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Fluorescence Spectra from Cancerous and Normal Human Breast and Lung Tissues

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Abstract—**The fluorescence spectra have been measured from native chromophores in malignant and normal human breast and lung tissues. The spectra profiles were found to be different in both species. In addition, one normal breast tissue exhibited Raman spectra.**

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 well-known 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. Their spectroscopy is sensitive to the microenvironment of pH, redox potential, bonding sites, polarity, ion concentration, etc. [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 spectrum region [5] from about 510 to 530 nm, and are part of coenzyme flavin adenine dinucleotide that is responsible for the oxidation reduction in the mitochondria [6]. The principal porphyrins fluorescence bands [3] lie between about 590 and 640 nm. Recently, Alfano and co-workers [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.

Raman spectroscopy is another optical tool to be used to probe changes in tissues. Resonance Raman scattering enhances certain vibrational modes of chromophores because it involves transitions within the electronic absorption band of the chromophores. In doing so, it has been used extensively by Spiro and others [9]–[10] to probe structural changes of many biological molecules, in particular, heme porphyrin proteins. The resonance Raman spectra of reduced and oxidized states of hemoglobin and cytochrome c contain frequency bands [9]–[10] ranging over 600–1700 cm^{-1}. The prominent bands in the Raman spectrum 1000–1650 cm^{-1} were assigned to the porphyrin ring modes. These heme molecules are responsible for the transport of oxygen in blood and the oxidation-reduction process which serves as the link in the extraction of energy from food. Heme porphyrins are also active sites in enzymes.

In this paper, our fluorescence spectroscopy research [7], [8] has been extended to human tissues. This work is needed to see if our previous observations in rats can be valid for human subjects. We show for the first time that there exist differences in the spectral profiles measured for the normal and malignant human breast and lung tissues.

Methods and Materials

The experimental apparatus used to obtain the fluorescence and Raman spectra from human breast and lung normal and malignant tissues has been detailed in our earlier paper [7], [8]. An argon ion laser beam at 488 and 457.9 nm was focused on the front surface of the tissue to a spot size of 200 μm. The laser power was set between 1 and 100 mW to keep the sensitivity between 1 and 2 mV for the detection system. The laser was chopped at 1000 Hz. The fluorescence from the front surface was collected and focused into the entrance slit of a double 1/2 m 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 spectral resolution is about 2 nm. 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.

Breast tissues obtained from two different individuals were used in this study. Normal and cancerous breast tissue (no. 1) was obtained from an 80 year old white female patient undergoing mastectomy. The breast cancer tissue was dissected free of connective tissue stroma and placed into a test tube. Histologic examination [Fig. 1(a)] revealed an infiltrating duct carcinoma with involvement of lymphatic channels and adjacent skin. The cells were pleomorphic with hyperchromatic nuclei and prominent nucleoli; many mitoses were seen. The normal breast tis-

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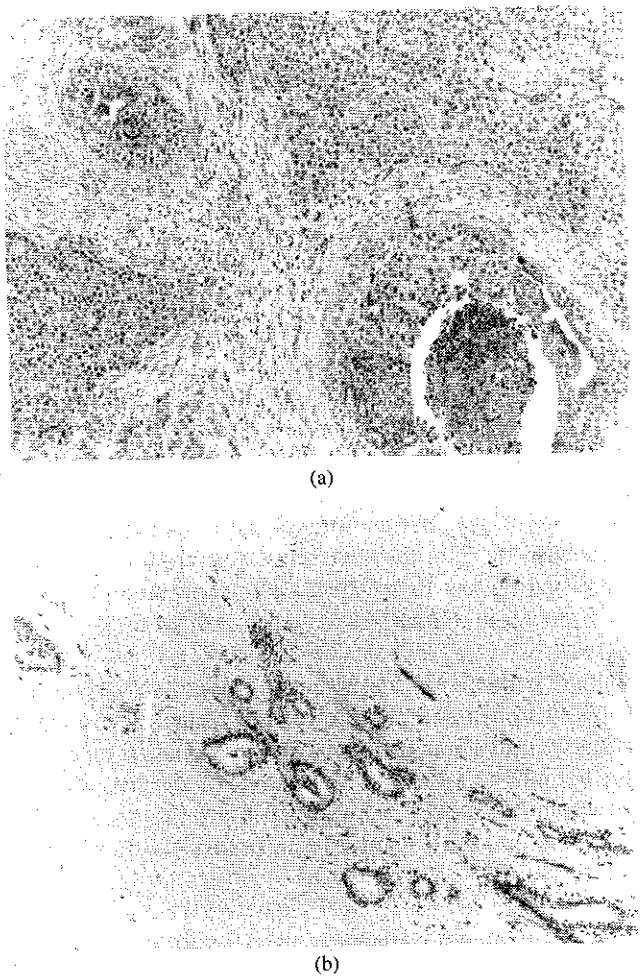

