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Int. Res. J. of Science & Engineering, 2017; Vol. 5 (6): 140-144
ISSN: 2322-0015

RESEARCH ARTICLE

Cancer diagnosis by Synchronous Luminescence Spectroscopy

Munde Bhaskar S

Department of Physics, K.K.M. College, Manwat- 431505 (India)
*Corresponding Author email: bhaskarmunde@yahoo.com

Manuscript Details
Received : 23.11.2017
Revised : 13.12.2017
Accepted: 30.12.2017
Published: 31.12.2017

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Munde Bhaskar S. Cancer diagnosis by Synchronous Luminescence Spectroscopy. *Int. Res. Journal of Science & Engineering,* 2017, 4(6): 140-144

ABSTRACT

We record the Synchronous Luminescence (SL) spectra of cancerous and normal tissues resected at biopsy from 05 patients. Two patients were suffering from breast cancer. One from cervical cancer, one from buccal mucosa cancer and one from utenes cancer. The fluorescence signal was recorded by scanning both the excitation and emission monochromator simultaneously at the same speed of 5 nm/s with a fixed wavelength separation of 40 nm and the emission was scanned in the 250-700 nm range. The SL spectra revealed more resolved spectral features, which is the characteristic of a composite system such as a tissue.

In all the SL spectra, the fluorescence intensity emitted from the cancerous sites is more than that from the normal sites at all the wavelengths in the 250-700 nm range. We calculate the intensity ratio of the cancerous to normal intensity and plotted against wavelength. At the peak positions, the intensity ratio ranges from 0.4 to 84 in different samples. This could discriminate the cancerous and normal tissue types. Distinct peaks around 300 nm and 350 nm and a broad emission band around 450 nm consisting of several narrow peaks characterized the SL spectra of cancerous tissues, while in all spectra from normal sites the intensity was maximum near 460 nm. The study shows that the SL spectroscopy provides a more efficient tool for early diagnosis of the cancer.

Key words: - Synchronous Luminescence (SL), cancer tissue, spectroscopy, early diagnosis of the cancer.

INTRODUCTION

Relative cancer incidence and mortality have globally increased in the past decade. Early diagnosis of cancer can increase the survival rate substantially and the current methods of screening have to be improved for this purpose. The methods of the spectroscopic techniques like Laser Induced Fluorescence (LIF), Synchronous Luminescence (SL) and Fourier Transform Infra-Red (FTIR) spectroscopy have been successfully employed to diagnose the cancer.(1-4).

In fluorescence spectroscopic technique, the discrimination potential depend on the various emission and excitation spectra, which could change the tissue morphology and composition due to the repeated exposure during the spectral measurements. Also the LIF spectra contain the overlapping spectral features. which is representative of a complex mixture of biomolecules. These drawbacks can be overcome to great extent by applying SL spectroscopy in which a single spectral measurement might reveal the required details regarding the tissue pathology.

Complete information on tissue fluorescence can also be obtained by recording excitation-emission matrices (EEM) (Vo-Dinh1981). This requires a series of fluorescence emission scans collected at sequential wavelengths at small wavelength increments. This is a tedious and time-consuming process. Therefore, most often EEM is used only to find the optimal excitation emission wavelengths for differentiating the tissue types. Emission spectra at one or few excitation wavelengths or excitation spectra corresponding to one or more emission wavelengths are then used for diagnosis purpose. Significantly more information on tissue fluorescence can be collected rapidly by recording the SL spectra. In this technique, the fluorescence signal is recorded by scanning both the excitation and emission monochromator simultaneously at the same speed of 5 nm/s with a fixed wavelength interval between the excitation and emission wavelengths. Since it takes the advantages of the absorption as well as the emission properties of a compound, it leads to considerable simplification in the measured fluorescence spectral profile. The SL spectra reveal a more resolved structure from a composite system like tissue in contrast to the generally featureless and broadband appearance of the conventional fluorescence spectra. (5-7)

In the present study, we record the SL spectra of cancerous and adjacent normal tissues resected at biopsy from 05 patients suffering from cancer of different organs. An attempt is made in the present work to discriminate the cancerous samples from normal samples by using intensity ratio at particular wavelengths by SL spectra.

