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Discrimination between normal and cancer cells by using spectral analysis of delayed luminescence.

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In our present studies, the time-resolved emission spectrum of delayed luminescence of cell cultures of human fibroblast and human melanoma have been measured using a sophisticated single photon device. Noticeable differences have been found both in the emission spectra, which are time dependent, and in the timing aspects of the different spectral components. This powerful and noninvasive technique can be applied in all fields of skin research, such as the investigation of skin abnormalities and to test the effect of products involved in regeneration, antiaging, and UV-light protection in order to prevent skin cancer. © *2005 American Institute of Physics*.
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Substantial efforts have been made in recent years to recognize early malignant changes in human tissues. It is well known that the traditional diagnostic techniques are very expensive, invasive, and almost unable to identify such pathologies in early stages. Various noninvasive optical techniques have been described in order to discriminate between normal and tumor tissue, including both spectrometric[1,2] and imaging[3,4] tools. All of these attempts have failed to replace the traditional biopsy technique from its leading role as the diagnostic reference. It is therefore of crucial importance to search for more sophisticated detection procedures.

In this respect several papers have recently demonstrated that the ultraweak delayed luminescence (DL) is closely connected to the differentiation stage of the biological system.[5–8] This connection has been justified suggesting a model[9] which connect DL emission to the formation of soliton states inside the quasi unidimensional polymeric chains that constitute the cytoskeleton. Moreover, recent reports on the DL from some solid state systems[10,11] have confirmed this close connection between DL and structure of the emitting systems.

Recently, we have established a very sensitive tool to study DL from biological systems after ultraviolet-A laser irradiation.[12] Here we report our results on the time-resolved spectral analysis of DL of human fibroblast and melanoma cells.

Well-defined cultured cell strains were used. Human white melanoma cells (CRL-1585) were purchased from the American Type Culture Collection (Manassas, VA); foreskin derived normal human fibroblasts (3229) were a gift from Dr. Robert Zimmerman (Boston, MA). Before measurement the cells were diluted in phosphate buffered saline up to a density of 4 million cells/ml and 150 μl of cell suspension was placed directly in the measurement chamber.

The measurements of the DL from cell cultures was performed by using an improved version of a previous setup.[12] The light source was a nitrogen laser (Laser Photonics LN 230C), characterized by a wavelength of 337 nm, a 5 ns pulse width, and an energy of 100 ± 5 μJ/pulse. Due to the very low intensity of DL, the spectral analysis has been performed by a set of broadband (about 80 nm full width at half maximum) interference filters (Thermo-Oriel 57530 / 57550 / 57590 / 57610 / 57630) characterized by maximum transmittance values at wavelengths of 460, 509, 567, 645, and 686 nm, respectively. During measurements the temperature of the sample was maintained at 35 ± 1 °C

It was previously shown that light-induced ultraweak photon emission relaxation dynamics are closely connected with the functional state of the investigated biological system.[5,13–17] A recent paper illustrates a remarkable difference between the intensities of the DL from normal fibroblasts and tumor cells.[18] Nevertheless the intensity is an extrinsic parameter depending on various factors which can lead to remarkable differences in the determination of ultraweak photon yield. Therefore it is important to include in the analysis other parameters, as the time trend and the spectral distribution of DL, in order to get further information.

Based on this aim we have determined, in the present studies, the time trend of the spectral components of the DL emission spectrum from human fibroblast and melanoma cells. These measurements have been repeated several times with distinct cell culture samples in order to increase the statistical significance. The results of these determination are depicted in Fig. 1. It appears that the time trends of the blue-green components are quite different from the orange-red components ones, especially at shorter times, so it can be concluded that the measured emission spectrum depends on the time window of the measurement.

