CHAPTER 7

SUMMARY AND CONCLUSION

7.1 INTRODUCTION

Cancer is a dreaded disease and is the second largest non-communicable disease and it has a sizable contribution in the total number of deaths. Early diagnosis and treatment may reduce patient morbidity and mortality, and therefore is of great clinical importance. Among the available techniques in screening the cancer, biophysical imaging techniques, biochemical techniques and biopsy are considered as the three major approaches in the diagnosis of cancer. Biochemical analysis is mainly based on the qualitative and quantitative determination of individual components considered to be the markers of an assumed disorder. To isolate an individual compound among many others in the complex mixture requires additional slow and costly processes. Though biopsy is considered as a reliable, direct method and gold standard in the clinical diagnosis of cancer, it is invasive, painful and also subjective. All these bring complication and artifacts (Dubayova et al 2003). In this context, it is worth to mention that the broad use of optical based diagnostic techniques has led to new approaches in medical diagnosis and new ways to treat diseases.

In particular, native fluorescence spectroscopy of tissues and body bio fluids with real time evaluation is one of the most sensitive methods for monitoring minor changes in the structure and microenvironment of the native fluorophores and hence in the discrimination of tumors. Differences in the native
fluorescence has been ascribed to various molecules such as tryptophan (Trp), tyrosine (Tyr), phenylalanine (Phe), indoxyl sulphate, pteridines and its derivatives reduced form of nicotinamide adenine dinucleotide (NADH), collagen, elastin, riboflavin and its derivatives and endogeneous porphyrins (Madhuri et al 2003).

Many applications of native fluorescence spectroscopy of biomolecules are reported on the characterization of cellular metabolic pathways and the discrimination of malignant from normal conditions of tissues. Pioneering work has been reported by R. R. Alfano et al (1984 & 1987) in discriminating the cancerous tissue from that of normal. Many groups have demonstrated the use of fluorescence spectroscopy to successfully detect various types and stages of cancer (Betz et al 1999, Saraswathy et al 2009, Kamath et al 2009, Chowdary et al 2009). However only limited studies have been reported on the applications of native fluorescence spectroscopy of biofluids in diagnostic oncology (Rabinowitz 1949, Leiner et al 1986, Madhuri et al 2003, Lualdi et al 2007).

In this regard, urine is considered as one of the diagnostically important biological fluid as it has many metabolites where number of them are natural fluorophores (Kusnir et al 2005). Urine analysis in clinical, biochemical analysis has a long history and is one of the oldest laboratory tests in medicine. The standard urine tests remain almost unchanged. Due to the presence of the fluorophores are in small amounts in urine their quantitative measurements is difficult. Therefore, developing a simple, rapid, and sensitive assay for determining these fluorophores in urine is necessary. The measurement of native fluorescence of urine, in principle, able to provide an indication of a number of health conditions, including urinary tract infection, proteinuria, renal disorder, nephritic syndrome, hepatopathy, and neuronal ceroid lipofuscinosis (Perinchery et al 2010). In the case of cancer diagnosis the literature on urine
fluorescence is surprisingly scarce in spite of its easy availability. As fluorescence spectroscopy has many advantages and complementary techniques, the present study is aimed to study the potential of steady state and time resolved native fluorescence of urine in discriminating the malignant subjects from that of normal subjects.

For this study, first voided urine samples of normal subjects and patients with oral and cervical cancer were analyzed by native fluorescence emission, excitation and synchronous luminescence spectroscopy and also time resolved fluorescence spectroscopy of different fluorophores present in the urine samples. By using linear discriminant analysis and principal components based linear discriminant analysis approaches, the spectral data were analysed under the following categories.

i) Normal vs Cancer subjects  
ii) Normal vs Early cancer subjects  
iii) Normal vs Advanced cancer subjects  
iv) Normal vs Oral cancer subjects  
v) Normal vs Cervical cancer subjects  

Both the statistical approaches were employed and the better results were discussed. The prominent features of the thesis work are listed below.

7.2 NATIVE FLUORESCENCE EMISSION SPECTROSCOPIC CHARACTERIZATION OF URINE OF NORMAL SUBJECTS AND CANCER PATIENTS

From the Excitation Emission matrices of human urine of eighty normal subjects and ninety cancer patients
It is observed that, there is a considerable difference in the EEMs topograms between the two groups. The 3D EEMs of normal subject shows that there is a maximum emission around 440 ± 10 nm at excitation 360 ± 10 nm. Whereas, cancer subjects has three peaks at 439 ± 10 nm, 513 ± 10 nm and 530 ± 10 nm at excitations 360 ± 10 nm, 383 ± 10 nm and 449 ± 10 nm respectively. The emission around 440 ± 10 nm may be ascribed to the presence of NADH, pteridine and its derivatives.

The additional peaks observed at longer wavelengths viz., 513 ± 10 nm and 530 ± 10 nm may be attributed to riboflavin and its metabolites. Similar observations were also made from the averaged 2D contour plots of normal and cancerous subjects.