METHODOLOGY

Two samples of human breast and one each of buccal mucosa cancer. cervical cancer and utenes cancer were obtained from Choithram hospital, Indore after resection at biopsy from 05 patients. Normal tissues were also collected from the adjacent normal sites of the abnormalities.

The tissue samples were stored in icebox and carried immediately for spectroscopic study. For fluorescence the tissue samples were taken out of icebox and kept at room temperature for about half an hour before taking any measurements. The samples were cut to the thickness of 2-3 mm and cross-sectional area of approximately 10 mm X 10 mm to fit in the sample holder.

A commercial spectrofluorometer (spex, USA, Fluorolog-II) was used to record the SL spectra. The fluorescence signal was recorded by scanning both the excitation and emission monochromators simultaneously at the same speed of 5 nm/s with a fixed wavelength separation of 40 nm between them. The bandpass of both the excitation and emission monochtomators was 2 nm wide. A xenon lamp of 450 W is used as the excitation source. The light from xenon lamp was incident perpendicular to the sample surface to a size approximately of 2 mm X 4 mm and the emitted light was collected at approximately 20^{0} angle with respect to the excitation light. Excitation intensity varied with wavelength but was always less than 40 $\mu W/mm^2$.

RESULTS AND DISCUSSION

The SL spectra of all the samples were recorded by simultaneously scanning both the emission and excitation wavelengths with a fixed interval between them and the emission was scanned in the 250-700 nm range. The SL spectra from two breast, one each of

buccal mucosa, cervical and uterus cancerous samples with corresponding normal samples are shown in figures 1(a) to 5(a) and the intensity ratio of cancerous to normal tissue versus wavelength are displayed in figure 1(b) to 5(b) respectively.

