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Steady State and Time-Resolved Laser Fluorescence from Normal and Tumor Lung and Breast Tissues

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ABSTRACT

The steady state and time-resolved laser spectra emitted from normal and tumor human lung and breast tissues have been measured. Differences exist in the spectra and lifetimes of normal and tumor tissues. These results may offer new diagnostic methods to determine cancer.

INTRODUCTION

Laser spectroscopy (1-4) provides a unique and sensitive approach to reveal changes in the physical and chemical properties that occur in healthy and abnormal cells in tissues. There are **intrinsic fluorophors** bound to proteins within cells that fluoresce in the visible spectral region. These native fluorophors display well-defined spectral features that characterize the local environment and state of the cells (1). Flavins are known to fluoresce and exhibit spectral changes when transforming from the oxidized to reduced state. Riboflavins are known to fluoresce in the visible spectral region (5,6) from about 510 to 530 nm. The principal porphyrins fluorescence bands (3) lie between about 590 to 640 nm. Recently, Alfano and coworkers (7-8) have established that the **fluorescence spectroscopy and relaxation times from malignant and normal rat tissues were different.** The differences were attributed to the transformation of local environment surrounding the fluorophors assigned to be flavins and porphyrins in the normal and cancerous rat tissues.

Recently we reported on spectral differences between human normal and cancerous tissues (9). In that report, only one laser wavelength at 488 nm was used to excite the samples. In the present paper, three different wavelengths were used to excite normal and tumor lung spectra. The kinetic results on lung tissues are also reported.

METHODS AND MATERIALS

The experimental apparatus used to obtain steady state and time-resolved fluorescence and Raman spectra from human breast and lung normal and malignant tissues has been detailed in our earlier report (7,8,9). An Argon ion laser beam

at 514.5 nm, 488 nm and 457.9 nm was focused on the front surface of the tissue to a spot size of 200 μm. The fluorescence from the front surface was collected and focused into the entrance slit of a double 1/2m grating scanning spectrometer (Spex Industries) blazed at 500 nm. A photomultiplier tube (Model RCA 7265, S-20) located at the exit slit of the spectrometer measured the intensity at different wavelengths. The output of the PMT was connected to a lock-in-amplifier (EG&G Princeton Applied Research) and an X-Y recorder combination to display each spectrum. Picosecond time resolved fluorescence measurements were performed using a streak camera system (8).

The tissue samples were solid chunks, not cut to any particular specificity and were a few millimeters thick. Each tissue sample was placed in a borosilicate glass test-tube with a cap for spectroscopic studies. The samples were run within two to four hours after removal on the same day of extraction. The measurements were made on at least three locations on each sample and on at least three samples to assure reproducibility.

RESULTS

Typical fluorescence spectra from normal (N) and tumor (T) human lung tissues photoexcited at 457.9 nm, 488 nm and 514.5 nm are shown in Fig.1 to Fig.3, respectively. The principal spectral peaks excited at 457.9 nm, 488 nm and 514.5 nm are located at 496 nm, 509 nm and 531 nm for normal tissues, respectively; and at 503 nm, 515 nm and 537 nm for tumor tissues, respectively. There is one subsidiary maximum located at about 606 nm in the normal lung tissue spectra. The tumor tissue spectra only showed a monotonic decrease with less structure. The spectral peaks and bandwidths of normal and tumor lung tissues have been summarized in Table I and Table II.

For comparison, we have included normal and tumor breast tissue spectra excited at 457.9 nm in Fig.4. The sharp spectral features are Raman lines.

The fluorescence profiles in time for spectral band at wavelength center at 600 nm excited by a picosecond 530 nm laser pulse are displayed in Fig.5. The profiles were found to be nonexponential in time. To fit these curves, a double exponential $I(t)=A_f e^{-(t/\tau_f)}+A_s e^{-(t/\tau_s)}$ was used. The A_f and A_s are the intensity amplitudes of the fast and slow components at t=0 and τ_f and τ_s are the decay times for the fast and slow components. The decay times are accurate within 25%. In Fig.5a, the fluorescence decay times of the fast and slow components from the normal of the lung were about 220 ps and 2650 ps, respectively. In Fig.5b, the fluorescence decay times of the fast and slow components for the tumor tissue of the lung were about 120 and 2600 ps, respectively.

