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Significant progress has been achieved to distinguish between cancerous and normal human breast and lung tissues by laser fluorescence spectroscopy. Several spectroscopic methods, such as pulse and CW laser fluorescence spectra, excitation spectra, and picosecond time-resolved kinetics were used to investigate cancer and normal tissues. The origin of the spectroscopic differences between cancerous and normal breast and lung tissues was explored experimentally by chemically-treated normal human tissues. A theoretical model to include self absorption by heme-molecules has been developed to understand the spectroscopic differences between cancerous and normal tissues. The studies of human tissue spectroscopy will lead to new insight into light biopsy technique to diagnose cancer. <i>Keywords:</i>					
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ANNUAL PROGRESS REPORT

Cancer Diagnosis by Laser Spectroscopy

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Program Director: Captain Charles Houston III

Contract Number: N0014-87-K-0431

Contract Period: Sept. 1, 1987--Oct. 1, 1990

Principal Investigator: R. R. Alfano

Researchers: G.C.Tang, Asima Pradhan, Wenling Sha,
J.Chen, C.H.Liu, R.R.Alfano

Institute: The City College of New York
Institute for Ultrafast Spectroscopy and Lasers

Research Foundation: 447240

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I. PROJECT GOALS

The objective of this project is to use optical spectroscopy to diagnoses cancer. In the past, spectroscopic work has been done on hematoporphyrin derivatives (HPD) which have been employed as fluorescent markers for cancer diagnosis and photoradiation therapy. These extrinsic fluorescent markers present themselves as foreign agents, and have been known to interact

with the normal cellular environment. There is a need to develop a new optical technique without the use of dyes or chemicals to detect pathological changes in malignant parts of tissues without interfering with the normal surroundings.

The intrinsic spectroscopic differences of normal and cancerous human tissues, in fluorescence, excitation and time-resolved spectra are being investigated and compared for salient spectroscopic features and fingerprints. This research is needed to develop the future generation of diagnostic-based devices for the early detection of cancer and may give information on the molecular changes occurring in the transformation of normal tissue to abnormal.

II. ACCOMPLISHMENTS

Significant accomplishments have been achieved during the past year:

(1). Steady state spectroscopy

Over the past year, previous steady state fluorescence studies on animal tissues photoexcited by a CW Argon laser have been extended to human tissues. Fluorescence spectra from normal and cancerous tissues from human breast, lung, kidney, ovary, skin, prostate and bladder have been measured. The breast and lung spectra have shown consistency. Normal breast tissue spectra show complex profiles having three distinct peaks at around 520nm, 550nm and 600nm while the cancer spectra show a smoothing of the profile with less structure. Most normal breast tissue spectra have Raman peaks while the cancer tissue spectra lack this feature. In the lung spectra, the normal show three distinct peaks, or in some cases two distinct peaks with the second peak



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- missing while its cancer counterpart spectrum is smooth. The cancer spectral peaks are either blue- or red-shifted, depending on the organ, as compared to the normal tissue main peaks. This could be due to the accumulation of positive or negative charge in the molecules. The steady state measurements have been done with excitation at three different wavelengths: 457.9nm, 488nm and 514.5nm and distinct differences are obtained at all three wavelengths.

To explore the possible origin of the spectroscopic differences, fluorescence experiments were performed on the normal tissues with blood extracted. The normal lung and breast tissue spectra were measured after they were placed in NH_4 solution for various lengths of time. The spectra from these tissues were measured after they had been taken out from the solution. It was found that the spectra from normal tissues with blood extracted have been significantly changed. The original fluorescence spectra from native normal tissues show two prominent subsidiary peaks at 555 nm and 600 nm. However, the spectral curves of the chemically-treated normal tissues with blood extracted become smoother as the length of times in the solution becomes longer. In addition, the spectral maxima of such treated normal breast tissues moves towards the peak of cancer tissue spectra. Both characteristics indicated the spectroscopic differences may be attributed to the reduction of hemoglobin in these tissues. Hemoglobins (Hb, HbO_2 , HbCO) have wide absorption bands, with three absorption bands peaked at 420 nm, 542 nm and 575 nm in the visible spectral region. The latter two absorption bands of hemoglobin can cause absorption dips in the fluorescence emitted from the normal tissues.

Therefore, self-absorption bands in fluorescence may cause the structure observed in the visible spectral region. The amount of self-absorption depends on the amount of hemoglobin in the fluorescence pathway. Therefore, a likely explanation for the fluorescence spectroscopic differences between normal and cancer breast and lung tissues is the amount of hemoglobins surrounding the fluorescence molecule in the samples causing self-absorption modifications in the fluorescence. The hemo-molecules may be natural markers for cancer. In order to be consistent with our observation, there must be less hemoglobin molecules (Hb, Hb₂O, HbCO) in cancer tissues which results in smoother spectra.