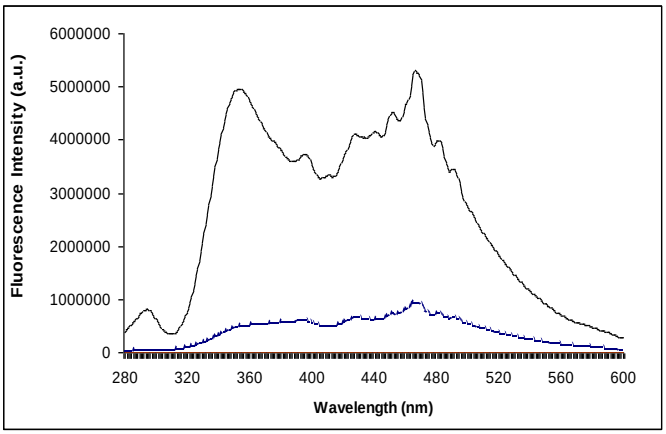

Figure 1 (a): SL spectra of cancer and normal tissue of Breast$_1$ Cancer

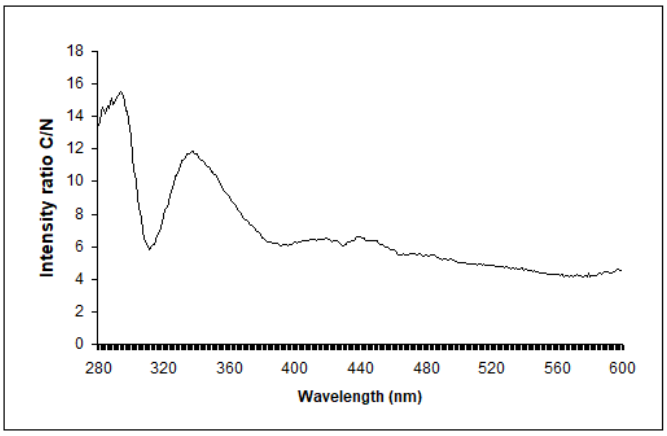

Figure 1 (b): Intensity ratio C/N of breast$_1$ cancer in SL spectra

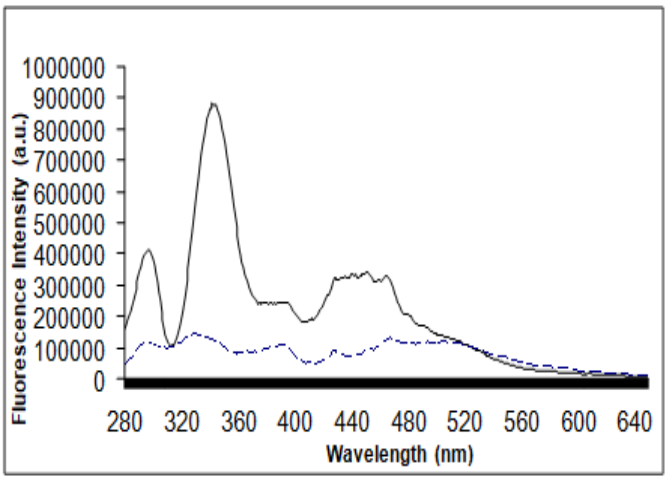

Figure 2 (a): SL spectra of cancer and normal tissue of Breast$_2$ cancer

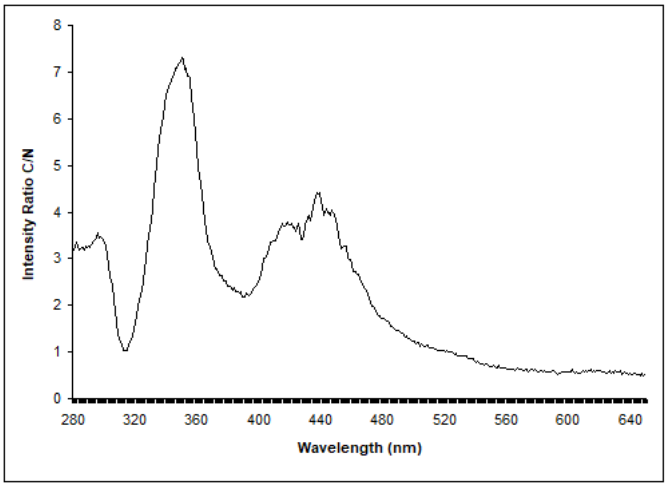

Figure 2 (b): Intensity ratio C/N of Breast$_2$ cancer in SL spectra

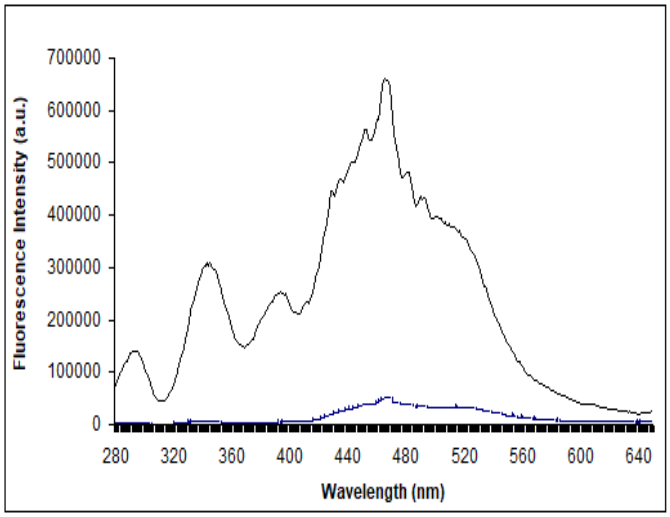

Figure 3 (a): SL spectra of cancer and normal tissue of Cervical Cancer

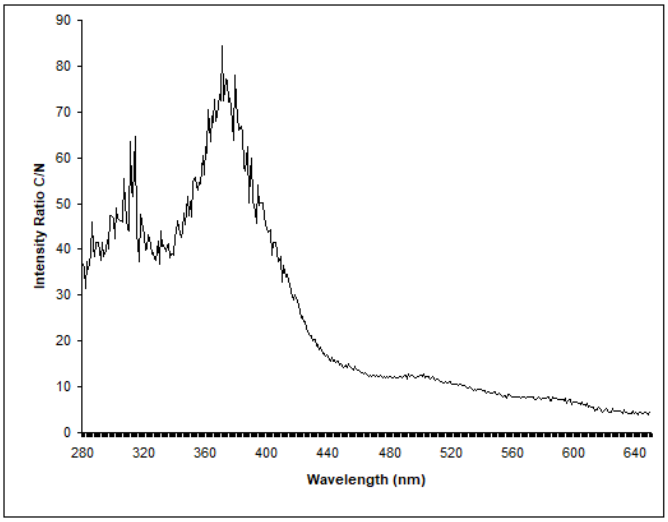

Figure 3 (b): Intensity ratio C/N of Cervical cancer in SL spectra

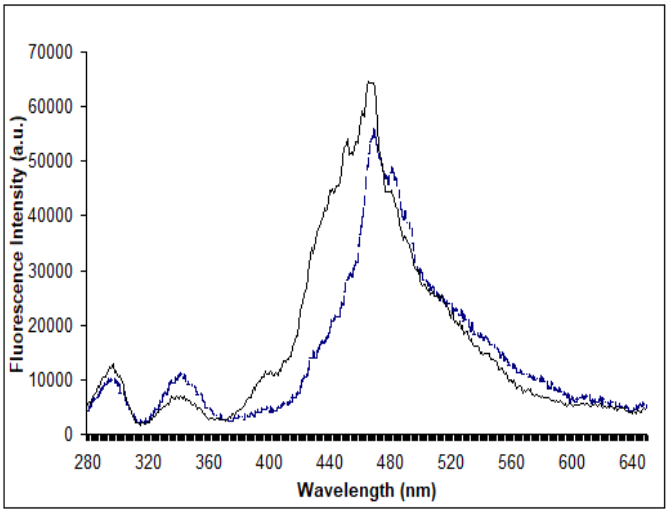

Figure 4 (a): SL spectra of cancer and normal tissue of Buccal Mucosa cancer

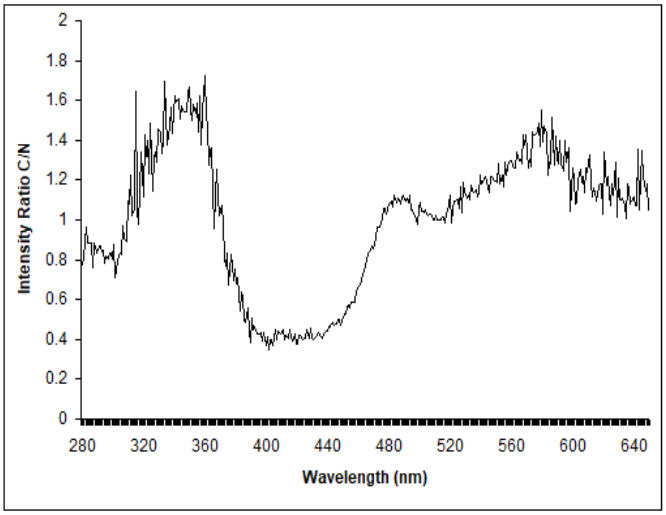

Figure 4 (b): Intensity ratio C/N of Buccal Mucosa cancer in SL spectra

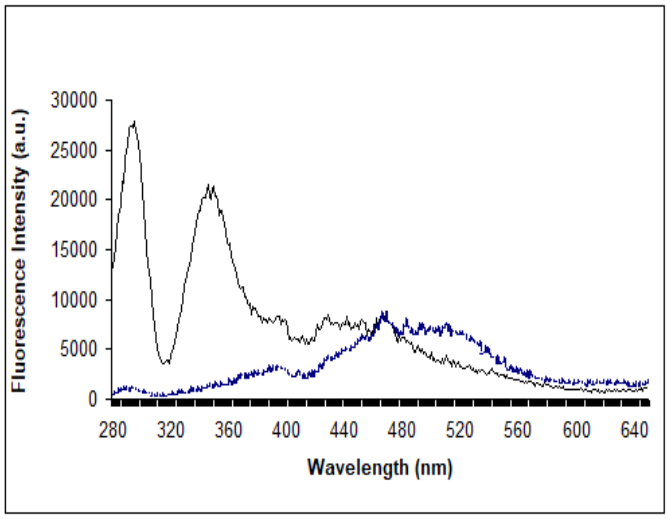

Figure 5 (a): SL spectra of cancer and normal tissue of Uterus cancer

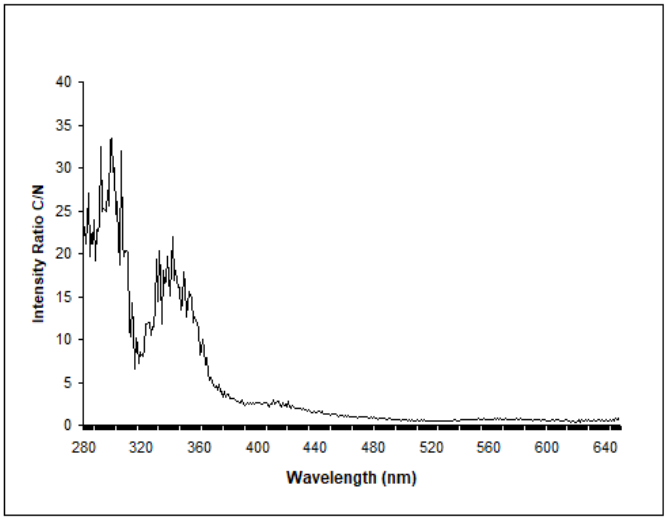

Figure 5 (b): Intensity ratio C/N of Uterus cancer in SL spectra

In all the SL spectra signification differences can be seen in the intensities of the different bands for the malignant and the normal tissue types. It has been found experimentally that the fluorescence spectra of a bio-molecule will change when it becomes abnormal. This is due to the modification of the fluorophore in abnormal tissue as compared to normal tissues. This results in spectroscopic differences between the two cases and helps in early diagnosis and treatment of the disease.