Fig. 1. (a) Sheets of pleomomorphic carcinoma cells in normal breast tissue no. 1 are seen, invading ducts and lymphatic channels. (b) Normal breast tissue no. 1, with involutional changes.

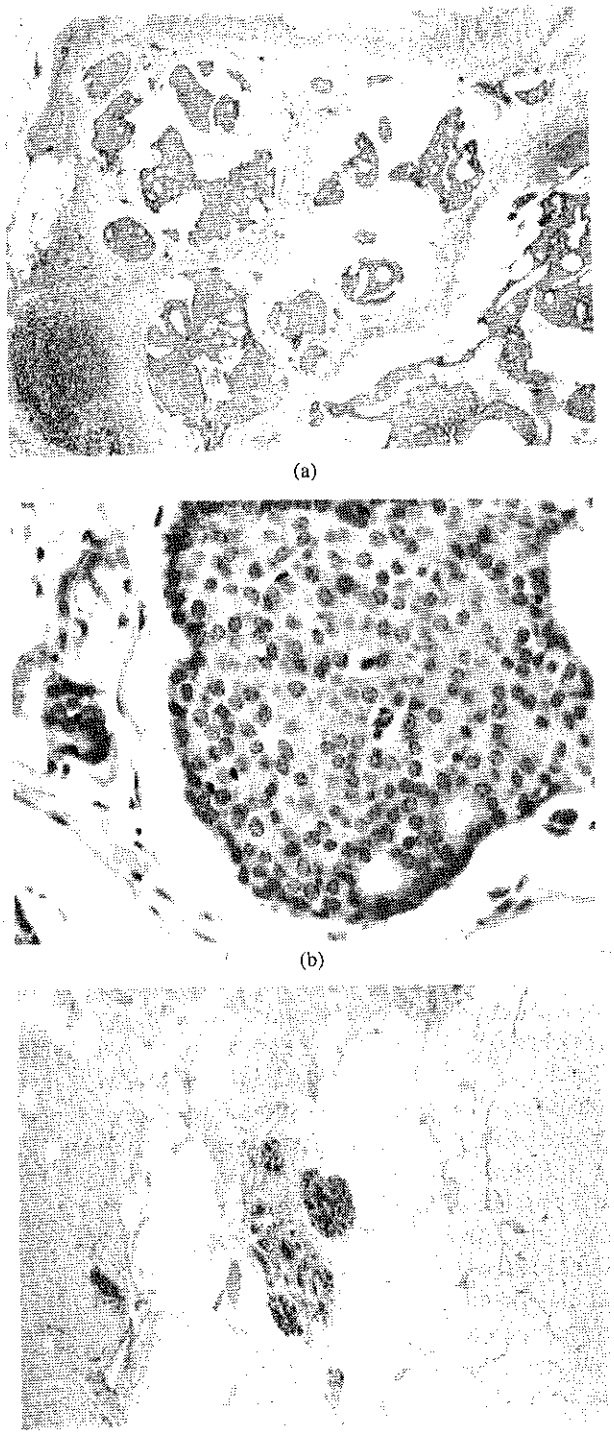

Fig. 2. (a) Histologic examination of the breast tumor no. 2 on low power (40×). (b) Histologic examination of the breast tumor no. 2 on high-power view (400×). (c) Histologic examination of the normal breast no. 2 on power view of 40×.

sue [Fig. 1(b)] showed involution of breast lobules and changes of fibrocystic disease with marked diffuse fibrosis.

