ABSTRACT

Cancer is a dreaded disease and the complete treatment of cancer is possible, only when it is detected at its very early stage. Although the conventional biopsy technique is still being considered as a golden standard method for the disease diagnosis, it is a tedious, time-consuming process and subjective. Diagnosis with nuclear radiation and X-rays may cause adverse side effects to the patients as well as medical personnel. In view of the possible harmful side effects of X-rays and nuclear radiation, medical community has been searching for an alternative and/or complementary technique to diagnose diseases without altering human body’s biochemistry.

Under these circumstances, the use of light for probing and imaging the biomedical media has attracted the attention of the scientists and clinicians, as it is safe, non-invasive, real time and inexpensive. This is because, the various properties of light together with the ways by which it interacts with biological tissues may provide multiple windows to peer inside body organs. Among the various light tissue interactions, the phenomena of fluorescence is being considered as a novel technique for biomedical applications as most of the tissue chromophores possess the fluorescence properties. Generally, the tissue characterization using fluorescence spectroscopy can be done by using either exogenous or endogenous fluorophores. The most frequently used exogenous porphyrin derivatives such as dihaematoporphyrin ether (DHE) and porfimer sodium, give rise to a characteristic red fluorescence when excited at the UV or near UV region. As the accumulation of these photosensitizing
substances occurs predominantly in pre-malignant and malignant areas, diseased tissues are characterized by higher fluorescence intensity in the red wavelength region compared with those of non-diseased tissues. The DHE uptake and fluorescence can be used in a prognostic manner to diagnose and determine the stage of transformation of individual lesions.

Recently, photophysical properties of native fluorophores and their structures have been considered as useful parameters to study the various pathological conditions and biochemical alterations at cellular and tissue levels and to distinguish the normal form from abnormal tissues. Differences in the native fluorescence have been ascribed to the presence of various molecules, such as tryptophan (Trp), tyrosine (Tyr), phenylalanine (Phe), nicotinamide adenine dinucleotide of reduced form (NADH), flavin-adenine dinucleotide (FAD), collagen, elastin and endogenous porphyrin present in cells and tissues. Among the various fluorophores, the fluorescence of collagen, elastin and more generally proteins are due to the presence of aromatic amino acids that are related to the structural arrangement of cells and tissues. The other fluorophores NADH, FAD and endogenous porphyrin are related to metabolic process are in connection with the onset of pathological conditions Many encouraging results have been reported in the diagnosis of various cancers.

Breast, cervical and oral cancers are the most common cause of death among women and men, in India. Although oral lesions are directly visible and easily detectable when compared with other organs, they continue to pose alarming health concern; the population at risk due to this cancer is ranked second in Asian countries and ranked number one in India because of the addiction of mankind to tobacco
products and alcohol. Patients are often left with severe cosmetic and functional difficulties resulting from this disease and its treatment. Similarly, cervical and breast cancers are considered to be the most common cause of death among women. These cancers go undetected in developing countries because of the high cost and a considerable lack of trained personnel and resources to screen and diagnose the disease. Although the causes for these cancers are unknown, several risk factors have been linked to the disease. Researchers are still trying to understand the actual role that these various factors may play in the development of various cancers. At this point, it is worth to note that improving the existing screening and detecting techniques of tissue abnormalities can enhance the number of cancer patients treated at the early stages.

In this context, the tissue optical spectroscopy has emerged as a new area in the field of medical diagnostics that has the potential to provide automated, cost effective screening of histo-chemical features of diseases. However, the field of optical biopsy is still under infant stage and requires a large-scale in-vitro and in-vivo studies in order to gain clinical acceptance. This is because, the exact reasons for the altered spectral signatures between normal and diseased tissues are not yet completely understood due to the complexity of tissue structure and its physicochemical nature.

Based on these, the present thesis is aimed with the following objectives:

i) Study the native fluorescence emission, excitation and synchronous luminescence spectral signatures of normal, pre-malignant and malignant breast, cervical and oral tissues under in vitro conditions, in the wavelength range 280 – 700 nm.
ii) *In situ* characterization of normal mucosa, mucosa of the oral cavity subjected to chronic smoking, premalignant lesions and malignant lesion of the oral cavity, by steady state fluorescence emission, excitation and synchronous luminescence spectroscopy.

iii) *In situ* characterization of normal and different tissue transformation conditions of the skin carcinogenesis induced in Swiss albino mice subjected to the topical application of DMBA.

iv) Analyzing the observed spectral characteristics by multivariate statistical analysis to develop an algorithm for the better discrimination of the normal from different pathological conditions of tissues.

In this regard, the present thesis is organized into seven chapters:

As the present work deals with the characterization of tissues by native fluorescence spectroscopic techniques, the first chapter of the thesis discusses the fundamentals of fluorescence spectroscopy of biomolecules at cellular and tissue levels, basis of cancer biology and an overview on the development of native fluorescence spectroscopy in diagnostic oncology.

The second chapter explains the different experimental setups, instruments and techniques adopted in the characterization of tissues. In particular, the different fluorescence spectroscopic techniques adopted are described. The application of different statistical methods, in the discrimination of cancer from normal tissues, are also explained in this chapter.
In the third and fourth chapter, native fluorescence characterization of breast and cervical tissues by fluorescence emission and excitation spectra in the UV-Visible region is discussed. Apart from the fluorescence emission and excitation spectra, the diagnostic potentiality of the synchronous luminescence spectroscopy is also explored. Multivariate statistical analysis was performed to identify the suitable wavelength of excitation and emission combinations for the better discrimination of the normal from pre-malignant conditions. Further, the results of the synchronous luminescence spectroscopy are compared with the fluorescence emission and excitation spectroscopy to identify the better technique.

As the incidence rate of oral cancer has rise to an alarming level in India, the applicability of the native fluorescence spectroscopy in the discrimination of the normal from pre-malignant, high-risk smokers and malignant oral cavity was extensively studied and are described in Chapter 5. In situ fluorescence emission, excitation and synchronous luminescence spectroscopy were measured using a fiber optic set up and the spectral characteristics were compared with each other. Multivariate statistical analysis was also performed for the measured data to identify the optimal wavelength of excitation and emission in the demarcation of the normal from pre-malignant and high-risk smokers under in vivo conditions.

Though very good differences in the spectral signature were observed from the in situ fluorescence emission, excitation and synchronous luminescence measurement of the oral cavity, one could not identify the spectral changes that occur during the process of tissue transformation from normal to malignant. In this context, a pilot study was carried out on the DMBA induced skin carcinogenesis in Swiss
albino mice. The fluorescence emission, excitation and synchronous luminescence spectra were recorded at different tissue transformation conditions. The spectral data were used in the statistical analysis to identify the optimal wavelength that gives better discrimination between normal and early stage cancers. The details of the observed results are discussed in Chapter 6.

Finally, a brief summary, the conclusions drawn from the entire work and the scope for future work are presented in the seventh chapter.