional optical techniques would be problematic as they exhibit a typical thickness of only $h \approx 3$ nm and weak extinction coefficients. For example, the peak value at $\lambda = 550$ nm in the reduced state spectrum is $k_t \approx 0.01$, which in a single pass transmission mode would correspond to 0.0003 absorbance units. The waveguide geometry provides a sensitivity enhancement of approximately 5,500.

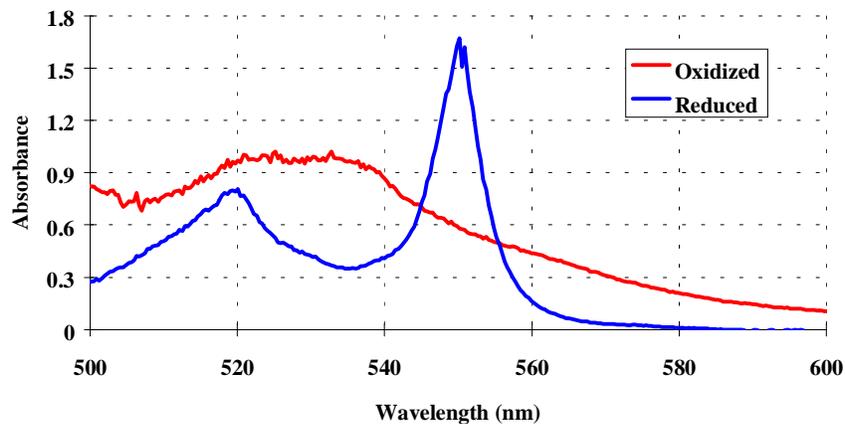


Fig. 7: Spectrum of cytochrome c adsorbed to the waveguide glass surface acquired.

In addition we used orthogonally polarized waveguide modes (TE and TM) over a wide spectral range to experimentally determine the angular orientation of the intrinsic porphyrin groups in a cytochrome c film immobilized on the waveguide surface. The dichroic ratio, defined as the ratio of absorbance in TE and TM polarization, is shown in Figure 8 over a broad spectral range. The dashed-blue lines (A) and (B) refer to the results of two independent and similar experiments for a monolayer of cytochrome c. The solid-red line describes the results calculated from an isotropic model for the molecular layer ($f_x = f_y = f_z = 1$). The data show that the angular orientation of the porphyrins is not random. In contrast the molecules have a preferential orientation when immobilized on the waveguide glass surface. By using Equations 1 and 2 the average angular orientation f_r of the dipoles can be determined from the experimental data.

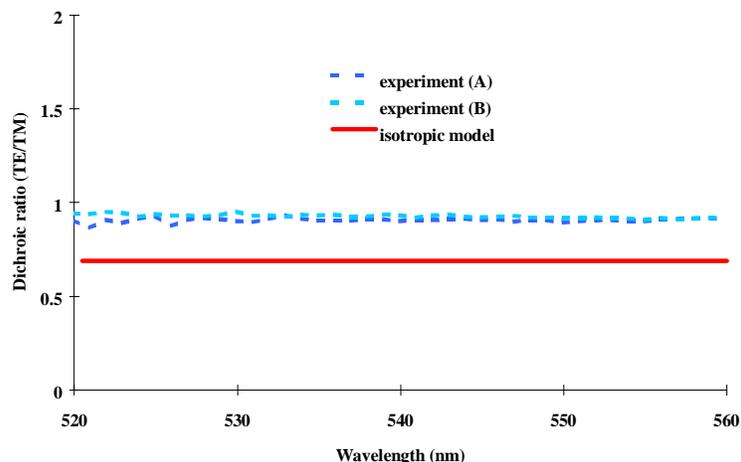


Fig. 8: Dichroic ratio over a broad spectral range for cytochrome c immobilized on a glass surface.

4. Conclusions

In this work, through the use of a broadband, multichannel, single-mode, and planar waveguide platform, we have extended the ability to measure polarized visible spectra of ultra-thin and weakly absorbing films. Absorbance spectroscopy is one of several experimental techniques that have been employed for studying structure and function in biomolecules such as heme protein. It is a powerful approach since the position, shape, and intensity of absorption bands are markers of ligand binding, oxidation state, and molecular conformation. The waveguide spectroscopy technique described here is expected to be a valuable tool towards the rational design and utilization of molecular films in new technological devices. In addition, the broad spectral range of this technique may be useful for spectroscopically resolving multiple analytes in chemical and biochemical integrated optical sensors.

Acknowledgments

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