Normal and cancerous breast tissue (no. 2) was obtained from a 64 year old white female undergoing mastectomy for a 2 cm tumor in the right breast. A fragment of the cancer as well as a fragment of normal breast were dissected and placed into separate sterile glass tubes. Histologic examination of the tumor on low power (40×) [Fig. 2(a)] showed an infiltrating mucinous (colloid) carcinoma of breast, composed of clusters of small uniform cells floating in a sea of mucin and traversed by fibrous trabeculae. High-power view (400×) [Fig. 2(b)] showed the cell clusters to better advantage. The tumor cells have a slightly bubbly cytoplasm, round nuclei with small nucleoli, and rare mitosis. Occasional glandular lumina are seen. Surrounding clear space represents extracellular mucin. Histologic examination of the uninvolved normal breast [Fig. 2(c)] (40×) shows atrophic lobules, fibrous tissue, and fat.

The lung tumor and normal tissue were obtained from a 77 year old white male undergoing left upper lobectomy for a rapidly enlarging endobronchial mass. A fragment of tumor tissue as well as a fragment of peripheral uninvolved lung tissue were placed in separate glass tubes for analysis. Histologic examination of the tumor on low power (40×) [Fig. 3(a)] revealed solid islands and sheets of undifferentiated carcinoma cells with large areas of tumor necrosis. On high power (400×) [Fig. 3(b)], tumor cells form cohesive sheets. They are pleomorphic with