From the difference graph, the positive deviations (characteristic maximum around 405/473 nm and 294/484 nm) may due to NADH, pteridine and its derivatives and a region of negative deviations (characteristic minimum around 383/530 nm) may be due to riboflavin and its metabolites.

Based on the observations made from EEMs, measurements of fluorescence emission spectra at excitation wavelengths 280, 350, 405 and 450 were carried out.

7.2.1 At 280 nm Excitation

The fluorescence emission intensity of cancer subjects is higher than that of normal subjects. The emission spectrum of normal subjects shows a prominent maximum at 396 nm and cancer patients at 422 nm with a red shift of 26 nm. In addition, cancer
patients exhibited a hump at 515 nm and the same was absent in the normal case.

- The Fluorescence emission intensity of normal subjects, early and advanced stage cancer patients are in the order of early cancer > advanced cancer > normal subjects. The normal subjects show a prominent maximum at 396 nm and both early and advanced cancer patients at 422 nm with a red shift of 26 nm from that of normal subjects. In addition to the emission maxima, small hump was observed at 515 nm for the early and advanced stage of cancer subjects and the same was absent in the normal subjects. Additional humps were observed at 450 nm, 480 nm and 490 nm from the emission spectrum of advanced stage cancer subjects which are found to be absent in both normal subjects and are not clear in the case of early stage cancer subjects.

- The fluorescence emission intensity for oral cancer patients is higher than that of normal subjects. Normal subjects show a prominent maximum at 396 nm and oral cancer patients at 414 nm with a red shift of 18 nm. In addition, oral cancer subjects shows humps around 515 nm and it is absent in the case of normal subjects.

- The fluorescence emission intensity for cervical cancer patients is three fold higher than that of normal subjects. Normal subjects show a prominent maximum at 398 nm and cervical cancer patients at 431 nm with a red shift of 33 nm. In addition, cervical cancer subjects shows humps around 515 nm and it is absent in the case of normal subjects.
The emission around 390 nm may be attributed to Indoxyl sulphate and the humps around 515 nm, 450 nm, 480 nm and 490 nm which may be due to the influence of flavins, NADH/Pteridines and bound NAD(P)H. The red shift observed and variation in the fluorescence intensity may be attributed to the difference in micro environment.

In all the cases, the difference spectrum shows positive difference below 405 nm which may be due to the increased contribution of indoxyl sulphate in the urine of normal subjects than that of cancer patients. At wavelengths higher than 405 nm, the contribution of NADH / Pteridine and flavins is relatively higher in the case of urine of cancer patients than normal subjects.

The fluorescence emission spectral data at 280 nm excitation was subjected to statistical analysis as discussed earlier. From the Figure 7.1, it is observed that the cancer patients are discriminated with a sensitivity of 60% and normal subjects with a specificity of 97.5%. Also, cervical cancer patients are discriminated with a sensitivity of 76.8% from that of normal subjects with a specificity of 95.5%. No statistical difference was observed across other groups.
Figure 7.1  Shows the percentage of sensitivity and specificity of the statistical analysis (a) original classification (b) leave one out cross validated classification, for the fluorescence emission spectral data at 280 nm excitation

7.2.2  At 350 nm Excitation

- The emission spectrum of normal subject's shows slightly higher fluorescence emission intensity around 430 nm than cancer patients and around 530 nm, the cancer subjects shows higher
fluorescence emission intensity than that of normal subjects. Emission maximum for both normal subjects and cancer patients occurred at 432 nm. In addition to the prominent maximum, cancer subjects exhibits a hump around 525 nm and the same is absent in the case of normal subjects.

- The fluorescence emission intensity around 430 nm varies in the order of early stage cancer > normal subjects > advanced stage cancer. The variation of fluorescence intensity at 515 nm is in the order of advanced stage > early stage > normal subjects. From the normalised spectrum, it is observed that the prominent emission maximum occurred at 432 nm for normal and early stage cancer subjects and at 430 nm for advance stage cancer patients.

- Around 440 nm the normal subjects exhibits higher fluorescence intensity than that of oral cancer patients. However, around 520 nm, cancer subjects exhibits higher fluorescence emission intensity than that of normal subjects. In addition to the prominent maximum at 440 nm, the spectrum of oral cancer patients exhibits a shoulder around 525 nm and the same is absent in the case of normal subjects.

- Normal subjects exhibit a prominent maximum around 429 nm and for cancer subjects it occurred at 432 nm. No significant difference was observed between normal subjects and cervical cancer patients around 430 nm. In addition, cancer subjects exhibits a shoulder around 520 nm and the same is absent in the case of normal subjects. Around 520 nm, cervical cancer subject exhibits higher fluorescence emission intensity than that of normal subjects.
The observed emission around 432 nm may be attributed to pteridine and its derivatives and/or NADH and hump around 525 nm observed for cancer patients may be attributed to flavins and its metabolites.

