BIOPHYSICAL APPLICATION OF THE OPTICAL WAVEGUIDE LIGHTMODE SPECTROSCOPY

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A thin transparent, dielectric film with refractive index higher than that of the surrounding is a good optical waveguide. The light in the waveguide can propagate only distinctly as transverse electric (TE) or transverse magnetic (TM) normal modes. Different normal modes have different effective refractive indices (defined as the ratio of the phase velocities of the light in vacuum and in the waveguide). Light can be coupled into the waveguide by an optical grating formed on the top of the thin film. One of the diffracted waves will propagate in the waveguide if the incident wave direction satisfies the incoupling equation

$$N = n_0 \sin a + l l \; / \; \Lambda \tag{1}$$

Here N is the effective refractive index, n_0 - refractive index of the air, a – incoupling angle, L - grating period, l - wavelength of the measuring light (laser), l - is the diffraction order (l = 0, ±1, ...) of the incoupled light.

The waveguide modes are very sensitive to the interfacial conditions and these opened the possibility of applications of planar waveguides as optical surface sensors. The sensor (optical chip) considered as a four-layer optical system: substrate, waveguide film, a thin adlayer and the cover medium (air or water solution). The adlayer modifies the incoupling conditions and measuring the incoupling angles one can get informations about the adlayer's optical parameters. The method is known as Optical Waveguide Lightmode Spectroscopy (OWLS). The adlayer can be a lipid bilayer, adsorbed molecules, etc. A linearized mode equation was derived for the four-layer planar optical waveguide [1]. A sensitive, high resolution OWLS equipment was designed and built in our laboratory. The special design allows to apply a micro-cuvette on the waveguide sensor, interchangeable lasers for measurements at different wavelengths and varying the temperature of the waveguide (20...35 ^{0}C).

Several biophysical applications of the OWLS method are presented in the following.

The effect of UV irradiation on uracil thin layer measured by OWLS [2]

The polycrystalline uracil thin layer (~100 nm) dosimeter is a well-established method to monitor the biological effects of the environmental ultraviolet (UV) radiation. It is based on the optical density (OD) decrease of the uracil layer in the UV-absorption band due to photodimerization of the crystal caused by UV-irradiation. It was shown that a corresponding change in the refractive index can be detected at a wavelength (630 nm) far from the UV-absorption band during UV irradiation. Uracil thin layer (~ 40 nm thick) was prepared on the waveguide sensor by a vacuum coating system. OWLS spectra were taken continuously at 630 nm (He-Ne laser) except during the UV irradiation. Both effective refractive indices decrease approximately exponentially with UV irradiation dose. Using the mode equation it is found that during UV irradiation the refractive indices decrease, but the optical anisotropy increases. The sensitivity of the OWLS method is about ten times higher than the OD method.

Effect of patterns and inhomogeneities on the surface of waveguides used for OWLS applications [3]

Patterns and inhomogeneities on the surface of the waveguide can produce broadening and fine structure in the incoupled light peak spectra. During cell spreading on the waveguide a broadening of the peaks is observed, while regular microstructures on the incoupling grating produce shifts and splitting of the peaks. Such splitting effect is used to monitor the lipid phase transition.

Lipid bilayer phase transition monitored by OWLS

Much work was devoted to study the phase transition in lipid bilayers using mainly calorimetric methods, X-ray and neutron-scattering. During the main phase transition the number of kink rotamers in the hydrocarbon chains increases leading, e.g. to the decrease of the thickness of the bilayer. It is interesting to raise the question how the optical parameters of the bilayer change during the phase transition. DMPC was chosen as its critical temperature is around 25^0C and a special differential technique was worked out to monitor the small changes. A lipid bilayer was prepared on the optical chip by Langmiur-Blodgett and Schaefer technique but only half of the chip was covered by the bilayer resulting in a sharp edge (line) of lipid coverage perpendicular to the grating on the chip. During OWLS measurement the laser beam was positioned to this interface monitoring the lipid and nonlipid region simultaneously. This gives double peaks in the spectrum. Lipid bilayers show optical anisotropy, which means that the TE and TM modes have different refractive indices. This makes the interpretation more difficult as OWLS measurements give two independent data (TE and TM modes effective refractive indices) but there are three unknown parameters (d_A, n_A^{TE}, n_A^{TM}). The problem could be solved making measurements at two different wavelengths (630 and 670 nm).

Most of the changes in bilayer thickness occur around 25 C^0. The ordinary refractive index (TE mode) decreases continuously around the phase transition but the extraordinary refractive index (part of the TM mode) shows a stronger decrease.

REFERENCES

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