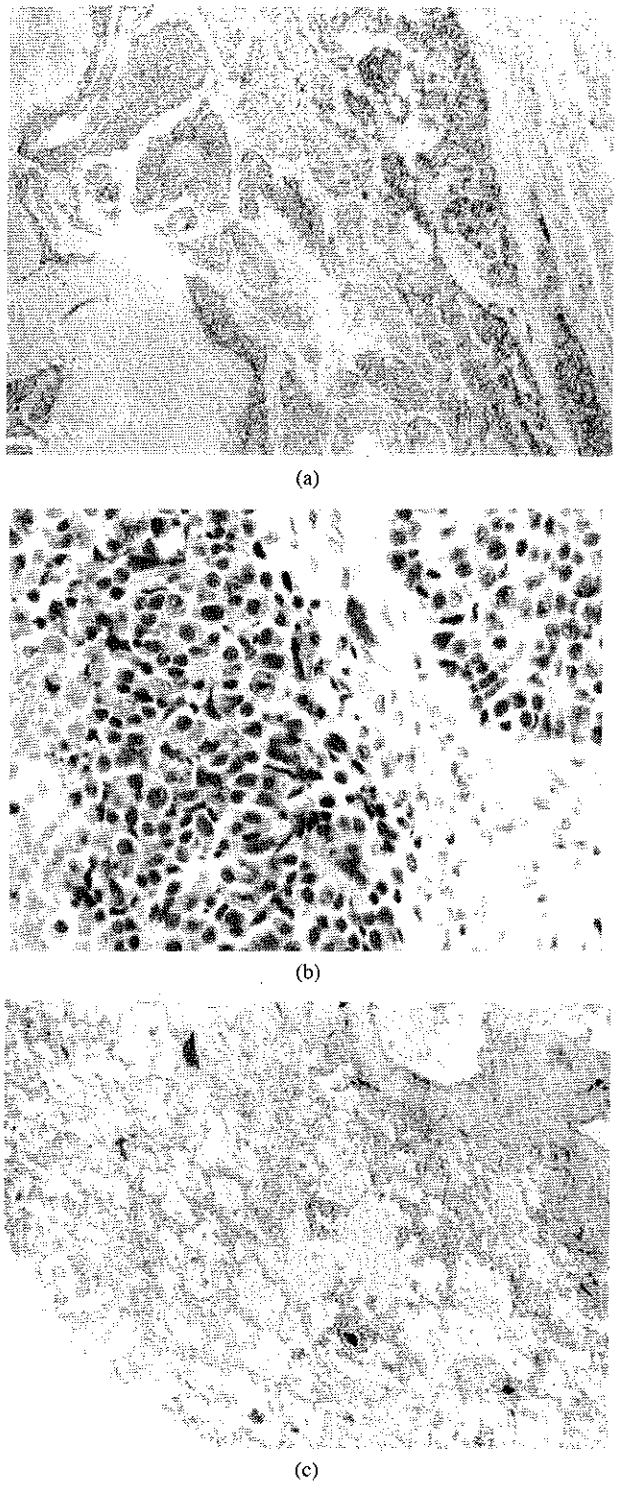

Fig. 3. (a) Histologic examination of the lung tumor on low power (40×). (b) Histologic examination of the lung tumor on high-power view (400×). (c) Histologic examination of the lung normal on power view of 40×.

moderate amounts of pink cytoplasm, irregular hyperchromatic nuclei, and numerous mitoses. No glandular or clear squamous differentiation was noted. The tumor therefore was diagnosed as undifferentiated carcinoma of lung. Histologic examination of uninvolved normal lung showed patent alveolar spaces, well aerated [Fig. 3(c)] (40×). Slight edema fluid and alveolar macrophages are seen in the alveolar lumina. The septae are thin with minimal chronic inflammation.

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 (Fisher Brand) for spectroscopic studies. The tubes had no emission. The samples were run within 2 h after removal on the same day of extraction. The measurements were made at least three locations on each sample to assure reproducibility.

Results

Typical fluorescence spectra from normal (N) and tumor (T) human breast tissues (no. 1) photoexcited at 488 nm are shown in Fig. 4, respectively. One notices the spectral profiles' shapes are different for the normal and tumor tissues. The principal spectral peaks are located at 514 and 517 nm for normal and tumor tissues, respectively. In the normal breast tissue spectra, there are two subsidiary maxima located at 556 and 592 nm. The tumor tissue spectrum showed a monotonic decrease with no clear subsidiary maxima. The location of the main peaks in this tissue varied at different locations—for a noncancerous sample from about 514 to 516 nm and for cancerous sample from about 517 to 519 nm.

Typical spectra from normal (N) and tumor (T) human breast tissues (no. 2) excited at 488 and 457.9 nm are displayed in Figs. 5 and 6, respectively. Again, one notices the apparent difference in the spectra between the normal and cancerous tissues. In this case, the spectra from the normal tissue also consist of narrow spike-like bands atop broad bands. The broad bands arise from fluorescence. The fluorescence curves for normal breast no. 2 are weaker than the fluorescence from breast no. 1 or lung tissues by about five-fold. The location of prominent maxima of the fluorescence bands displayed in Fig. 5 for normal (N) and tumor (T) are at ~ 521 and ~ 518 nm, respectively. The subsidiary peaks are located at 556 and 592 nm. For Fig. 6, the peaks' positions of the main broad bands are located at 509 and 501 nm for normal and tumor tissues, respectively. After the main peak, the tumor tissue spectra shows a monotonic decrease with a weak band centered about 592 nm. The normal tissues clearly exhibit stronger secondary maxima at about 556 and 592 nm in comparison to the tumor spectra [Figs. 5(b) and 6(b)].

By comparing the locations of the narrow spike-like bands of the spectra shown in Figs. 5 and 6 relative to the laser wavelengths, one concludes that these bands arise from resonance Raman scattering. For the normal tissue 2 [Fig. 5(a)], the Raman lines are shifted by about 1037, 1243, 1588, 2761, and 2963 cm^{-1} from the laser lines.