To reveal the relationship between the fluorescence and self-absorption spectra from the normal tissues, a theoretical model is being developed. Analytical expressions for fluorescence with self-absorption have been given by Forster theory. We are calculating the intrinsic fluorescence spectra of normal tissues with and without self-absorption. Numerical results are being calculated for fluorescence spectra of normal and cancerous tissues with five kinds of hemo compounds absorption spectra. To confirm our theoretical model, we are comparing the calculated results with the experimental results. So far, it appears that the calculated results are in good agreement with the experimental spectral curves. A reasonable explanation to account for the spectral profile differences between the normal and cancer tissue is that the fluorescence spectra with structure are caused by stronger self-absorption of hemo-group makers in normal tissues than in cancer tissues, at least for excised tis-

sues.

(2). The time-resolved fluorescence spectroscopy

The time-resolved spectra have been measured at 530 nm and 350 nm excitation to study the kinetic behaviour of the involved molecules. The measurements obtained using a Nd-glass laser and a streak camera system showed two decay times, a slow and a fast decay time using 530nm excitation. The fast component of the cancer tissues is faster (120ps) than those of the normal tissues (220ps) while the long component (2600ps) is almost the same. These experiments were done on human lung and breast tissues. The shorter fast component of the cancer tissues implies that there could be more non-radiative pathways than in normal tissues. Further work on fluorescence depolarization will shed more light on this work. In addition, the wavelength dependence will also be investigated.

(3). Pulsed laser spectroscopy

Pulsed laser spectroscopy has also been used to obtain more distinct peak shifts in the cancer tissues as compared to the normal tissues when excited at 351nm or at 527 nm.

Picosecond pulse fluorescence measurements for both normal and cancer tissues of breast and lung were performed using a picosecond mode-locked Nd-glass laser, harmonic generators, and a spectrometer coupled to an OMA II system. Single 8 ps- 527 nm and 351 nm pulses were used to excite the samples.

The main spectral peak of cancer breast tissue is located at 464 nm, and the normal tissue spectrum peak is located at 500 nm when the samples were

- excited at 351 nm. A large blue shift of 36 nm from the normal to the cancer spectra was observed. The cancer tissue spectrum profiles are smoother than the normal tissue. The normal tissue spectra have a shoulder and a wide valley around 490 nm. The cancer tissues emit more light at shorter wavelengths as compared to the normal tissues. The observed spectral difference between normal and cancer tissue can also be attributed to greater self-absorption of the hemoglobins in the normal tissue as compared to the cancer tissues. The normal tissue has a strong absorption band from 380 nm to 480 nm due to the hemoglobins. This strong absorption band can give rise to stronger self-absorption of fluorescence at shorter wavelengths in the intrinsic fluorescence in normal tissues. Therefore, the net fluorescence peak shifts towards longer wavelength. The converse of this statement is that the cancer tissue spectra shifts towards the shorter wavelength.

Similar results were obtained for lung tissues.

Further work is under way to check the consistency of these results.

(4). Excitation spectra

A major advance in cancer diagnosis occurred when the excitation spectra was measured for normal and cancerous tissues.

The excitation spectra of tumor and normal tissues measured at emission wavelengths 520nm, 550nm and 600nm corresponding to the three peaks in the fluorescence spectra of normal tissues have been obtained. The overall excitation spectra showed wide uv and visible bands. The normal spectra showed three peaks within the uv band while the tumor spectra showed a smooth uv

band as well as a larger visible band. These spectroscopic differences were attributed to changes in the electronic structures of fluorophors in the cancer tissues and to indicate self-absorption of hemoglobins in normal tissues than in cancer tissues.

In addition, to locate the source of the spectroscopic features, spectra from cancer cells and components of cell are being studied. Profiles of kidney tumor cells show smoothening. Normal cells are yet to be studied for comparison.

III. FUTURE PLANS

Our future plans are:

- a) The spectra from organs other than lung and breast will be studied for consistency.
- b) Time-resolved and pulsed laser fluorescence spectra will be obtained for more types of tissues.
- c) Fluorescence depolarization kinetic experiments will be performed to study the non-radiative processes such as energy transfer and rotational diffusion.
- d) Fluorescence from cells and cell components will be measured and compared for normal and tumor cells.
- e) Statistics of these methods will be investigated.
- f) Setup collaborators with more hospitals such as Doctors Hospital in New York to collect more well characterized samples.

