

## **Detection & Diagnosis Of Malignancy By Laser Induced Fluorescence Spectra (Lif)**

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### **Abstract**

The Laser induced fluorescence spectroscopy (LIF) is a new, novel & promising tool in the diagnosis of diseases. It plays very important role in study of the muscles of the organs of different living beings. The LIF spectroscopic is less invasive compared to other methods. It is also clear that the study of intrinsic fluorescence spectra would help in differentiating diseased tissues from normal ones. Fluorescence spectroscopy can reveal physical and chemical changes in fluorophores.

### **Keywords**

LIF, invasive, fluorophores.

### **Literature Review:**

- 1) P.K. Gupta et al <sup>(1)</sup> reported that the integrated fluorescence intensity for cancerous tissues was always observed to be much higher compared to normal and benign breast tissue sample.
- 2) S.K. Mujumdar et al <sup>(2)</sup> have studied that the overall fluorescence yield was observed to be higher for cancerous breast tissue compared to the normal ones.

3)S.K.Mujumdar et al <sup>(3)</sup> concluded that intensity at the peak at 290 nm and 330 nm wavelengths for the cancerous tissue was significantly larger than that for the soft benign tumor and normal tissue.

4)P.K.Gupta et al <sup>(4)</sup> reported that even with the 488 nm excitation the spectrally integrated fluorescence intensity was considerably higher for cancerous as compared to benign tumor or normal tissue.

5)V.B.Kartha et al <sup>(5)</sup> showed that excitation in the wavelength range 315 –350 nm shows maximum difference between normal and malignant tissue. While excitation with 425-450 nm may provide suitable reference in view of this continuous wave He-Cd laser simultaneously emitting at 325nm and 442 nm may provide the best source for discrimination between normal and malignant tissue.

## **INTRODUCTION:**

The light induced fluorescence spectroscopy (LIF) plays very important role in study of the muscles of the organs of different living beings. The use of LIF spectroscopy in the diagnosis of diseases is a new, novel and promising tool for the medical field. Fluorescence spectroscopy can reveal physical and chemical changes in fluorophores. The LIF spectroscopic is less invasive compared to other methods. When a tissue in a body is said to be affected by a cancer it undergoes few physical and chemical changes. Intrinsic fluorescence of the molecules, which play an important role in biological activity, might give large amount of information about the health of muscles or organs. It is also clear that the study of intrinsic fluorescence spectra would help in differentiating diseased (here malignant) tissues from normal ones.

## **PREPARATION OF SAMPLE:**

We have collected some Samples; the collected samples are from the different organs of human being having different ages. The samples were collected with the help and permission of doctors.

The samples were collected with the help of doctors. After performing surgical operation the samples were preserved in icebox immediately to carry to laboratory for recording fluorescence spectra of these tissues. We have collected a part of the malignant tissues (squamous cell carcinoma) as well as source part of non-malignant tissue of the different organs of the human body. The collected samples are cleaned and the blood was completely removed using distilled water. These samples were confirmed histopathologically. All the samples showed presence of carcinoma (malignancy).

The collected samples were preserved in icebox because cell do not break or decay at low temperature and their chemical properties are maintained. The physiological saline was added to the samples as antibiotics and its temperature was continuously maintained. Those collected squamous cell carcinoma was placed in falcon plastic culture dish containing 2 ml distilled saline water in order to prepare them for the experiment for recording the fluorescence spectra.

### **Experimental Set-up**

Figure 2.1 shows the optical diagram of general purpose of spectrofluorometer. spectrofluorometer ( Hitachi model no. F2000) was used for measuring the fluorescence spectra of tissues of different organs of human body. This instrument has a xenon lamp as the source of exciting light; such lamps are generally useful because of their high intensity at all wavelengths ranging upward from 200 to 800 nm. The light from the xenon lamp is made to fall on a grating. The excitation wavelength is changed only with this grating. The selected wavelength of light is passed through a slit after which it falls on the sample which is taken in a four side polished quartz cuvette. Light is scattered from the cell and sample also exhibits some fluorescence. This light is then passed through another slit and is made to fall on a grating. This grating is useful in identifying the fluorescence wavelength of the sample. The instrument shown is equipped with monochromator to select both the excitation and emission wavelength. The excitation and emission monochromator are placed  $90^{\circ}$  to each other. The excitation monochromator contains two gratings, which increases the purity of the exciting wavelength. In addition these monochromator use concave gratings. From the grating another slit is used to prevent stray radiation and then the light is made to fall on the photomultiplier tube. The PMT detects the fluorescence, which is quantified with appropriate electronic device. The output is usually presented in a graphical form.