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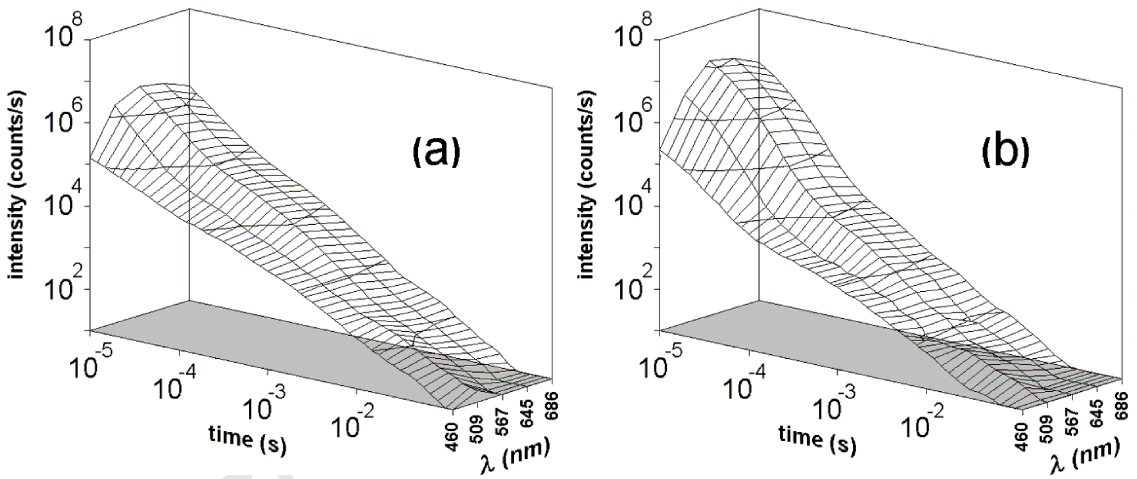

FIG. 1. Intensity of DL as a function of time and emission wavelength (a) human fibroblasts (b) human melanoma cells.

As it regards the time trends, it is well known that relaxation from non-equilibrium state toward equilibrium of complex systems can be approximated by a power law, being such an approximation consistent with the idea of a distribution for relaxation kinetics. In particular Fig. 1 shows that in our investigation the trends of the experimental data exhibit a multimodal behavior according to the equation

$$I(t) = \frac{I_1}{\left(1+\frac{t}{t_1}\right)^{m_1}} + \frac{I_2}{\left(1+\frac{t}{t_2}\right)^{m_2}} + \frac{I_3}{\left(1+\frac{t}{t_3}\right)^{m_3}} + \cdots, \quad (1)$$

which reflects a distribution of exponential decays represented by the sum of several Gamma distributions.[19] In this performance, the choice of the parameters is quite arbitrary if they are not connected to some well-determined physical process. So we preferred to extract, from the experimental data, some information with a clear physical meaning in order to use them to compare the DL from different biological systems.

The intensity $I(t)$ of luminescence is related to the number of excited level $n(t)$ which decay, in a radiative way, at time t by the expression $I(t)=-dn(t)/dt$. Starting from the experimental values of the intensities I_i at time t_i, we can determine the values of the dimensionless function

$$P(t) = \left(\frac{dn}{n}\right) \Big/ \left(\frac{dt}{t}\right) = \frac{I(t)t}{n(t)}, \quad (2)$$

where

$$n(t) = \int_t^\infty I(t')dt'. \quad (3)$$

Due to the smoothing procedure used in order to reduce random noise,[5] experimental points are sampled such that it results $\Delta t_i/t_i=constant$, so that values P_i of the $P(t)$ function evaluated according to Eq. (2) are proportional to the experimental probability that Δn_i levels of the n_i excited ones decay radiatively.

It appears that the $P(t)$ function curves depend on the emission wavelength and/or the nature of the biological system. It was found (data not shown) that the $P(t)$ curves relative to the spectral components with emission wavelength $\lambda_{emiss} > 550$ nm are identical inside the experimental errors. These findings confirm what was obtained in some vegetable systems whose emission spectrum are characterized by λ_{emiss} greater than 600 nm.[16] Furthermore they show again that DL seems to be a quite general observation which is present with similar characteristic in all living systems, at least in a well-defined wavelength range. Due to their similarity the $P(t)$ curves of the spectral components at 567, 645, and 686 nm will be reported by their average value.

