LASER INDUCED FLUORESCENCE SPECTROSCOPY FOR DIAGNOSIS OF CANCER

Cancer is one of the most dreaded diseases of our time and has a very high incidence. Many cancers are curable if detected early. Development of techniques to detect cancer at an early stage, before the disease becomes difficult to manage, is therefore an urgent current need. One of the techniques being actively pursued for this purpose is laser induced fluorescence (LIF) spectroscopy. This promising technique offers several important advantages like, a very high intrinsic sensitivity, suitability for detecting small superficial tumors not detectable by other techniques such as x-ray diagnostics and the use of non-ionizing radiation for diagnostics which avoids the risks associated with the use of ionizing radiations. Further, the diagnostics can be made near real time and insitu whereby no tissue needs to be removed and the tissue diagnostics by this technique can be easily automated facilitating use by less skilled personnel and mass screening. Extensive studies have therefore been carried out using both human tissue removed at surgery or biopsy and induced tumour in animals to evaluate the potential of this technique for discriminating cancerous and precancerous tissues from normal. The results have been very encouraging and are motivating considerable efforts to realise the in-vivo diagnostic potential of the technique. The quantitative biochemical information available by this technique on the tissue should also prove to be very valuable for understanding the causes of the disease and choosing the right therapy.

Considering the tremendous potential of the LIF technique for effective management of cancer, studies on the use of this technique for discriminating cancerous and precancerous tissue from normal have been started at CAT. We provide here an overview of the technique and also of the work being carried out at CAT.

When light is incident on a tissue it is absorbed, transmitted or scattered by the tissue. Most of the scattered light is scattered elastically i.e. without any change in frequency. The scattered light also has a very weak component which is scattered in-elastically i.e. with a change in frequency via processes like fluorescence, Raman scattering etc. The inelastically scattered light is characteristic of the chemical composition and morphology of the tissue and thus can help monitor normal to cancerous transition in the tissue. Since the inelastically scattered light is a very small fraction of incident light at a given frequency or a given band of frequency, practical applications require use of a high spectral brightness source i.e. lasers.

The fluorescence technique is very sensitive and is widely used for monitoring substances at trace concentrations. As an example the LIF based uranium fluorometer developed at CAT (See CAT Newsletter Dec. 1989) can detect uranium in aqueous solutions to a concentration of 0.1 ppb. Further, since the ultraviolet or near ultraviolet light typically used for exciting native tissue fluorescence has a very small penetration depth in the tissue (a few hundred microns) the probe volume can be made very small. This makes LIF based methods ideal for the detection of small superficial lesions, only a few cell layers thick. Lesions deep inside the tissue are, for the same reason, difficult to diagnose by optical methods. LIF has been used for diagnosing cancer in two ways. The approach initially pursued involves monitoring fluorescence from an appropriate drug administered to the patient. The selective retention of this drug in tumor is exploited for diagnosis as well as therapy of tumors. Although this technique is quite well developed it suffers from the drawback of drug induced photosensitization of the skin necessitating the patient to avoid light for a few weeks. Efforts have therefore been made to reduce the quantity of the administered drug and improve fluorescence detection techniques for diagnostics and also to see if the fluorescence from the native tissue itself (auto fluorescence), which was an unwanted interference in these studies, could be used for diagnostics. Investigations showed significant differences in the autofluorescence spectra of normal, precancerous and cancerous tissues and this has stimulated considerable interest in the use of autofluorescence spectroscopy for tissue diagnostics.

A schematic of the experimental arrangement being used at CAT for autofluorescence spectroscopy of tissues is shown in fig.1. It uses a home-built pulsed N_2 laser the output of which is coupled to an optical fiber (core diameter 400 μm) via a dichroic mirror which reflects N_2 laser radiation (337nm) and transmits longer wavelength fluorescence output. The power of the laser pulse is monitored by a beam splitter-photodiode combination. The fluorescence from the tissue, kept in contact with the fibre, is collected by the same fiber and imaged on the entrance slit of a scanning monochromator. The wavelength dispersed light at the exit slit of the monochromator is detected by a photomultiplier tube detector. A microprocessor based system developed at CAT is being used for on-line acquisition of N_2 laser power and fluorescence spectral data.