### **EXCITATION AND EMISSION SPECTRA:**

We are interested to record both excitation and emission spectra. An emission spectrum is the wavelength distribution of the emission measured with a single constant excitation wavelength.

Conversely an excitation spectrum is the distribution of excitation intensity at a single emission wavelength. Such spectra can be presented on either a wavelength scale or a wave number scale.

For an ideal instrument the directly recorded emission spectra would represent the photon flux emitted at each wavelength over a wavelength interval determined by the slit width and dispersion of the emission monochromater.

Similarly the excitation spectrum would represent the relative quantum yields and emission spectra are independent of excitation wavelength. Hence the excitation spectra of a fluorophore are typically super impossible on its absorption spectrum. Even under ideal circumstances, such correspondence requires the presence of only a single type of fluorophore and the absence of other complicating factors such as a non-linear response resulting from a high optical density of the sample.<sup>(4)</sup>

## **RESULTS AND DISCUSSIONS:**

The block diagram of experimental setup used to study the effect of laser radiation on bacteria is shown in figure 1. The experiment consists of ultraviolet nitrogen laser, the quartz-focusing lens, the sample holder and Bunsen Burners. This shows that diseases caused by the bacteria may be treated using nitrogen laser beam if the beam is carefully utilized. The beam was carried from the source to the lesion by an optical fiber. A calculated and proper dose of the beam was given to the patients and the patients, who were not giving response to any type of drugs, were completely brought on the track of recovery.

By using the experimental set up of LIF Spectroscopy we have recorded fluorescence spectra of normal muscles of various organs. Out of the recorded spectra few samples show interesting results. We select human samples and recorded the spectra. The same spectra are used for the detail study and analysis.

We record the spectra of different parts of the affected portion of the different organs of human beings.

The spectra of cancer and normal tissues recorded by the experiments are and displayed in figure 2.1(a) and 2.1(b). These spectra are recorded without any integration. The spectra of some cancerous samples tissue show lot of fluctuation and it is difficult to interpret them. In order to get more information the average spectra have been recorded by recording fluorescence spectra from several laser pulses by keeping same sample before the laser beam. The average intension measurements gave considerable information about the samples than we decide to take the average spectra of the samples and here after we started recording the average spectra only.

The fluorescence spectrum at 290 nm excitation shows one primary emission peak around 330 nm which may be attributed to the aromatic amino <sup>(3)</sup> acid tryptophan and four additional peak around 390 nm, 450 nm, 468 nm, 488 nm and 490 nm which may be attributed due to presence of elastin, the Coenzyme Nicotinamide Adenine Dinucleotide (reduced from NADH) and Coenzyme, Flavin Adenine Dinucleotide (FAD). <sup>(5)</sup>

For 280 nm excitation, all spectra shows primary emission peak around 333 nm due to the presence of tryptophan. It is also observed that the fluorescence intensity due to tryptophan increases with increasing concentration of different organs to human body tissues. <sup>(2)</sup>

All the LIF spectra show peak intensity near 340 nm. The intensity of LIF spectrum around 340 nm of cancerous tissues is more than that of normal tissues. <sup>(1)</sup> This is due to presence of tryptophan. Cancerous tissues have more intensity because cancer cell have more concentration of tryptophan, NADH and FAD. <sup>(5)</sup>

The fluorescence spectra show exciting feature at about 340 nm. The normal tissue show small peak near 340 nm where as the cancerous tissues show big peak near 340 nm.

Light included fluorescence spectra (LIF) of samples are displayed in figure. The spectra of different organs of human body is as shown in the given figure 2.2, from the observation of the spectra we see that the muscles in the form of peaks and deeps have exhibited various features. It seems if the spectra are studied and analyzed in details, some part of the spectra emitted by cancerous tissues and that given by normal tissues can be easily distinguished.

## **CONCLUSIONS:**

The features of LIF spectra of the normal and cancerous tissues show that the cancerous tissues can be very easily distinguished from normal tissues. The cancerous tissues in different stages might show different features and therefore we are sure that the different stages of cancer.