DISCUSSION

From Fig.1 and Table 1, the experimental results using 457.9 nm excitation for lung tissues indicate about 7 nm spectral red shift from normal to tumor lung tissue spectrum. A red shift of 6 nm was also observed using 488 nm and 514.5 nm excitation (Fig. 2 and 3 and Table 1). However, a blue shift was observed in rat kidney tissue (7,8). The tumor lung tissue spectra are much smoother than normal lung tissue spectra. The lack of structure at 605 nm in tumor tissue may be associated with the decrease in porphyrins.

The observed 6 nm red shifts of the main peak and the smoothing of spectra for tumor lung tissues are attributed to the physiological and biochemical transformation of the lung cells from the normal tissues. Previous fluorescence studies have shown that when proteins containing fluorophors gain positive (negative) charge ions, their spectral maxima have been blue (red) shifted (10). Hence, the observed red shifts in the main maxima of the human lung tumor spectra suggest an accumulation of negative charge ions in the malignant cells' intracellular environment. One may conjecture that the most likely fluorophors giving rise to the observed spectral signature at about 520 nm are the flavins in the mitochondria. The spectral feature at 605 nm may be associated with porphyrins.

The flavin band observed for the normal breast tissue near the 509 nm peak

Table I

Spectral Peaks and Bandwidths of Normal and Tumor Human Lung Tissues

Sample	λ_{exc}(nm)	P_1(nm)	P_2(nm)	P_3(nm)	$\Delta\lambda/2$(nm)
L-N	457.9	496	---	606	38
L-T	457.9	503	---	---	41
L-N	488	509	---	604	37
L-T	488	515	---	---	53
L-N	514.5	531	---	605	94
L-T	514.5	537	---	---	87

Table II

Normalized Intensities of Spectra of Normal and Tumor Human Lung Tissues

Sample	λ_{exc}(nm)	I_1	I_2	I_3
L-N	457.9	1	0.25	0.21
L-T	457.9	1	0.38	0.17
L-N	488	1	0.37	0.28
L-T	488	1	0.57	0.29
L-N	514.5	1	0.77	0.75
L-T	514.5	1	0.93	0.73

