Study of Multilamellar Films of Photoreceptor Membrane by Photon-Correlation Spectroscopy Combined with Integrated Optics

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A new method combining integrated optics and photon-correlation spectroscopy was developed to study dynamic behavior in thin films and at interfaces. As a first application, the lateral diffusion of rhodopsin, the visual protein in photoreceptor membrane, was studied.

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Photon-correlation spectroscopy (PCS) is widely used to study the dynamics of samples of interest by analyzing the temporal autocorrelation function of fluctuations in the light scattered quasielastically from them.[1,2] While behavior such as macromolecular diffusion in bulk solutions is routinely investigated using PCS, and fluorescence-correlation spectroscopy has recently been used to study surface kinetics,[3] conventional PCS methodology cannot be used, for example, to study boundary regions occurring at solid-liquid interfaces and in thin films. We report here the development and first application of a new light-scattering method combining PCS and integrated optics[4] (IO) to probe dynamic behavior at interfaces within distances of the order of the wavelength of light, λ. The recent application of integrated optics to Brillouin[5] and Raman spectroscopy[6,7] demonstrated the value of combining integrated optics with spectroscopic techniques and PCS-IO now extends this capability for studying interfacial and thin film dynamics into the range accessible to modern PCS instrumentation, viz., $1-10^{-8}$ s.

The samples chosen for this first study were thin, multilamellar films of photoreceptor membrane (PRM) that with humidification retained many of the important photochemical characteristics of the native membrane.[8,9] The possibility of studying the diffusive behavior of the PRM pigment protein, rhodopsin, was particularly appealing since diffusively mediated intramembrane rhodopsin interactions are considered to play an important role in vision.[10,11] The investigation of membrane protein diffusion more generally by means of PCS-IO offers important advantages since proteins are not labeled and photoabsorption is avoided by a proper choice of laser line. By contrast, fluorescence studies of membrane protein diffusion[12,13] require protein labeling and interpretation of results can be complicated by label effects on protein motion, undesirable photochemical events, etc. With PCS-IO, lateral distances in the sample of the order of λ are probed, a useful feature since λ is intermediate between the ~ 1-μm diffusion studied by photobleaching–fluorescence-recovery techniques and the highly localized protein motion probed by polarized flash photometry.[14]

The PCS-IO experimental setup is illustrated in Fig. 1. The waveguide consisted of a sample film as superstrate, a Corning 7059 glass film as core, and a Pyrex slide as substrate.[15] Approximately 5 mW of 6471 Å laser light was coupled into the guide using a fluid index-matched to the sample to insure single-mode TE_1 propagation. Photoabsorption was avoided because 6471 Å is well above the sample absorption band.[9] Illumina-

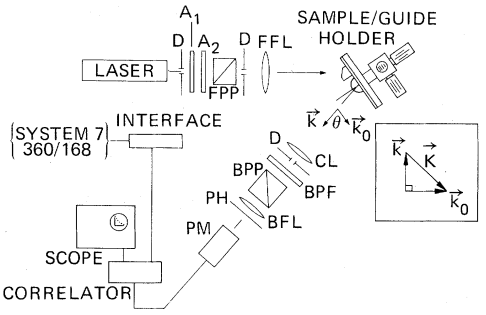

FIG. 1. Experimental apparatus. A_1 and A_2 are neutral density filters and D are diaphragms. FPP and BPP are prism polarizers while FFL and BFL are focusing and CL the collecting lenses. BPF is a narrow-band pass filter. The experimental scattering geometry is shown in the inset.

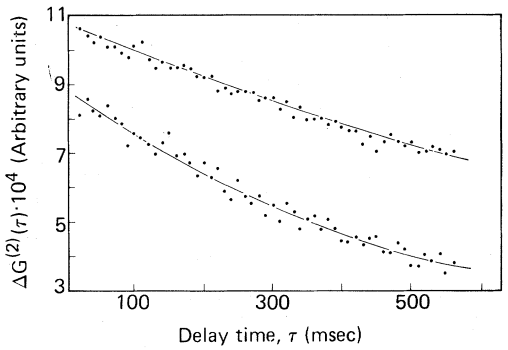

FIG. 2. Two representative baseline-corrected autocorrelation spectra of photoreceptor membrane films. The slow relaxation corresponds to run 3 in the third sample ($D_L = 3.78\times 10^{-11}$ cm^2/s) while the faster relaxation corresponds to run 4 in the first sample ($D_L = 7.52 \times 10^{-11}$ cm^2/s) (see table).

ting the sample with the evanescent portion of the guided light allowed a well-defined 0.5-μm-thick region of the sample close to the sample/core interface to be probed[15] by a relatively weak portion ($\approx$ 1 mW) of the guided light. Perhaps more importantly for PCS measurements, the incident wave vector of the evanescent light, $\vec{k}_0$, was single-valued and well defined.[16]