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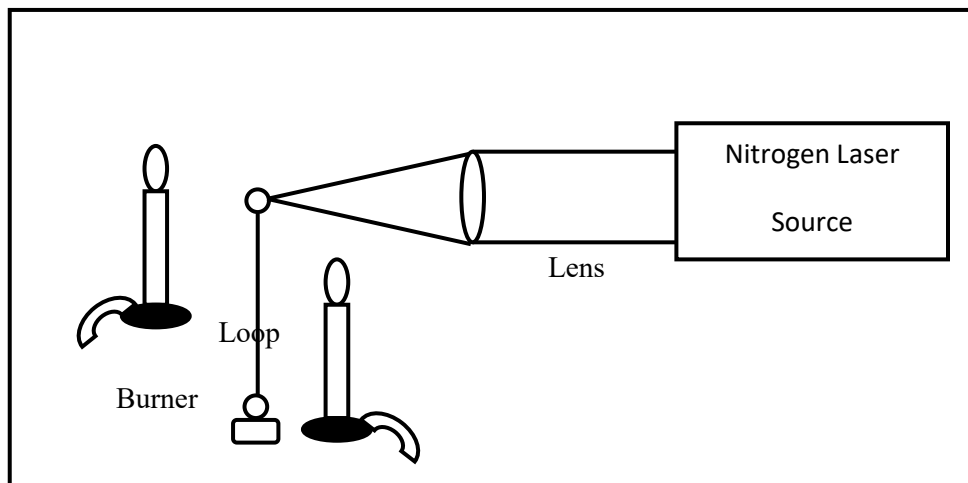
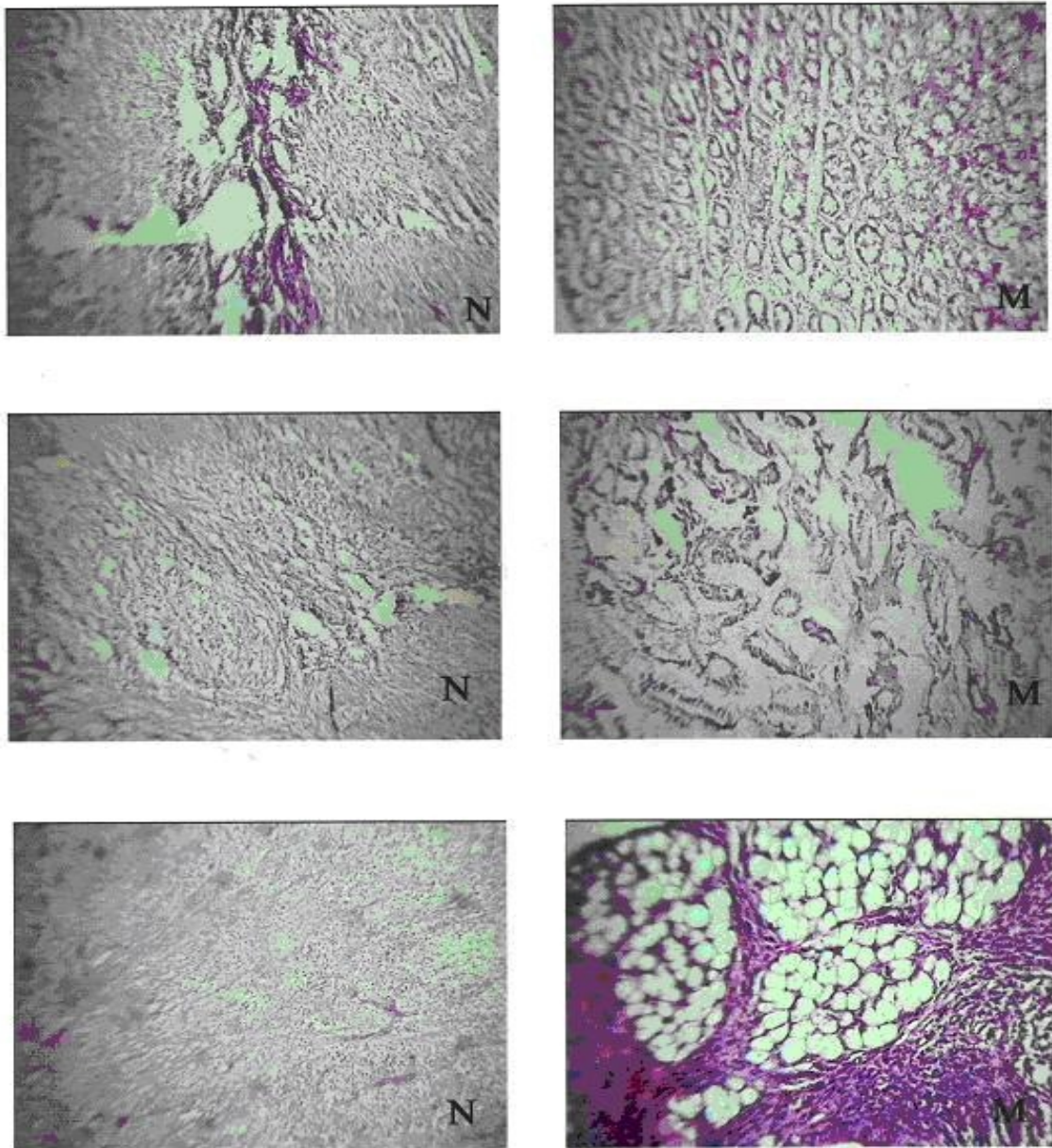


Fig.1: Experimental Setup of spectrofluorometer.

