

The Light Guide Effect of Planar Lipid Bilayers and Its Application to Bilayer Spectroscopy

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The light guide properties of planar lipid bilayers offer a novel approach to bilayer spectroscopy. Potential applications are outlined.

Hitherto, the influence of an electric field on the shape and structure of lipid bilayers has been studied predominantly via capacitance measurements. These sum up contributions of different origin. Optical techniques can discriminate between some of these effects originating from different parts of the lipid film. The poor resolution, however, restricts the application to the torus transition region and lenses [1, 2]. Phase contrast or interferometric methods working in normal incidence with respect to the bilayer's plane are limited in resolution by the signal to noise ratio since the phase modulations generated by schlieren within the electrolyte exceed the modulation of the bilayer (50 Å in thickness) by many orders of magnitude.

The novel optical technique presented is based on the light guiding properties of a planar lipid film. It profits from the long path of interaction between the light and the film being equal to the diameter of the aperture (mm-range). Along this path the microscopic effects are summed up yielding information on dynamic properties of the lipid film (electric relaxation processes, conformational changes). The method is suited for scattering experiments since a high intensity wave travels along the film. The light is guided along the bilayer even through curvatures. Thus, the volume of interaction is always defined. The method reveals the lateral properties of the film and is therefore expected to contribute to the knowledge of its anisotropic properties. The technique is, in principle, applicable to any film with a refraction index higher than the one of the adjacent medium.

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Wave guide equations of the bilayer

The bilayer with a thickness $2d$ and an index of refraction n_2 is immersed in an electrolyte, n_1 (Figure 1). Assuming isotropic and loss-free media,

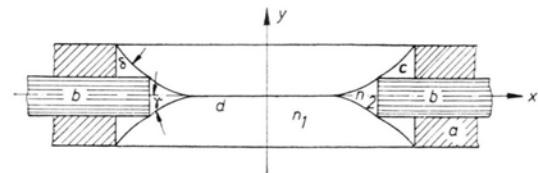


Fig. 1. Cross sectional view of a lipid membrane. a: separator foil, b: glass fiber, c: torus volume, d: bimolecular area, γ : contact angle between torus and bilayer, δ : contact angle between torus and separator, n_1 : index of refraction of the electrolyte, n_2 : index of refraction of the lipid material.

the solutions of the Maxwell equations for this geometry were given in optical notation [3]. Light of vacuum wavelength λ_0 travels in a center wave and a surface wave with common phase velocity along the x -axis. The center wave propagating inside the bilayer shows a symmetric cosine distribution in amplitude with respect to the plane $y = 0$,

$$E_{z\text{center}} = A \exp[i(2\pi N/\lambda_0)x] \cdot \cos[2\pi/\lambda_0(n_2^2 - N^2)^{1/2}y]. \quad (1)$$

The surface wave travelling outside the bilayer is exponentially attenuated in y -direction.

$$E_{z\text{surface}} = \exp[(i2\pi N/\lambda_0)N x] - (2\pi/\lambda_0)(N^2 - n_1^2)^{1/2}y]. \quad (2)$$

The amplitude A in (1) is chosen to satisfy the continuity at $y = \pm d$ and to normalize $E_{z\text{surface}}$.

$$A = \exp[(2\pi/\lambda_0)(N^2 - n_1^2)^{1/2}d]/\cos[2\pi/\lambda_0(n_2^2 - N^2)^{1/2}d]. \quad (3)$$

The common phase velocity of the center and surface waves results in an index of refraction N which is attributed to the wave guide system. N is determined by a transcendental equation which is derived from satisfying the continuity conditions for tangential E at the phase boundaries $y = \pm d$.

$$d/\lambda_0 = 1/[2\pi(n_2^2 - N^2)^{1/2}] \cdot \tan^{-1}(N^2 - n_1^2)^{1/2}/(n_2^2 - N^2)^{1/2}. \quad (4)$$

An examination of (4) leads to the following conclusions. Waves bound to the bilayer can only exist if n_2 is larger than n_1 . Since d/λ_0 is very small

only lowest modes with even symmetry (cosine-distribution) can propagate along the bilayer, and N is close to n_1 .