The films used in these studies were formed by depositing bovine retinal rod outer-segment disks onto microscope cover slips using the isopotential spin-dry centrifugation technique.[8,9] These films consisted of $\sim 10^3$ membrane layers, were highly oriented, and were typically 10–20 μm thick. After humidification at 20–23 °C and 100% relative humidity for 35 min to 3.5 h, films were firmly pressed face down onto glass waveguide cores, which produced large, optically clear regions of arbitrary shape in each sample. To prevent dehydration, spaces between cover slip edges and guide cores were sealed with Vaseline on three sides and with the coupling prism and index-matching fluid on the fourth. [For comparison purposes, some films were bleached by room light while others were not (see Table I).] Light scattered from optically clear regions at 90° to the guided light (Fig. 1) was collected within about one coherence solid angle and amplified, and discriminated photomultiplier pulses were then processed by a digital correlator. Incoming photodetection counting rates were scaled to levels appropriate to scattered-light intensities and correlator times. While strong scattering from stationary sample inhomogeneities resulted in large autocorrelation function baselines and heterodyning of the scattered light,[17] the exponential character of evolving autocorrelation "spectra" above baseline was readily monitored on an oscilloscope. When an occasional sample became dehydrated during the course of these measurements, this exponential part decreased correspondingly, ultimately disappearing into the baseline. Occasionally, inactive sample regions were also found that yielded strictly flat spectra. Results for scattering from active regions were fitted with the sum of one, two, or three exponentials plus baseline and in eighteen of twenty

TABLE I. Photoreceptor membrane PCS/guided wave study results.

$10^{11}D_L$ (cm^2/sec)	Humidification (h)	Bleached
5.12 ± 2.80	0.6	No
11.12 ± 4.80	0.6	No
8.88 ± 2.40	0.6	No
7.52 ± 1.50	0.6	No
8.16 ± 1.78	1.0	No
2.12 ± 0.84	1.0	No
6.06 ± 0.86	1.0	No
5.10 ± 2.26	1.0	No
9.84 ± 2.02	3.5	Yes
11.42 ± 2.02	3.5	Yes
3.78 ± 1.20	3.5	Yes
5.40 ± 2.48	3.5	Yes
9.14 ± 1.70	3.5	Yes
4.58 ± 0.96	3.0	Yes
1.84 ± 0.58	3.0	Yes
4.46 ± 1.80	3.0	Yes
5.68 ± 1.38	3.0	Yes
3.20 ± 1.18	3.0	Yes

cases were best fitted by one exponential. These eighteen spectra were then analyzed further. Two representative spectra are presented in Fig. 2.

The dynamic behavior of thin films of alternating strata of water and phospholipid bilayer—a system devoid of proteins—was recently investigated using forced Rayleigh light scattering.[18] The area, thickness, and bilayer spacing of samples studied were comparable to those employed here. While scattering from both thermal and hydrodynamic inhomogeneities was observed, no evidence for any dynamic mode of the kind observed in PRM was reported. Furthermore, the influence of extraneous vibrations on spectra from PRM films was ruled out on the basis of the flat spectra observed for inactive and dehydrated films and because spectral decay times for active regions were found to be independent of sample region size and shape. These results and the high proportion ($\gtrsim$ 80 wt.% of all protein) and high density ($\cong$60-Å spacing) of PRM rhodopsin suggest that spontaneous rhodopsin concentration fluctuations were primarily responsible for light scattering in PRM. Exponential spectra, then, resulted from the formation and relaxation of these fluctuations via protein diffusion.[1,2,17] The characteristic $1/e$ time, τ, of the spectral decay was then related to the two-dimensional rhodopsin mutual lateral diffusion coefficient, D_L, and to the scattering wave vector, $\vec{K}$, by[1,2,17]

$$\tau = (0.5 D_L K^2)^{-1}, \quad (1)$$

where the factor 0.5 accounts for the projection of K onto the membrane plane (Fig. 1, inset) and $K = |\vec{k} - \vec{k}_0| = 4\pi n \sin(\theta/2)\lambda_0$ with $\lambda_0 = 6471$ Å, $\theta = 90°$, and $n = 1.499$, the measured refractive index of a humidified PRM film at room temperature and 6328 Å.[19] It is seen that a length in the sample of $2\pi/K \approx 2000$ Å was probed in these measurements. Rhodopsin diffusion was therefore measured in regions smaller than the rod outer-segment disks used to make sample films—a capability unique to this new method.

D_L values calculated using Eq. (1) are presented in Table I. Each group of values represents a different sample and different values within a group represent different sample regions. Though $D_L \approx 10^{-11}$ cm^2/s here, the reported tracer lateral diffusion coefficients for intact frog and mud puppy rods at room temperature were $\sim 10^{-9}$ cm^2/s.[20] The slow diffusion seen here is most likely due to rhodopsin aggregation. This interpretation is supported by recent x-ray diffraction and electron microscopic data from compacted films of the kind studied here,[21] which exhibited regions of lipid/protein segregation and unit-cell dimensions distinctly less than those of native rod cells. Presumably, then, inactive regions were those depleted in rhodopsin, and D_L variations resulted from inhomogeneities including protein/lipid segregation. These results are also in accord with those of Schindler *et al.*[22] who recently reported the room-temperature tracer lateral diffusion coefficient of the *Escherischia coli* matrix protein in humidified, reconstituted, multibilayer membrane films to be $\leq 10^{-12}$ cm^2/s. This slow diffusion is also thought to stem from protein aggregation. The *E. coli* matrix protein molecular weight and concentration were 36 000 (Ref. 23) and 40% (Ref. 22) while the corresponding PRM rhodopsin values are 37 000 and 40%,[10,24] and functional analogies between these two systems have been postulated.[23]

Finally, it is seen (see Table I) that photobleaching did not affect D_L results indicating that further photoinduced aggregation did not occur.

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