Typical spectra from normal (N) and tumor (T) human lung tissues excited at 488 nm are displayed in Fig. 7. Once again, one notices the difference in the spectral profile between normal and cancerous tissues. The principal spectral peaks for normal and cancerous lung tissue are located at 512 and 520 nm, respectively. In the normal

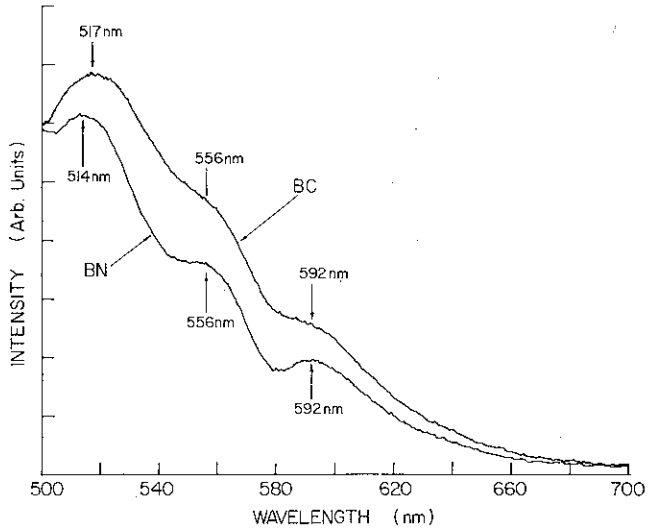

Fig. 4. Fluorescence spectra of human breast tissue no. 1: labeled *BN* = normal (20 mW) and *BT* = tumor (20 mW) excited by 488 nm, 2 mV sensitivity.

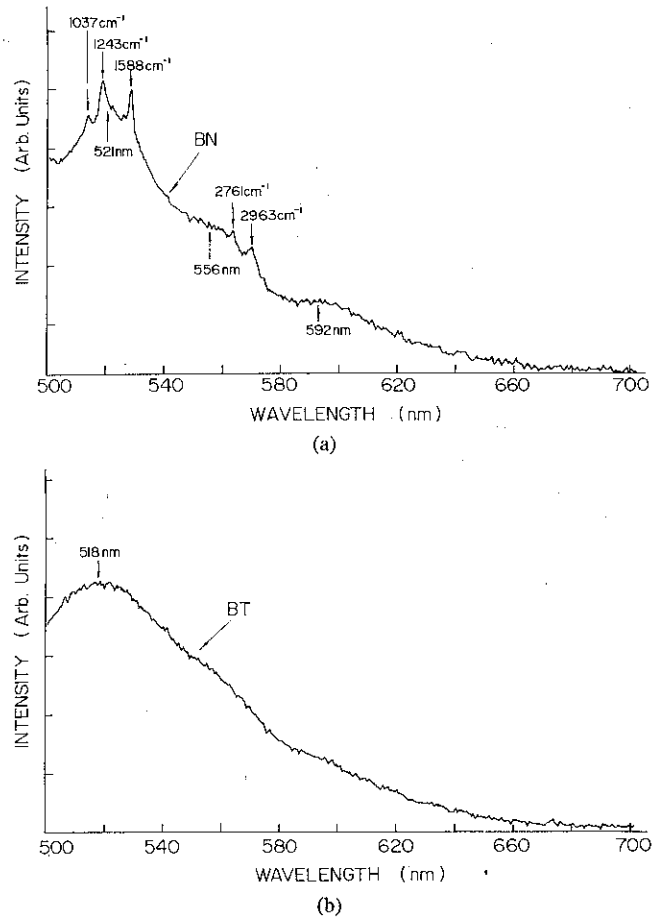

Fig. 5. Fluorescence and Raman spectra of human breast tissue no. 2: labeled (a) *BN* = normal (50 mW) and (b) *BT* = tumor (6.5 mW) excited by 488 nm, 1 mV sensitivity.

lung tissue, there are two subsidiary maxima clearly located at 554 and 600 nm. The tumor tissue spectrum shows a structureless and smoother profile with no clear subsidiary maxima. The peaks at 554 and 600 nm are clearly reduced.