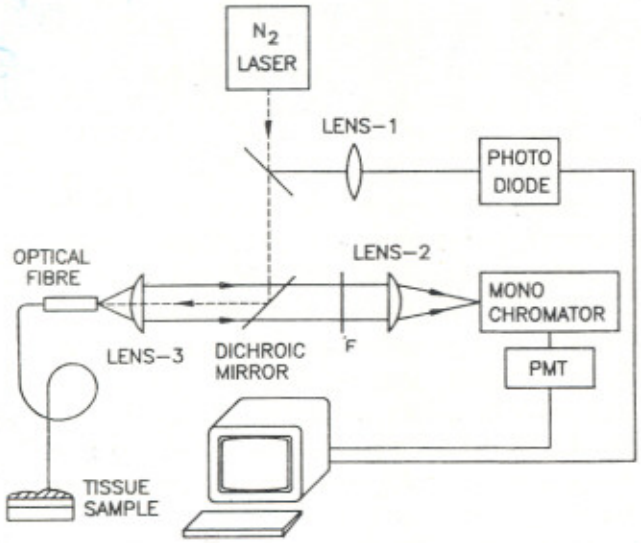

Fig.1: A schematic diagram of the experimental set-up for autofluorescence spectroscopy of tissues.

Autofluorescence spectra were recorded from pathologically characterized tissue samples obtained from Choithram Hospital and Research Centre, Indore, after removal at surgery or biopsy from patients with cancer. The spectroscopic experiments were performed within 4 to 24 hours of tissue resection and the specimen were stored in ice until study.

Typical autofluorescence spectra from uterine tissue are shown in fig.2. All N_2 laser excited fluorescence spectra (from both normal and cancerous tissues) are characterized by two major emission bands, one relatively narrow shorter wavelength band peaking at about 390 nm and the other comparatively broader long wavelength band peaking around 460 nm. Some malignant tissue spectra also showed a pronounced red emission band with peak in the region 630-660 nm. This red band was not observed in any normal tissue sample investigated by us.

The observed fluorescence spectrum is a superposition of the spectra from a number of fluorescing substances present in the tissue which can be excited by N_2 laser. The spectra are also influenced by absorption and scattering of the excitation light as well as the fluorescence emission by the tissue. The blood present in tissue is known to strongly affect the recorded fluorescence spectra by its strong absorption bands in the range 380-600 nm. The fluorophores responsible for the 390 nm band are believed to be structural proteins (collagen and elastin) and for the broad 460 nm band the co-enzymes NADH/NADPH, pyridoxal-5 phosphate, flavins etc with the contribution from NADH being dominant. The red emission band is ascribed to endogenous porphyrins which are selectively retained in cancerous tissues and may be considered a strong indicator of malignancy. From fig.2, the spectral intensity distribution of the fluorescence spectra from normal and cancerous tissues can be seen to have differences even when the red band is missing in the cancerous tissue spectra.

The differences in the spectra of cancerous and normal tissue may arise due to biochemical or morphological changes in the tissue as it undergoes normal to cancerous transition. The present understanding of these is not very satisfactory. It is pertinent to note here that although some researchers have reported a general decrease in blue-green fluorescence from cancerous tissues we observe this decrease from only those cancerous sites which also gave red fluorescence band. Since porphyrins responsible for red fluorescence have absorption bands located around 400nm, our observation suggests that reabsorption of blue fluorescence may be a reason for the observed decrease.

To establish whether the observed differences in the spectra from normal and cancerous tissue are statistically significant and to facilitate diagnosis, the differences in the spectra were quantified by forming a discrimination score on the basis of which the tissue can be characterized as normal, precancerous or cancerous. This score can be based on the value of fluorescence intensities at specific wavelengths and/or the ratio of intensities at specific wavelength pairs or wavelength bands chosen appropriately. Instead of intensity, the ratio of intensities was used since the absolute intensity can get affected by several unavoidable factors like the nature of contact between tissue and fiber, variations in excitation and collection geometry etc.

For discrimination purpose the malignant tissue spectra were grouped into two categories. First category (C-R) comprised of cancerous tissue spectra in which red emission band was present and the second category (C-NR) comprised of cancerous tissue spectra in which the red emission band was not present. For use as input parameters for the discrimination function we selected six intensity ratios. Two of the parameters used were the ratio of intensities at peaks of the 460 nm (B) and 390 nm (V) bands, represented by R_P (B/V) and the ratio of integrated intensities over the wavelength bands 420 nm - 580 nm (B) and 360 nm to 420 nm (V), represented by $R_{B/V}$. These were

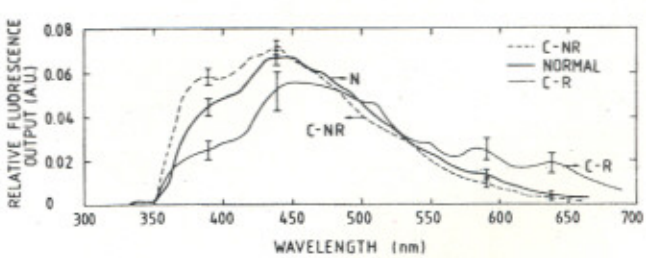

Fig.2: Spectra from normal (N), and cancerous site with red flouresence (C-R) and with no red fluorescence (C-NR) . Normal and C-NR spectrum are average of spectra from 45 sites, and C-R spectrum is the average of spectra from 25 sites.

