Conference on Lasers and Electro-Optics

1993 Technical Digest Series
Volume 11

Conference Edition

Summaries of papers presented at the
Conference on
Lasers and Electro-Optics
May 2–7, 1993
Baltimore, Maryland

Sponsored by
Optical Society of America
IEEE/Lasers and Electro-Optics Society

in cooperation with
Quantum Electronics Division of the European Physical Society
Japanese Quantum Electronics Joint Group

Optical Society of America
2010 Massachusetts Avenue, NW
Washington, DC 20036–1023

CTuN84 Multicomponent fluorescence lifetimes for dye-liposome complexes using time-correlated photon counting

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Liposomes are vesicles of synthetic phospholipids and comprise one of the most promising and diverse systems for the targeted delivery of drugs. Photosensitive substances enclosed in liposomes can be released by heating the liposome environment utilizing radiation. We have adopted the idea of "direct laser heating" and utilized it for short-pulsed localized laser excitation of liposomes containing sulforhodamine dye.

Time-correlated photon counting experiments were performed to obtain fluorescence lifetimes for encapsulated and solution-phase sulforhodamine dye. A frequency-doubled, CW mode-locked Nd:YAG laser at 532 nm was used directly to excite fluorescence in one set of measurements, while it was utilized to pump a Spectra Physics (Model 3500) dye laser for tunable excitation with shorter pulse durations for additional experiments. An ITT 4129 microchannel plate detector was used in conjunction with a fast constant fraction discriminator (Tennelec TC454). The response function of the system obtained by detecting light from a scattering solution was typically 70 ps FWHM.

Fluorescence decays involving the liposome-dye complexes had to be fit to the sums of three exponentials. The data were corrected for the differential nonlinearity of the time-to-amplitude converter (TAC). To study polarization effects, primary data were collected at polarization angles perpendicular, parallel, and at 54.7° (magic angle) relative to the exciting polarization.

A comparison of the percentages of the three lifetime components for various dye concentrations provided a measure of the relative contributions from rather weak membrane-bound complexes (longest lifetime component), partial-quenching (intermediate lifetime), and full quenching (shortest lifetime) processes involving the sulforhodamine dye molecules. The quenching is typical of Forster energy transfer. The implications of the lifetime data for energy transfer and quenching will be presented.

The use of selective polarization allowed us to study the effects of molecular reorientation in solution. The sample was excited with a vertically-polarized laser and either the vertical or horizontally-polarized emission was collected, together with data at the magic angle (54.7^0). When the collection of sulforhodamine chromophores is illuminated with laser light, an anisotropy occurs because only those molecules with a component of the absorption transition moment parallel to the polarization vector of the incident radiation will be excited. The effects of plane-polarized exciting radiation will be discussed in terms of the relative contributions from the three lifetime components for different concentrations of sulforhodamine dye present within the liposomes.

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CTuN85 Scattering and reradiation of diffuse photon density waves by spherical inhomogeneities

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Diffuse photon density waves are just beginning to receive attention as a result of intrinsic interest in their phenomenology, and because of their propensity to provide information about the dense random media through which they propagate,[1] such as human tissue and organs. Briefly, diffuse photon density waves are scalar, overdamped, traveling waves of light energy density. They will propagate through any medium in which the transport of light energy density is governed by the diffusion equation. These traveling waves are brought about by introducing an amplitude-modulated source into a turbid medium. This produces a macroscopic ripple of brightness that is microscopically composed of photons undergoing a random walk. The wavelength of the disturbance corresponds to the root-mean-square distance traveled by a photon during a modulation period, and can therefore be altered by changing the modulation frequency or D.

In this work, we demonstrate some general physical attributes of these waves. In particular, we investigate the scattering and reradiation of diffuse photon density waves by objects within otherwise turbid homogeneous media.[2]

In Fig. 1, we exhibit the conversion or transduction of a diffuse photon density wave from one optical and diffusive wavelength to another. This is done by illuminating an object containing a dye that absorbed light at our source wavelength of 780 nm (dotted lines) and reradiated it at a red-shifted wavelength of 830 nm (solid lines). In some sense, this represents a "fluorescence" of the DPDW. In the process, the object becomes a source for the reradiated DPDW. Localization of the object is accomplished by determining the source center of the reradiated wavefronts.

Our second set of experiments demonstrates the scattering of these waves from purely absorptive or dispersive spheres. We have found that measurement of the wavefront distortions due to the purely absorptive sphere can be predicted by a simple diffraction model in which the sphere is approximated by a disk of the same diameter (see Fig. 2). The pure dispersive case is a bit more complex since we would expect a combination of diffraction and refraction to shape the wavefront distortion. We have found that a ray optic model works well when the object is less (see Fig. 3), but the diffraction model is required in the other limit.

The findings presented here represent two different approaches to the detection of localized inhomogeneities within turbid media. Further work is underway to exploit these results for the purposes of medical imaging.

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1. For example, M. A. O'Leary, D. A. Boas, B. Chance, A. G. Yodh, "Refraction of Diffuse Photon Density Waves," Phys. Rev. Let. 69, 2658–2661; J. Fishkin, E. Gratton, J. Opt. Soc. Am. A, in press.
2. In our experiments, we use a turbid medium known as Intralipid. Refer to M. A. O'Leary, *et al.*

CTuN86 Intensity attenuation of the transmitted light pulse at various arrival time intervals through biological tissues

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The time-resolved method is important to image hidden objects in highly scattering media by detecting the early arriving (snake) photons of the transmitted light pulse, which traverse through the medium in small zigzag paths near the forward direction. The late arriving (diffuse) photons, which undergo random walks and are scattered in all directions, are eliminated by time gating. The earlier the photons arrive, the better will be the spatial resolution of the image, but the intensity is correspondingly lower. In this presentation, the intensity attenuation of the transmitted snake photons through uncompressed and compressed biological tissues at different arrival time intervals are measured and analyzed by the diffusion theory.

An ultrafast laser beam (100-fs/625 nm/82 MHz pulse repetition rate/10 mW power) was focused to a 100 μm spot on the biological tissues placed between two glass slides. The transmitted pulse within a 1-mm diameter pinhole was collected by a lens, time-resolved by a streak camera, and detected by a Silicon Intensifier Target. Experiments were performed using large slabs of chicken breast tissues of various thicknesses up to 70 mm. Tissues were compressed by pushing the two glass slides closer to each other. This method is used in x-ray mammography. The transmitted pulse profiles were fitted by the diffusion theory using a standard numerical fitting program. The intensity of the photons arriving at different time intervals are numerically integrated.