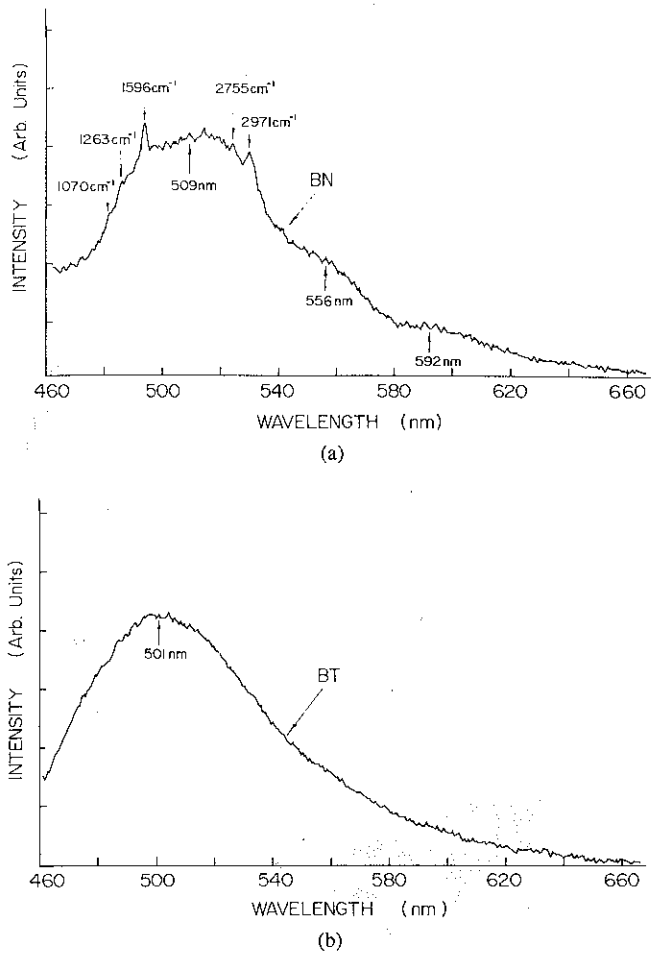

Fig. 6. Fluorescence and Raman spectra of human breast tissue no. 2: labeled (a) *BN* = normal (20 mW) and (b) *BT* = tumor (1.6 mW) excited by 457.9 nm, 1 mV sensitivity.

Discussion

Natural fluorophors within intact cells emit visible luminescence and Raman spectra and display a unique set of spectral features which characterize the state of cells making up the tissues. Depending on the environment, flavins and porphyrins are known to fluoresce in 510–530 nm and 590–640 nm regions, respectively [3], [5], [6]. The porphyrin ring of heme proteins exhibit vibrational mode frequencies in the range of 1000–1650 cm^{-1} [9], [10].

Our experimental results using 488 nm excitation for breast tissue no. 1 indicate about 3 nm spectral red shift from normal to tumor breast tissue spectrum, and for breast tissue no. 2, about a 3 nm blue shift from normal to tumor breast tissue spectrum. These shifts are almost within our experimental error. A definite blue shift of 8 nm was observed using 457 nm excitation (Fig. 6). A blue shift was observed in rat kidney tissue [7], [8]. The tumor breast tissue spectra are much smoother than normal breast tissue spectra. This was observed in both breast tissues. A similar trend was also observed in rat kidney tissue [7], [8]. The lack of structure at 592 nm in tumor tissue spectra may be associated with reduction of fluorophors, in particular porphyrins, responsible for these spectral fea-