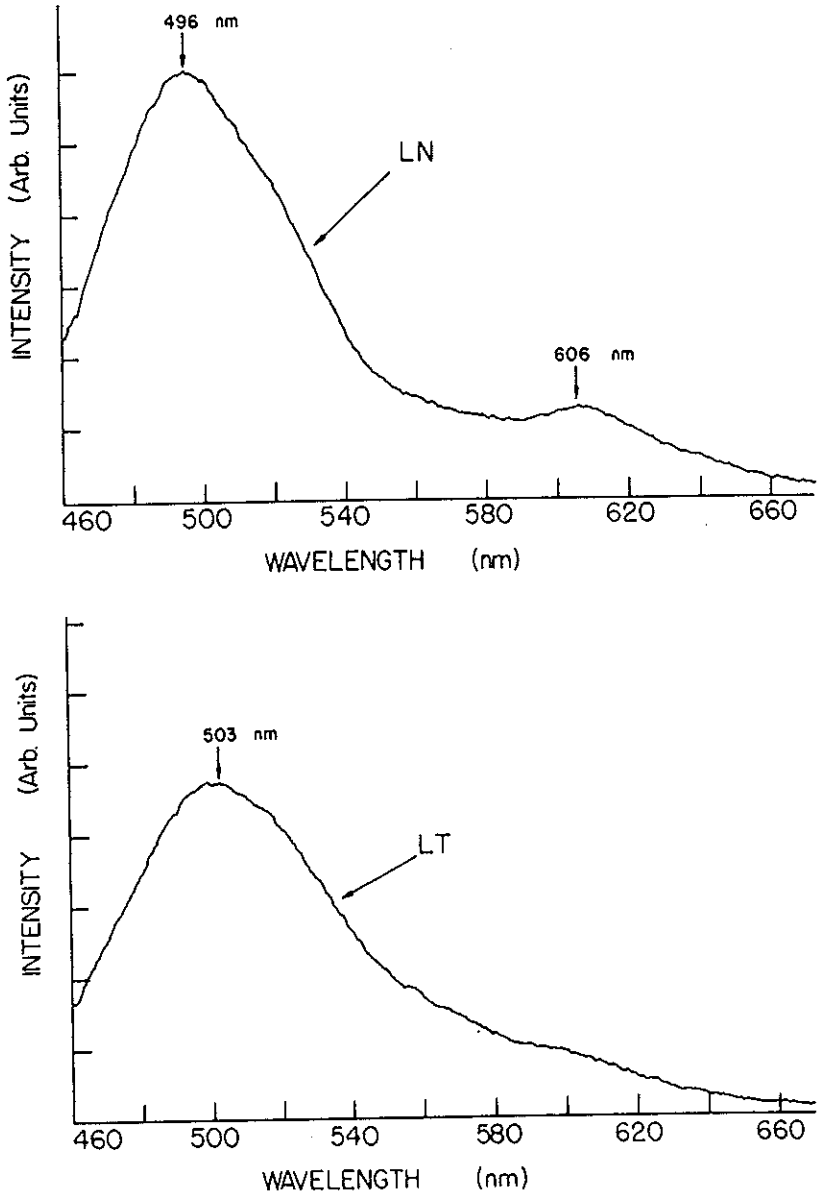

Figure 1: Steady state laser fluorescence spectra of human lung tissues: labeled (a) LN=normal (17mw) and (b) LT=tumor (10mw) excited by 457.9 nm,2 mv sensitivity.

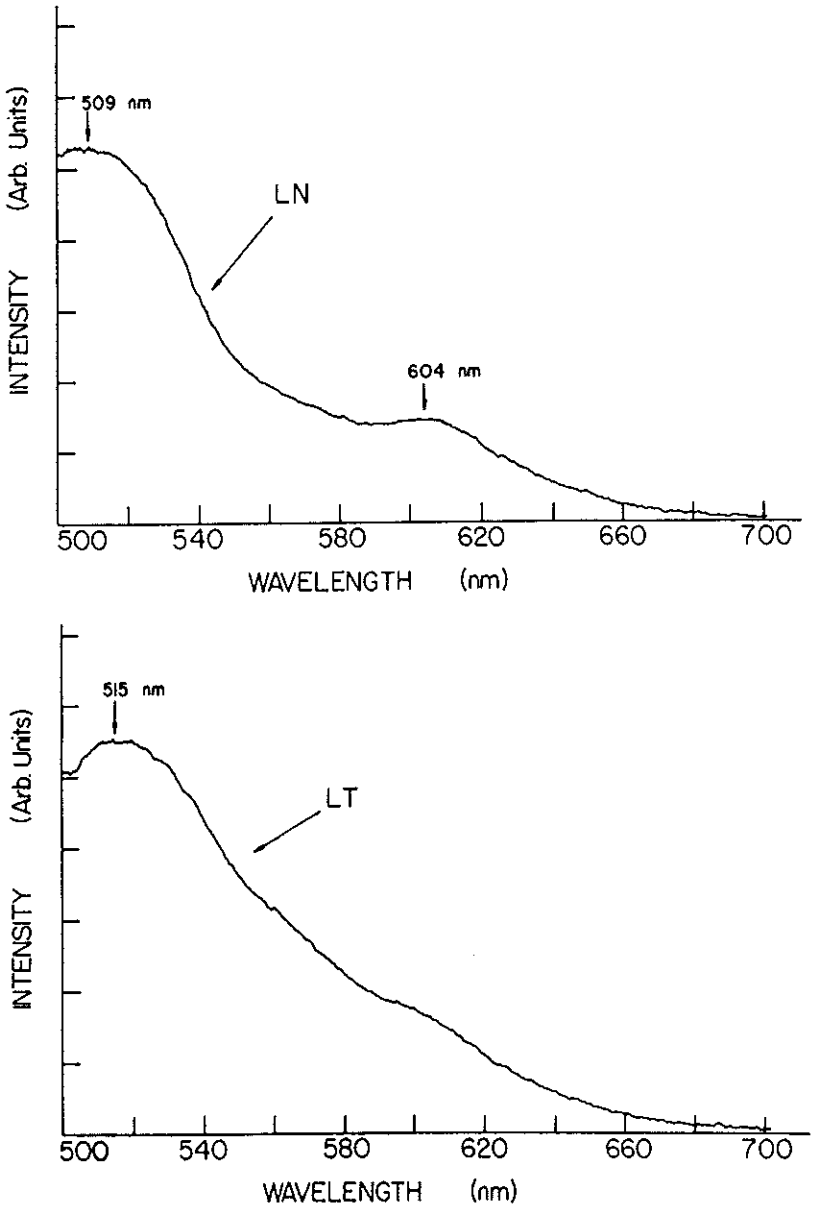

Figure 2: Steady state laser fluorescence spectra of human lung tissues: labeled (a) LN=normal (70mw) and (b) LT=tumor (25mw) excited by 488 nm,2 mv sensitivity.