Table-1: Predictive values for different diagnostic parameters for uterine tissues.

Discrimination goal	Diagnostic parameter	Sensitivity (%)	Specificity (%)	PVP (%)	PVN (%)
N from C-NR	$R_{B/V}$	86.7	87.8	87.6	86.8
N from C-R	$R_{B/V}$	87.8	97.8	94.6	94.6
N from C-R	$R_{R/V}$	100	100	100	100
N from C-NR	MVLR score (4 variables)	88.9	87.8	87.9	88.8
N from C-R	- do -	100	100	100	100

Table-2: Discrimination results for colonic and breast tissues.

Tissue type	Diagnostic parameter	Sensitivity (%)	Specificity (%)	PVP (%)	PVN (%)
Colon	MVLR score (4 variables)	94	92.3	92.4	93.8
Breast	MVLR score (4 variables)	92	81.2	86.4	88.6

chosen because the relative collagen and NADH fluorescence components are seen to be different for cancerous and non-cancerous tissue from uterus, colon etc. For the same reason, for tissues showing red fluorescence band, the ratio of intensities in the bands 580 nm to 680 nm (R) and 360 nm to 420 nm (V) represented by $R_{R/V}$ was also used as a parameter. Further, with a view to take care of the likely interference from blood absorption, the ratio of intensities at three wavelength pairs of equal blood absorption were used as input parameters. A stepwise multivariate linear regression (MVLR) analysis was used for selecting the significant of these input parameters and their regression co-efficients which maximized the discrimination between the two tissue types. The cut-off point for discrimination was chosen to be the average of the mean scores for normal and C-NR or C-R as the case may be. These correctly identified 80 of the 90 malignant sites and 79 out of the 90 normal sites investigated. The mean value of the ratio variables for each tissue type was also calculated and discrimination power of these in discriminating normal and malignant tissue spectra was tested by applying Student's t-test. The predictive value for these parameters and of MVLR score using the average of mean values for cancerous and non-cancerous tissues as cut-off value is tabulated in Table 1. The table lists the sensitivity, specificity, predictive value positive (PVP) and predictive value negative (PVN) towards uterine cancerous tissue with red fluorescence band (C-R) and without red fluorescence band (C-NR). Sensitivity towards cancerous tissue is defined as the conditional probability of predicting cancerous tissue as cancerous. Specificity towards cancerous tissue is defined as the conditional probability of predicting non-cancerous tissue as non-cancerous. The predictive value positive term is the conditional probability that predicted cancerous tissue is truly cancerous. The predictive value negative refers to conditional probability that predicted non-cancerous tissue is truly non-cancerous. A scatter plot for the MVLR score for cancerous and non-cancerous tissues is shown in fig 3. A paired comparison of the parameter values for cancerous and normal tissue from the same patient led to marginally improved predictive values with both sensitivity and specifity values being 87.8%.

The spectra from other tissues show similar features but with some differences which nevertheless are important for diagnosis. Thus for colonic tissue the same discrimination indices with a different set of cut-off values could be used for diagnostics with similar sensitivity and specificity as for uterine tissue. However, for breast tissue these indices did not prove to be that useful and new indices were required. These results suggest that the spectroscopic diagnostics algorithm may not be general, but organ specific. The discrimination results obtained for colonic and breast tissues are shown in table 2.

The transportability of in-vitro discrimination functions for in-vivo studies is an important issue needing con-