The overall result of this analysis is depicted in Fig. 2, where large errors at longer time are due to the numerical evaluation of Eq. (3). It appears that, in the longer time region ($t > 100$ μs), the various $P(t)$ trends tend to have similar values (inside the experimental errors), both for normal and tumor cells, so one can hardly distinguish between them. In contrast, in the shorter time region ($t < 100$ μs), the curves relative to fibroblasts and melanoma cells are quite different and dependent on λ_{emiss}. As a matter of fact, the $P(t)$ values of melanoma cells are about twice as that of fibroblasts for the spectral components with $\lambda_{emiss}=509$ nm and $\lambda_{emiss}=550$ nm. Moreover, in contrast to fibroblasts, in melanoma cells these components exhibit a marked maximum at about 30 μs.

Another intrinsic parameter that could be used to develop a strategy of diagnostic discrimination is based on the measurement of emission spectra.

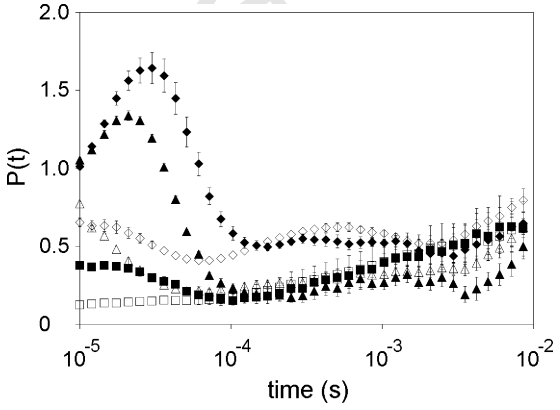

FIG. 2. $P(t)$ curves for the spectral components of human fibroblasts (HF) and human melanoma (HM) cell cultures. (□) HF, $\lambda_{emiss}=460$ nm, (■) HM, $\lambda_{emiss}=460$ nm, (△) HF, $\lambda_{emiss}=509$ nm, (▲) HM, $\lambda_{emiss}=509$ nm, (◇) HF, $\lambda_{emiss}>550$ nm, (◆) HM, $\lambda_{emiss}>550$ nm. Markers are average values of five different samples; bars represent the standard deviations (where not reported, errors are smaller than marker size).

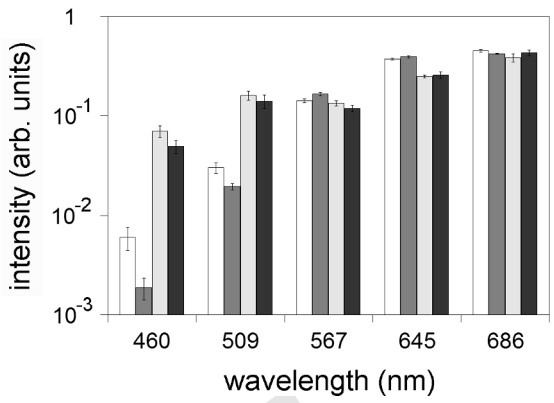

FIG. 3. Emission spectra of human fibroblast (HF) and human melanoma (HM) cells calculated averaging the actual spectra in two different time intervals. (white) HF, 10–50 μs; (dark grey) HM, 10–50 μs; (grey) HF, 10–50 ms (black) HM, 10–50 ms).

Figure 3 shows the emission spectra of fibroblast and melanoma cells, calculated on the average of the actual spectra in two different time intervals, 10–50 μs and 10–50 ms, respectively. It is clearly shown that the emission spectrum depends strongly on the time window in which the measurement is performed. In particular the two spectra seem to be quite different in the shorter time region, while, on increasing the time range, it seems they will converge to similar values. This difference between the relative intensities of the various spectral components could be used in order to distinguish the normal cells cultures from the cancer cells. For instance, if the ratio is calculated between every spectral component and the component $\lambda_{emiss}=567$ nm in the shorter time interval (10–50 μs), the differences for fibroblast and melanoma cells values are significant with a reliability of 99.3, 99.9, 99.8 for the 460, 509, and 686 nm components, respectively.

In summary, in the present studies it is suggested to use DL as a parameter for cell identification. In particular, our results shows that DL is a powerful non-invasive tool to determine biophysical changes within normal and tumor cells. It is also found that the differences between the DL strongly depended on the time and the energy intervals in which measurements are performed. For this reason, our foreseen future developments will concern also the improvement of instrumentation with the aim to collect simultaneously the luminescence in several spectral bands, extended up to 1 μm, and to reduce the time delay of acquisition up to 100 ns.

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