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delay. All spectra were displayed appropriately to reveal the time course of the fluorescence spectra. For every average spectrum, linear regression analysis was performed and the best fit was found to have the form:

$$y(x;t)=k(t)(x-330)^{a(t)}\exp[b(t)(x-330)].$$

The coefficients of the best fit are shown in Table 1. The characteristic whole fluorescence spectrum decay of collagen and elastin are revealed. It was found that both collagen and elastin fluoresce for ~more than 25 ns after the laser pulse. Our electro-optical design seems to be able to resolve the time course of the fluorescence spectrum during a 175 ns laser pulse. This type of additional spectroscopic information may have applications in tissue characterization and pharmacokinetics.

*Also with Biomedical Engineering Department, University of Southern California, Los Angeles, California 90089-1551*

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3:15pm

#### CTuK5 Time-gated imaging of murine tumors using fluorescence of HpD and phthalocyanine

R. Cubeddu, P. Taroni, G. Valentini, G. Canti

*C.E.Q.S.E - C.N.R., Dipartimento di Fisica, Politecnico di Milano, Piazza L. da Vinci, 32 Milano, Italy*

Fluorescence imaging can be applied to localize a specific endogenous or exogenous fluorophore in organic tissues. This technique is specially promising for tumor detection since some compounds localize in malignant tissues in a higher concentration than in surrounding healthy ones.<sup>1,2</sup> Continuous wavelength fluorescence imaging suffers from drawbacks that hamper its application; for example, sharp cut-off filters must be used to suppress the excitation light and narrowband filters are often required to separate the fluorescence of interest from background fluorescence and stray light. Further, the measurements must be done in absolute darkness. To overcome these limitations, we propose an acquisition technique that operates in the time domain.<sup>3,4</sup> An intensified video-camera acquires only the fluorescence light that falls within a short time window, suitably delayed with respect to the pulsed laser excitation. The width of the acquisition window can be set down to 5 ns. The experimental set-up includes a nitrogen-pumped dye laser that generates sub-nanosecond pulses at a tunable wavelength, a precision pulse/delay generator to set the required synchronization, and an image processing system placed in a computer. Real time image enhancement is available through a dedicated software.

The measurements have been performed on experimental tumors induced on the back of some mice. The fluorescence of the tumor area was acquired after the incorporation of an exogenous photosensitizer. Two approaches have been considered.

In the first set of experiments, the photosensitizer most used in clinical practice, in other words, the hematoporphyrin derivative (HpD), has been employed. It can be effectively excited at 400 nm and emits a strong fluorescence with a lifetime of about 15 ns, whereas the tissue autofluorescence mainly extinguishes within 3-5 ns.<sup>5</sup> In this case, the tumor was discriminated from the background fluorescence by acquiring the emission of the suspected portion of tissue after a delay of 20 ns with respect to the excitation and using a gate width of 50 ns (Fig. 1). These parameters provide the optimal compromise between selectivity and signal to noise ratio. In fact, a longer delay would lead to an excessive reduction of the signal.

Alternatively, a second generation photosensitizer (aluminum disulfonated phthalocyanine) was injected into the mice. It was excited using red light (620 nm), which does not produce a significant autofluorescence.<sup>6</sup> In this case, the gate proves useful to cut the excitation light, whose wavelength is near to the Phthalocyanine emission spectrum (680 nm) and whose intensity is orders of magnitude higher than that of the fluorescence. Fig. 2 shows an example of an image acquired using the gate feature.

As a conclusion, we can say that several benefits derive from the time-gated approach. First of all is the possibility to operate under normal illumination, which is especially appreciable in the prospect of applying this technique for clinical examinations. Moreover, the rejection of excitation light and the capability of separating fluorescence signals with time constants that differ for few nanoseconds are key factors in achieving fluorescence images with a good contrast.

*\*Dipartimento di Farmacologia, Università di Milano, Via Vanvitelli, 32 Milano, Italy*

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3:30pm

#### CTuK6 Release of liposome contents by pulsed laser excitation of membrane-associated dyes

P. Misra, D. L. VanderMeulen, K. G. Spears<sup>†</sup>  
*Laser Spectroscopy Laboratory, Department of Physics & Astronomy, Howard University, Washington, DC 20059*

Liposomes are vesicles comprised of phospholipid bilayer membranes enclosing an aqueous internal space. Liposomes are useful for study as a model system to investigate the effects of laser radiation at the molecular level, since their structure and properties are similar to natural membranes and vesicles. Such quantitative data assist in achieving a better fundamental understanding of the processes involving energy transfer from the electronic and vibrational states as it relates to procedures such as laser surgery or selective photo-induced biological modifications at the cellular or subcellular level (either for research or clinical treatment). In addition, lipid vesicles are under clinical study as potential carriers for the delivery of therapeutic agents.

In the first phase of our studies, our data demonstrated for the first time that the contents of liposomes containing high concentrations of xanthene dyes (for example, sulforhodamine) can be efficiently released by single pulses of laser excitation. Data were obtained for the dependence of dye release on several interdependent variables (dye concentration, liposome size and composition, laser excitation parameters and initial temperature of the system). Quantitative analyses of results with nanosecond and picosecond laser pulses were consistent with a photo-induced localized thermal mechanism for the lysis of liposomes.

The use of membrane-associated dyes was investigated as a potentially advantageous alternative, since a smaller dye-lipid ratio may be required compared to the use of dyes internalized in the aqueous space; moreover, there would also be less interference in the internal volume with the encapsulation of active compounds. The dyes initially selected for testing were those known to possess a high efficiency for conversion of absorbed excitation energy to heat and whose absorbance characteristics matched available laser frequencies. From among the nonfluorescent triphenylmethanes (for example brilliant blue G) or thiazins (for example methylene blue), dyes were identified that have a high coefficient for partitioning into the bilayer membrane, as demonstrated by spectral shifts and chromatography. Of those tested, the most efficient photo-induced release of liposomes was achieved with methylene blue, which also has the advantage of being FDA-approved. Laser excitation of methylene blue-liposomes with two 8 ns pulses at 532 nm resulted in 80% liposomal release at a level of dye incorporation (mol dye / mol lipid) at least 10-20 fold less than that necessary for release of encapsulated sulforhodamine under the same conditions.

*<sup>†</sup>Chicago Institute of NeuroSurgery & NeuroResearch, Chicago, Illinois 60614;*