For example a bilayer of a thickness $2d = 50.4 \text{ \AA}$ prepared from a lipid material with a bulk index of refraction $n_2 = 1.4$, and surrounded by an aqueous electrolyte (10^{-3} M KCl , $n_1 = 1.34$), shows an index of refraction $N = 1.34001$ and the attenuation factor $2\pi/\lambda_0(N^2 - n_1^2)^{1/2}$ in (2) is $6.52 \cdot 10^{-6} \text{ \AA}^{-1}$ when light with 5000 \AA wavelength propagates along the bilayer.

The light is coupled from and to the bilayer by optical fibers being immersed into the Plateau-Gibbs border, the torus volume surrounding the circular bilayer area. The cross sectional view of the torus volume (Fig. 1) is similar to a horn shaped coupler which reduces in thickness from the diameter of the fiber to the bimolecular dimensions. The shape of the torus is determined by the contact angle δ of the torus-separator interface and the contact angle γ of the bimolecular film with the torus. The coupling properties are predominantly governed by the torus transition region which is defined by an increase in thickness from d to about $\lambda/2$. The same thickness interval has been shown to be strongly subjected to electrostrictive forces [4]. Thus, electrostrictive effects of the torus transition region can be easily observed by monitoring the transmitted light intensity as a function of the electrical potential applied to the lipid film. Intensity modulation of -18% with time constants of 30 ms are observed when applying a 120 mV pulse. Coupling efficiencies (light power fed into the torus versus power coupled to the wave guide) of 10^{-3} are observed experimentally, which are in reasonable agreement with the theory [5]. The surface waves travelling outside the film are refracted into the torus with some inclination to the fiber axis. Thus the surface waves excite higher propagation modes than the center wave running in parallel to the fiber axis. The intensity carried by the surface waves can be reduced in favour of the center wave by mode discrimination in the fiber [6].

The light guide properties have experimentally been verified by guiding the light along a slightly spherically bulged film, avoiding direct view between the input and output fiber. The transmission of the lipid assembly including coupling efficiencies of fibers and the torus is approximately 10^{-6} [7].

Besides the torus transition region, the lipid bilayer is also subjected to electrostrictive effects. Fortunately the time constant observed for torus compression is slower by a factor of hundred [8], thus the torus remains unaffected during the bilayer changes in thickness. The intensity carried by the center wave decreases with a compression of d , whereas the intensity transported by the surface wave, increases. Depending on the amount of surface wave discrimination by the torus-fiber assembly a net intensity modulation at the output fiber is observed showing time constants in the $100 \mu\text{s}$ range. The corresponding amplitudes of the thickness modulations are in the 1 \AA range. The initial thickness at zero field is calculated from the specific capacitance; all other parameters can be experimentally deduced by observing the modulation as a function of the wavelength of light propagated along the film [8].

In general, light which passes the lipid film is modulated in amplitude, phase and polarization by scattering processes with the anisotropic film constituents. Due to the physical and optical anisotropy, the magnitude and the direction of the dipole moments induced depend on the orientation of molecules with respect to the incident light wave. Therefore, the scattered light radiated by the induced dipole moments gives information on the molecular motion. Information on the scattering process is demodulated from the light frequencies by coherent mixing. Two types of fluctuations are detectable with their frequency spectra: thermally induced and current induced fluctuations where the local field of an ion passing the film forces the surrounding lipids to change their equilibrium orientation.

Absorption measurements on chromophors dissolved in the lipid film also profit from this technique since the wave guide properties are still maintained though the index of refraction of the film becomes complex as Kane and Osterberg [3] have shown by perturbation analysis. In contrast to ideal wave guides, the absorption is enhanced by a factor of ten [9], thus recommending the technique for weakly absorbing or poorly soluble chromophors.

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