**Cancer diagnosis by Laser Induced Fluorescence Spectroscopy**



**Fig. 2.2 Stains of normal (N) and malignant (M) tissues  
Top: Rectum Cancer, Middle: Stomach cancer and  
Bottom: Breast Cancer**

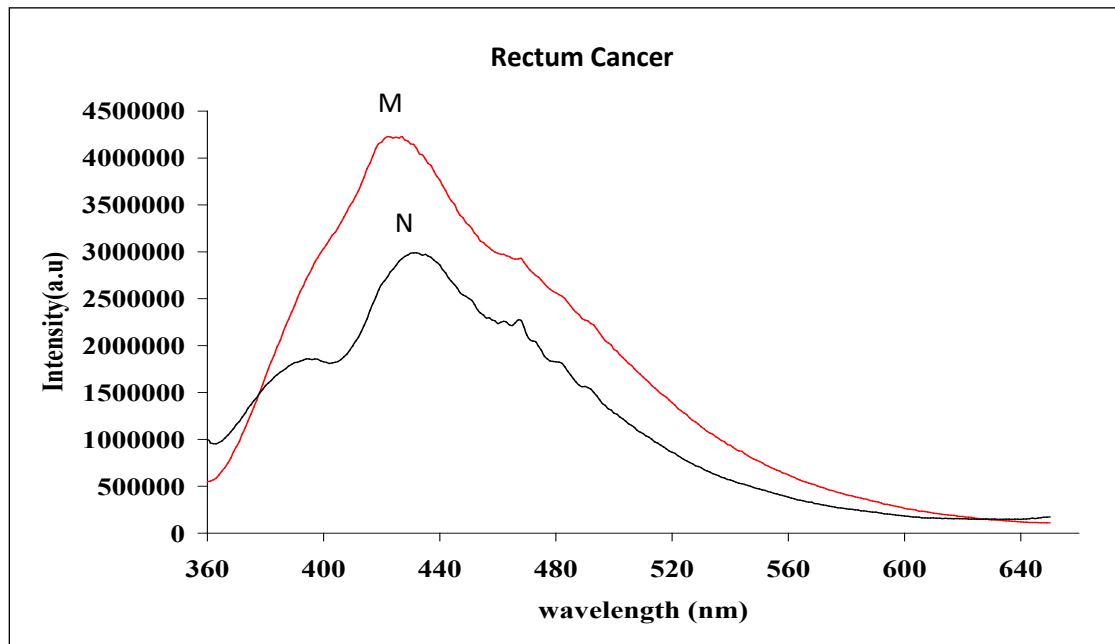


Fig.2.1 (a) L.I.F spectra of cancer and normal tissues of Rectum Cancer

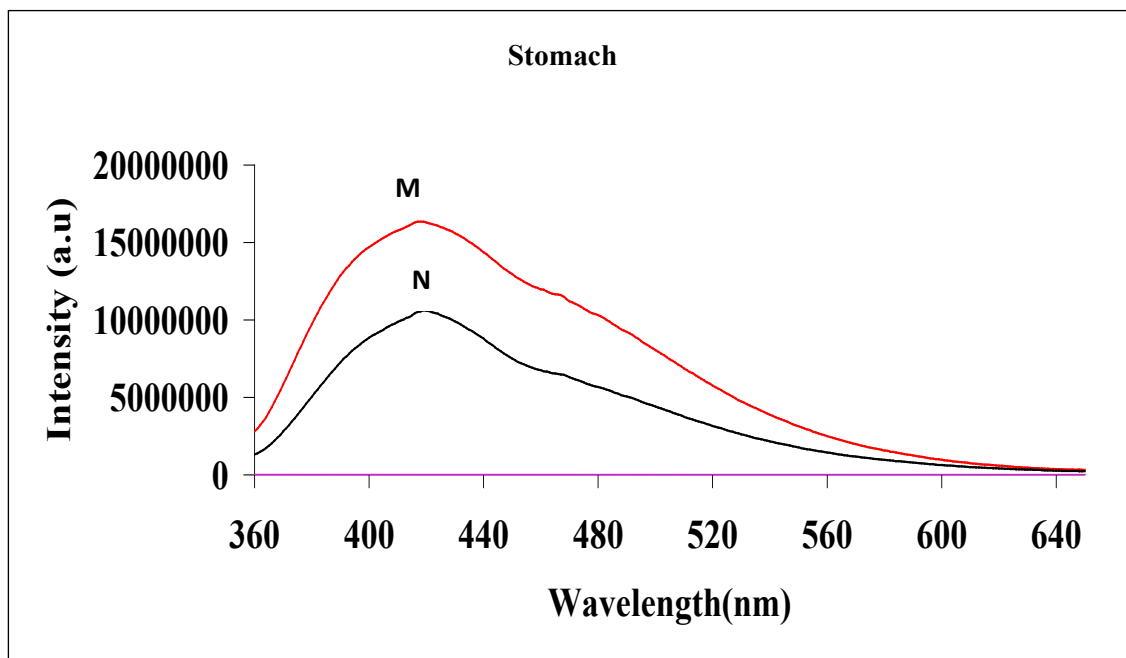


Fig.2.1 (b) L.I.F spectra of cancer and normal tissues of Stomach Cancer.



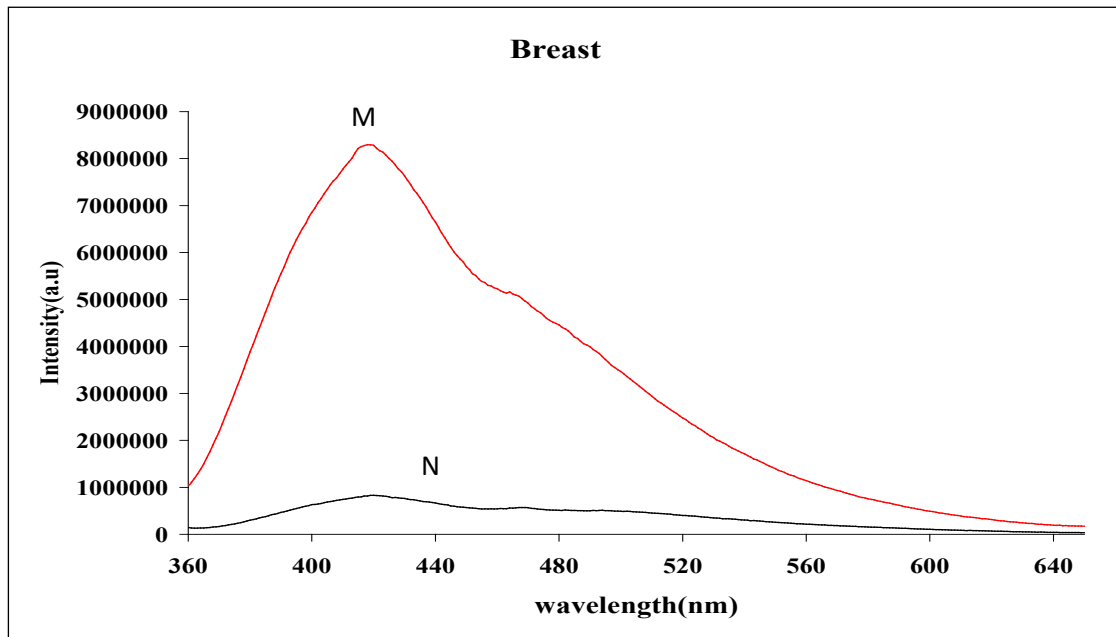


Fig.2.1 (c) L.I.F spectra of cancer and normal tissues of breast cancer.

### Biographies:

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**Mrs. Manasi Vyankatesh Ghamande:** Mrs.Manasi Vyankatesh Ghamande is working as Assistant Professor in Vishwakarma Institute of Technology, Pune, since 1991. Mrs.Manasi Vyankatesh Ghamande has presented and published more than sixty papers in an International Conferences. Out of these papers, for six papers she has received Best Paper Award. Published and filed four patents till date. She was chairperson for three International Conferences and as Session Chair for ten Conferences. She has received Active Young Researcher Award, received Most Promising Mentor Award, received Well Appraised Teacher-2017 Award, received IARDO Best Teacher Award 2018. Received Innovative Researcher and Dedicated Academician (Chemist) Award.