IV. PUBLICATIONS

- (1) "Fluorescence Spectra from Cancerous and Normal Human Breast and Lung Tissues." R.R.Alfano, G.C. Tang, A.Pradhan, W.Lam, D.S.J.Choy, E.Opher, IEEE QE-23, 10:1806-1811, 1987.
- (2) "Steady State and Time-Resolved Laser Fluorescence from Normal and Tumor Lung and Breast Tissues." R.R.Alfano, G.C.Tang, A.Pradhan and MBleich, D.S.J.Choy and E.Opher, Journal of Tumor Marker Oncology, vol.3, 165-174,1988.
- (3) "Laser Spectra from Human Normal and Tumor Lung and Breast Tissues." R.R.Alfano, G.C.Tang, A.Pradhan, MBleich, D.S.J.Choy and S.J.Wahl, To be published in The American Institute of Physics Conference Proceedings Series for the 3rd International Laser Science Conference.
- (4) "Excitation Spectra from Native Normal and Cancerous Human Breast Tissues." G.C.Tang, A.Pradhan and R.R.Alfano, Submitted to Photochemistry and Photobiology on Feb. 11, 1988.
- (5) "Spectroscopic Studies of Human Cancer and Normal Lung and Breast tissues." G.C.Tang, A.Pradham, R.R.Alfano, Submitted to Lasers in Surgery and Medicine on April 29, 1988.
- (6) "Investigation of Optical Spectroscopy of Cancerous and Normal Human Tissues." Asima Pradhan, G. C. Tang and R. R. Alfano, To be published in Journal of the Electrochemical Society, on Oct.9,1988.
- (7) "Optical Spectroscopic Diagnosis of Cancer and Normal Breast Tissues." R.

R. Alfano, Asima Pradhan and G. C. Tang, Submitted to Journal of Optical Society of America B, on Nov. 10, 1988.

(8) "Light Sheds Light on Cancer-Optical Laser Spectroscopy of Human Cancer and Normal Lung and Breast Tissues." R. R. Alfano, G. C. Tang and Asima Pradhan, Submitted to New York Academy of Medicine, Nov.30, 1988.

(9) "Pulse and CW Laser Fluorescence Spectroscopy from Cancer and Chemically Treated Normal Breast and Lung Tissues." G. C. Tang, Asima Pradhan, Wenling Sha, J. Chen, C. H. Liu, S. J. Wahl and R. R. Alfano, Submitted to Applied Optics, on Dec.7,1988.

V. PRESENTATIONS

(1) "Fluorescence Spectra from Cancerous and Normal Human Breast and Lung Tissues." R.R.Alfano, G.C.Tang, A.Pradhan, D.S.J.Choy and E.Opher, **Invited talk** , OSA Annual Meeting, Rochester, NY, Oct.18-23, (1987).

(2) "Laser Fluorescence Spectra from Cancerous and Normal Human Breast and Lung Tissues." R.R.Alfano, G.C.Tang, D.S.J.Choy and E.Opher, **Invited Paper**, Bulletin of the American Physical Society 32, 1602, Third International Laser Science Conference, September (1987).

(3) "Laser Fluorescence Spectra from cancerous and Normal Human Breast and Lung Tissues." R.R.Alfano, G.C.Tang, A.Pradhan, M.Bleich, D.S.J.Choy and E.Opher, **4th International Conference on Human Tumor Markers**, New York, August 25-28, (1987).

(4) "Ultrafast Spectroscopy, Lasers, Techniques, Clocks, and Applications" (on Cancer), R.R.Alfano, Rensselaer Polytechnic Institute, March 9, 1988.

(5) "Frontier of Ultrafast Laser Technology and Supercontinuum." (Medical) R.R.Alfano, **Plenary invited talk** , International Conference on Lasers '87, 7-12 Dec. 1987, Lake Tahoe, Nevada.

(6) "Spectroscopic Studies of Human Cancer and Normal Lung and Breast Tissues." G.C.Tang, A.Pradham, R.R.Alfano, 8th Annual Meeting of The American Society for Laser Medicine and Surgery, April 25-27, 1988, Dallas,Texas.

(7) "Light Sheds Light on Cancer-Optical Laser Spectroscopy of Human Cancer and Normal Lung and Breast Tissues." R. R. Alfano, **Invited talk** , New York Academy of Medicine, May 10,1988.

(8) "Light in The Diagnosis of Cancer." R.R.Alfano, **Invited talk** , Society for Urology and Engineering, June 1-2,1988, Boston, Massachusetts.

(9) "Fluorescence and Time-Resolved Spectroscopy of Normal and Cancerous Tissues." A.Pradham, **Invited talk** , Society for Urology and Engineering, June 1-2, 1988, Boston, Massachusettes.

(10) "Excitation Spectra from Normal and cancerous Breast Tissues." Society for Urology and Engineering, G.C.Tang, **Invited talk** , June 1-2, 1988, Boston, Massachusettes.

(11) "Investigation of Optical Spectroscopy of Cancerous and Normal Human

Tissues." Pradhan Asima, Electrochemical Society Symposium on Nonlinear Optics and Ultrafast Phenomena, Chicago, Illinois, Oct.9-14, 1988.

(12) "Optical Properties of Cancerous and Normal Human Breast and Lung tissues." G.C.Tang, OPTICON88, Santa Clara, California, Oct.30-Nov.4, 1988.