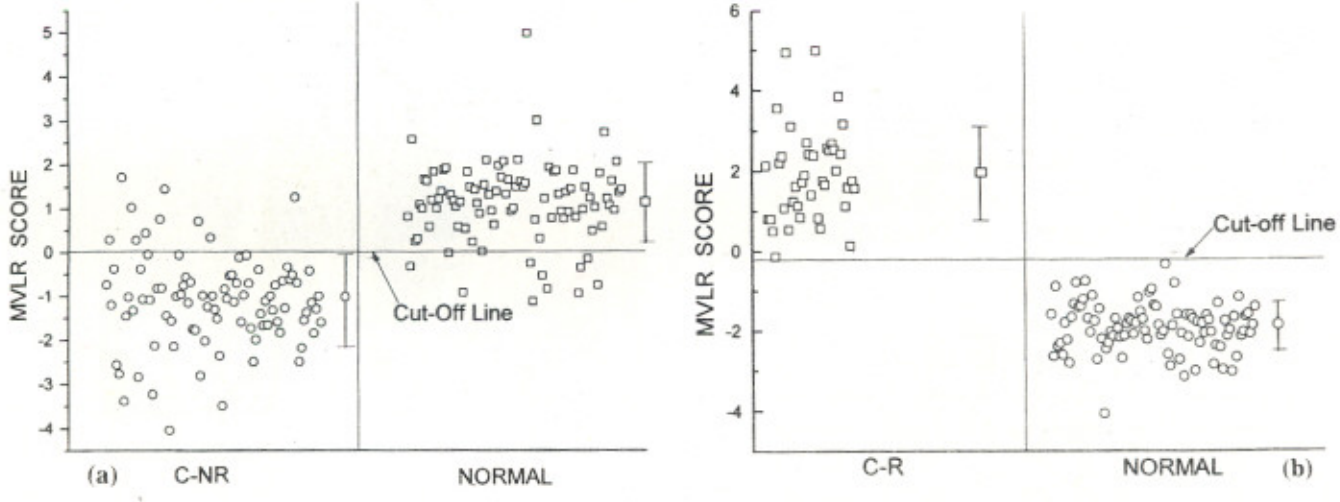

Fig.3 : Scatter plot of the 4-variable MVLR score from normal and cancerous sites (a) without red fluorescence and (b) with red fluorescence. The bar shows mean value ± standard deviation

firmation. Recent studies done elsewhere have shown that applying regression co-efficients optimized for in-vitro measurements gives poorer results compared to MVLR analysis of in-vivo spectra, thus emphasising the need for in-vivo studies. These in-vivo studies have also lived upto the expectation that the LIF technique can diagnose cancer and more significantly can distinguish between precancerous lesions from non-cancerous abnormal tissues with accuracies greater than endoscopist's visual assessment and nearly comparable to the histological assessment.

To sum up laser induced auto fluorescence spectroscopy holds considerable promise for early diagnosis of cancer. In the in-vitro studies carried out at CAT, on human tissues resected at surgery or biopsy from various organs, the technique has been verified to provide good discrimination between cancerous and non-cancerous tissues. With these encouraging results more detailed in vitro studies as well as in-vivo studies required for clinical exploitation are now being planned.

P K Gupta and S K Majumder

CONFERENCES / WORKSHOPS

INCOVAST - 95

An International Conference on Vacuum Science and Technology and SRS Vacuum Systems (INCOVAST-95) was organised at CAT during January 30 to February 2, 1995. The conference was organised jointly by CAT and Indian Vacuum Society (IVS). It was attended by 200 participants of which 30 scientists were from countries like USA, Japan, France, UK, Switzerland, Russia and Korea.

Dr Alain Poncet, CERN, Geneva delivered the key note address and gave an account of the latest developments in the field of vacuum systems for Synchrotron Radiation Sources. Dr R J Reid, Daresbury Laboratory, UK was present as the official representative of International Union for Vacuum, Science and Techniques and applications. Topics such as light source vacuum systems, vacuum pumps and components, electron beam equipment, thin films, and novel vacuum facilities, were covered in 23 invited and 120 contributed papers.

The conference was inaugurated by Shri A N Prasad, Director, BARC. Dr D D Bhawalkar, Director, CAT presided over the inaugural function. Shri J K N Sharma, President, IVS gave a brief account of the activities of IVS. Shri S S Ramamurthi, CAT was the Chairman of the organising committee. Shri A K Sinha, BARC was the convenor while Shri A S Raja Rao, CAT was the co-convenor.

Modern Trends in Computer Technology (MTCT-95)

A two day National Seminar on Modern Trends in Computer Technology (MTCT-95) was conducted jointly by CAT and the Computer Society of India (Indore Chapter) during February 23-24, 1995. The seminar was inaugurated by Dr S S Kapoor, Director, Physics and E & I Groups, BARC, who spoke about the development of parallel machines in India. The inaugural function was presided over by Dr D D Bhawalkar, Director, CAT, who spoke about the importance of computers in office automation.

The seminar was attended by 55 registered participants. Twenty three papers were presented in the seminar along with seven invited talks. These covered a wide range of modern trends like multimedia technology, networks and data communications, parallel processing, automation and software engineering, text to speech systems, hand writing recognition, image processing, on-line mirroring of databases etc. The seminar convenor was Shri A K Gupta and the seminar coordinator was Shri Anil Rawat, both from CAT.

Dr S S Kapoor and Dr D D Bhawalkar inauguarating the Seminar.