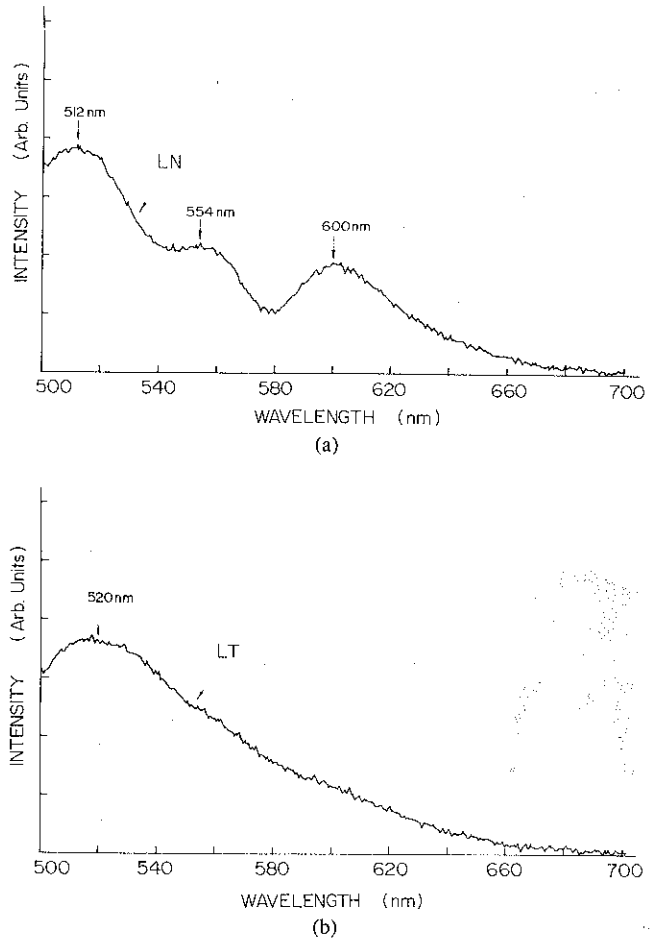

Fig. 7. Fluorescence spectra of human lung tissue: labeled (a) *LN* = normal (10 mW) and (b) *LT* = tumor (10 mW) excited by 488 nm, 1 mV sensitivity.

tures in normal tissues near 592 nm. These features can be used to determine cancer in breasts.

The observed 8 nm blue shifts of the main peak and the smoothing of spectra for tumor breast tissue are attributed to the physiological and biochemical transformation of the breast cells from the normal tissue. Previous fluorescence studies have shown that when protein containing fluorophors gain positive (negative) charge ions, their spectral maxima have been blue (red) shifted [11]. Hence, the observed blue shifts in the main maxima of the human breast tumor spectra suggest an accumulation of positive charge ions in the malignant cells' intracellular environment. Both the peaks of the normal and tumor breast tissues fluoresce between 514 and 520 nm over a bandwidth of 50 nm. One may conjecture that the most likely fluorophors giving rise to the observed spectral signature are the flavins in the mitochondria. The spectral feature at 592 nm may be associated with porphyrins. The origin of the 556 nm feature is unknown, but may be tentatively assigned to melanins [12] or keratins [7].

The broad 600 nm band and narrow spike-like Raman bands from 1000 to 1650 cm^{-1} in the normal breast tissues (no. 2) can be assigned to heme proteins and porphyrins [3], [9], [10]. So far, no spike-like bands have been measured from the cancer tissues. This assignment is consistent with the intensity reduction observed for the 600 nm band and larger fluorescence intensity from tumor tissues. Since only one of the two different normal breast tissues exhibited Raman peaks, the porphyrins may be either dissociated or uncoupled to the binding sites in this case. The 2761 and 2963 cm^{-1} are most likely CH_3 modes. The fluorescence intensity of fivefold more in the other breast (no. 1) and lung tissues overwhelms the observation of any Raman bands in those tissues.

The flavin band observed for the normal lung tissue near the 512 nm peak exhibits a red shift of about 8 nm in the tumor tissue (see Fig. 7) spectra. This is opposite to the blue shift observed for rat kidney and breast tissues. This red shift may suggest a buildup of negative ions [11] in the lung tumors. The 554 and 600 nm subsidiary bands are similar to breast tissues bands discussed in the previous paragraph and most likely arise from the same type of molecules. The lack of structure in the porphyrin spectral region of the cancerous lung tissues is consistent with spectra measured from breast and rat 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 510–520 nm with subsidiary bands at about 556 and 590–600 nm. Depending on the tissue, 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]. It appears there is a reduction in the density of porphyrin molecules in the cancer cells of human breast and lung and rat kidney tissues.

Much more research will be required to characterize the spectral features of other human malignant and benign tumors and normal tissues. *It should be pointed out that only two pairs of cancerous and normal breast and lung [13] samples of human-derived tissues have been employed, and that no distinction between malignant versus 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 optical fiber fluorescence and Raman spectroscopic instruments for the diagnostics that offer high speed and spatial resolution for *in vitro*, *in vivo*, and surgical use [14].

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Elana Opher, photograph and biography not available at the time of publication.