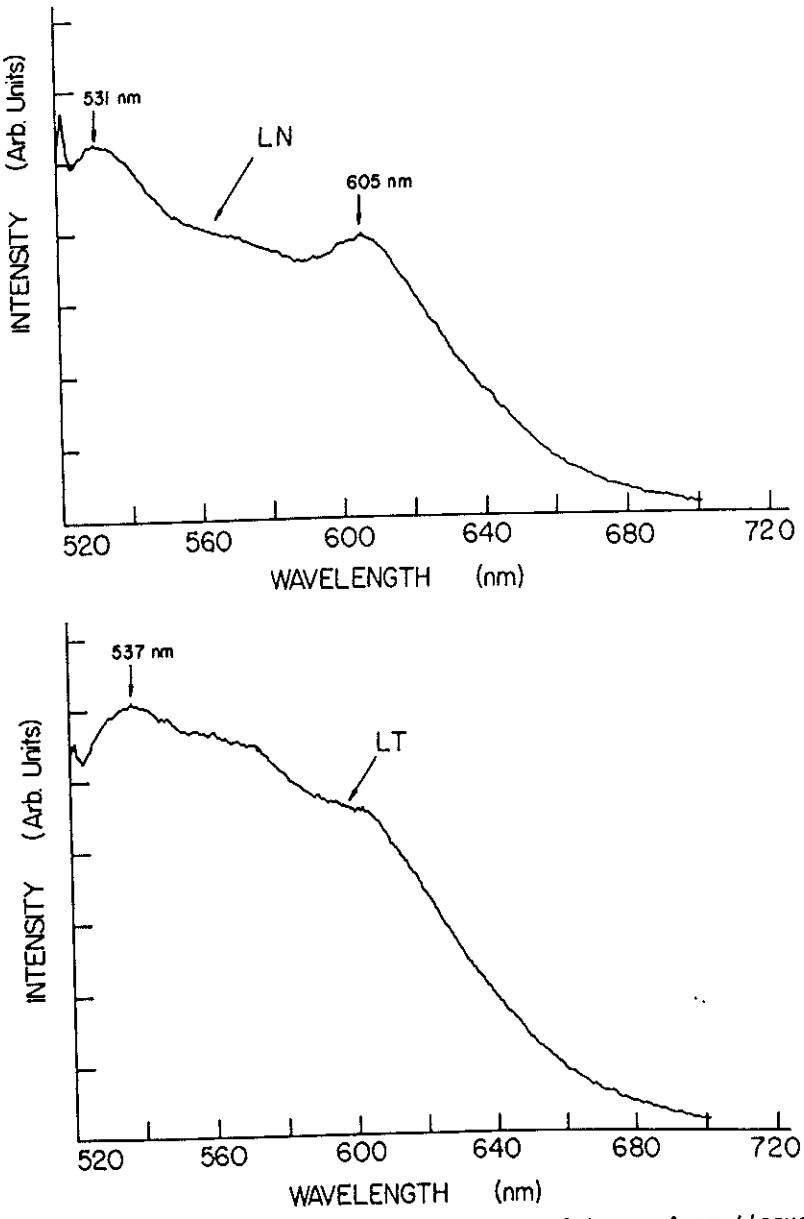

Figure 3: Steady state laser fluorescence spectra of human lung tissues: labeled (a) LN=normal (100mw) and (b) LT=tumor (100mw) excited by 514.5 nm,2mv sensitivity.