Figures 1 and 2 show the SL spectra for breast cancer tissues of different samples. There is large difference in intensities emitted from cancerous tissues in two samples. This may be due to the difference in degree of malignancies of two samples. In all the SL spectra, significant, differences the sample with more malignancy will emit the large there intensity and the intensity ratio with the normal tissue will be more and this could easily discriminate the malignancy level in the samples.

In both the breast cancerous samples distinct peaks around 290 nm and 350 nm and a broad emission band around 450 nm consisting of several narrow peaks were observed. In one of the samples of breast cancer, the intensity ratio is about 7.4 at 350nm.

In cervical sample, in addition to the distinct peals around 290 nm, 350 nm and a broad emission band around 450 nm, a peak around 400 nm is observed. In cervical cancer the intensity ratio is in between 30 and 84 for the wavelength range 250-400 nm and the highest ratio being 84 at 375 nm wavelength.

In buccal mucosa sample, distinct peaks around 300, 325, 375 and 425 nm and broad emission band around 450 nm is observed. The highest intensity ratio is 1.7 at 360 nm.

In uterus cancer sample, the distinct peaks around 300, 350 nm and a broad band around 450 nm is observed. The highest intensity ratio is 33 at 300 nm. In all the samples one each of Brest, cervical, buccal mucosa and uterus cancer, the intensity ratio is more in the wavelength range 300-375 nm and these ratios ranges between 1.7-8.4
Using these intensity ratios, one can perform the mapping of the cancer-affected portion by using suitable laser wavelength around 350 nm.

CONCLUSION

Differences in the SL spectra of cancerous and normal tissue types may arise due to biochemical or morphological changes between the tissue types. The three narrow spectral bands around 300, 350 and 400 nm in SL spectra of cervical, buccal mucosa and utenes cancer tissues may be attributed to tryptophan, the structural proteins and NADH respectively. The broad band around 450 nm may be due to the presence of number of fluorophores like pyridoxal phosphates, carotenes and lipopigments etc. The fine structure seen in the wavelength range 450-500 nm appears to be due to stray xenon light reaching the exit slit of the excitation monochromator.

From the analysis of the results, it is obvious that SL spectra can give more information than emission spectrum at single excitation wavelength and absorption spectrum at various wavelengths and therefore, it can be used as a more efficient tool for the early diagnosis of cancer.

Conflicts of interest: The authors stated that no conflicts of interest.

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