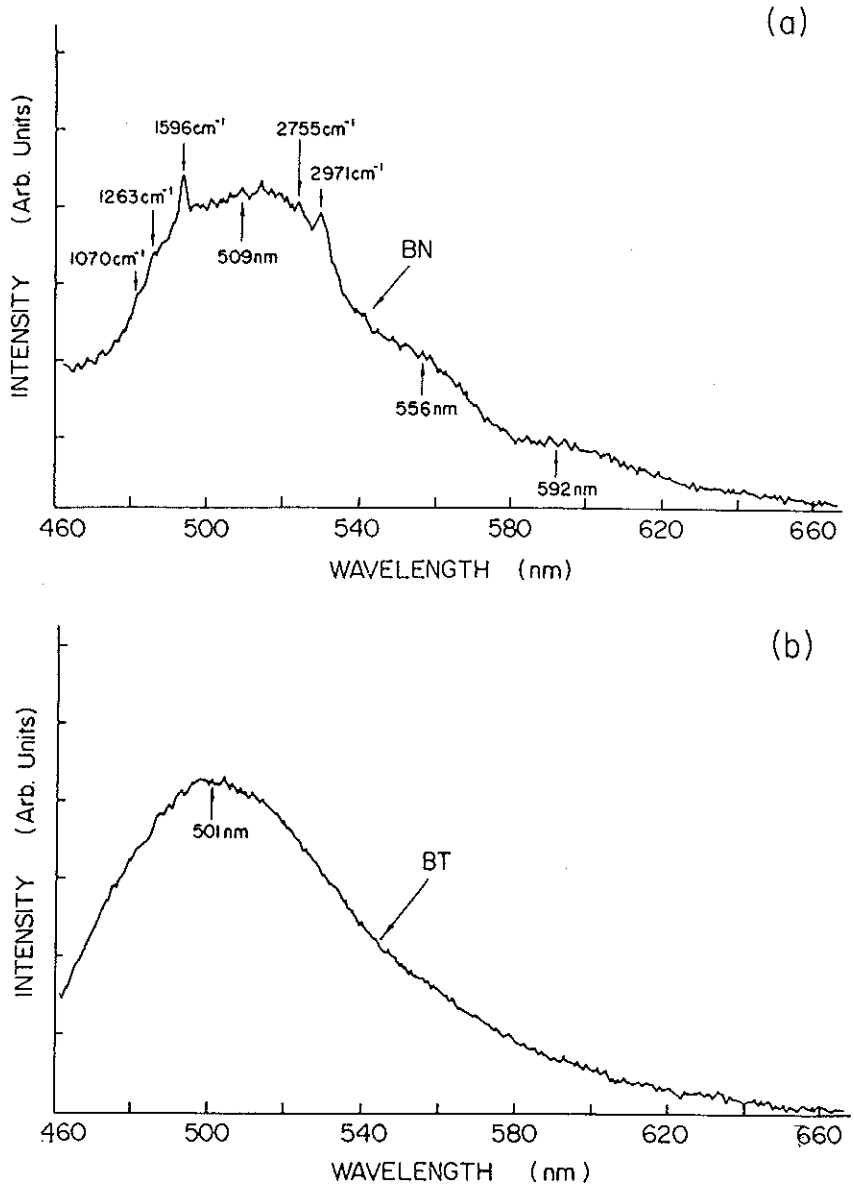

Figure 4: Fluorescence and Raman spectra of human breast tissue : labeled (a) BN=normal (20 mw) and (b) BT=tumor (1.6 mw) excited by 457.9 nm, 1 mV sensitivity.

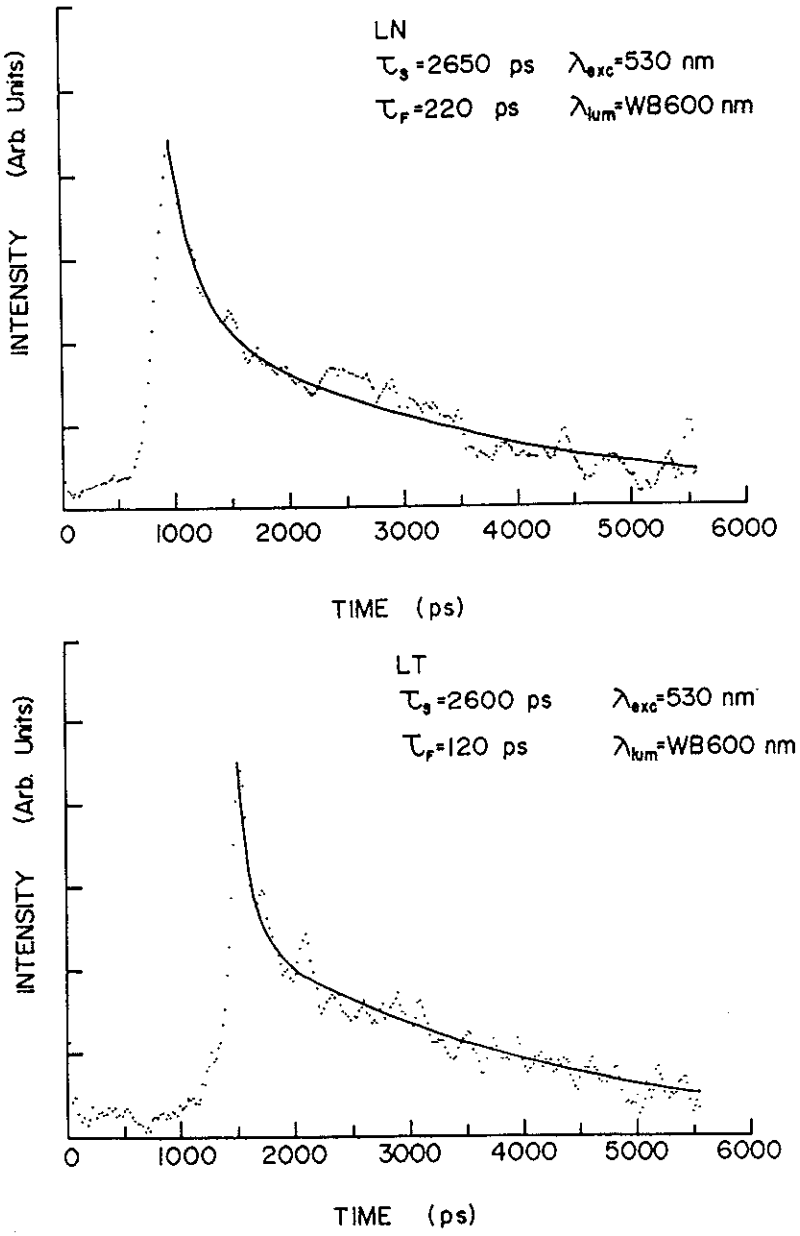

Figure 5: kinetic fluorescence profiles from human lung tissues at the wavelength center at 600 nm (bandwidth 40 nm) excited by 530 nm picosecond pulse. (a) From normal lung tissue τ_f=200 ps, τ_s=2650 ps. (b) From tumor lung tissue τ_f=120 ps, τ_s=2600 ps.

exhibits a blue shift of about 8 nm in the tumor tissue (see Fig.4) spectra. This is opposite to the red shift observed for lung tissues. This blue shift may suggest a buildup of positive ions (11) in the breast tumors. The original of the 555 nm feature is being investigated but may be tentatively assigned to melanins (11) or keratins (7). The lack of structure in the porphyrin spectral region of the cancerous breast tissues is consistent with spectra measured from lung cancer tissues.

The **salient spectral features** which are **common to both the normal breast and lung tissues** are the locations of the main peaks at about 496 to 515 nm with subsidiary bands at about 555 nm and 590 to 605 nm. Depending on the tissues, the main peaks of the cancer spectra are shifted either to blue or red of the normal tissue peaks. Furthermore, the cancerous breast and lung tissues exhibit fluorescence profiles with markedly less structure in the porphyrin region. This feature was also observed in rat kidney tissues (7,8). From lack of structure in the tumor tissue spectral region, it appears there may be a reduction in the density of porphyrin molecules in the cancer cells of human breast and lung and rat kidney tissues, or buildup of the 550 nm feature.

The time-resolved fluorescence shows that the decay time of tumor tissues is faster than that of normal tissues. This suggests an enhancement of the nonradiative processes in tumors.

Much more research will be required to characterize the spectral features of human malignant and benign tumors and normal tissues. It should be pointed out that limited number of cancerous and normal breast and lung samples of human-derived tissues have been employed, and that no distinction between malignant vs benign tumors was attempted. This is the next stage of study. If neoplastic tissues have clearly-defined and reproducible fluorescence properties, this spectroscopy approach may provide an immediate and "light-biopsy" diagnosis of cancer in vivo and could even be considered as a substitute for needle biopsy or some similar cytologic examination. One can then clearly envision a strategic defense against disease in humans using fluorescence and spectroscopic endoscopic instruments for diagnostics that offer high speed and spatial resolution for in vitro, in vivo and surgical use (12).

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