From the difference spectrum of all the cases, it is observed that the urine of cancer patients exhibits a positive difference around 390 nm and a negative difference around 525 nm. This shows that, at 390 nm the fluorescence intensity of indoxyl sulphate is slightly more for normal subjects than that of cancer patients. However, around 520 nm, the contribution of flavins is more for cancer patients than that of normal subjects.

The fluorescence emission spectral data at 350 nm excitation was subjected to statistical analysis and Figure 7.2 shows the percentage of statistical significance. From the figure, it is observed that the discrimination of cervical cancer subjects from normal subjects yields a good sensitivity when compared to other groups. In all the cases, the specificity to detect normal subjects is good and the sensitivity in the discrimination of cancer patients is low.
Figure 7.2 Shows the percentage of sensitivity and specificity of the statistical analysis (a) original classification (b) leave one out cross validated classification, for the fluorescence emission spectral data at 350 nm excitation.

7.2.3 At 405 nm Excitation

- Normal subjects show a primary peak around 475 nm, a hump around 617 nm 580 nm. Whereas, cancer patients show a major
peak around 520 nm and hump around 617 nm. Also, a small hump around 580 nm was observed in the emission spectrum of normal subjects and the same is absent in the spectrum of cancer patients. The overall fluorescence intensity around 520 nm is higher for cancer patients than normal subjects and little difference was observed around 480 nm.

- The Fluorescence emission intensity of normal subjects, early stage and advanced stage cancer patients varies in the order of advanced stage cancer > early stage cancer > normal subjects. Early and advanced stage cancer patients show a prominent maximum around 520 with hump around 617 nm. Whereas, normal subjects show a prominent maximum around 480 nm with humps around 580 nm and 617 nm.

- Normal subjects show a primary peak around 475 nm, a hump around 617 nm 580 nm. Whereas, oral cancer patients show a major peak around 520 nm and hump around 617 nm. Also, a small hump around 580 nm was observed in the emission spectrum of normal subjects and the same is absent in the spectrum of oral cancer patients. The overall fluorescence intensity around is higher for oral cancer patients than normal subjects.

- Similar observation was made from the emission spectrum of normal subjects and cervical cancer patients. However, the emission intensity of cervical cancer is much higher that of normal subjects.

- The observed peak around 475 nm may be attributed to NAD(P)H, pteridines, the humps observed around 580 nm and 617 nm may be due porphyrins and the peak around 520 nm may
be due to flavin. Also, it is interesting to note that the hump observed at 580 nm in the case of normal subjects is absent in cancer patients.

- Difference spectrum shows positive difference in the wavelength region below 507 nm and negative difference in the region above 507 nm. This indicates that the urine of normal subjects exhibits more NAD(P)H and pteridines fluorescence than cancer patients. However, the negative difference indicates increased contribution of flavins for cancer subjects than that of normal subjects.

- The fluorescence emission spectral data at 405 nm excitation was subjected to statistical analysis and figure 7.3 shows the percentage of statistical significance. From the figure, it is observed that the discrimination of cervical cancer subjects from normal subjects yields a sensitivity of 92.8% and specificity of 95.%% . In all the cases, the specificity to detect normal subjects is above 90% and the sensitivity in the discrimination of cancer patients is in the order of cervical cancer > overall cancer > early stage cancer > oral cancer > advanced stage cancer.
Figure 7.3 Shows the percentage of sensitivity and specificity of the statistical analysis (a) original classification (b) leave one out cross validated classification, for the fluorescence emission spectral data at 405 nm excitation

7.2.4 At 450 nm Excitation

- Cancer patients exhibit a many fold increase in the fluorescence emission intensity than that of normal subjects. Further, it is observed that the emission maximum occurred at 524 nm and 526 nm for normal and cancer subjects respectively.
- The emission maximum occurred at 524 nm for normal subjects and at 526 nm for early and advanced cancer subjects and the fluorescence intensity varies in the order of normal subjects < early cancer < advanced cancer.

- Normal subjects and patients of oral and cervical cancer show broad fluorescence emission spectrum in the wavelength region 500 nm to 600 nm with emission maximum at 524 nm for normal subjects and at 526 nm for oral and cervical cancer patients. The fluorescence emission intensity of oral and cervical cancer is greater than normal subjects.

- The observed maximum around 525 nm may be attributed to flavins and from the difference spectrum of all the cases, it is observed that the difference around 480 nm may be attributed to NAD(P)H which is maximum for normal subjects than cancer patients and the difference due to flavin and porphyrin is maximum for cancer patients than normal subjects in the wavelength region above 525 nm.

The fluorescence emission spectral data at 450 nm excitation was subjected to statistical analysis and figure 7.4 shows the percentage of statistical significance. Here, again the discrimination of cervical cancer subjects from normal subjects yields a good sensitivity when compared to other groups. In all the cases, the specificity to detect normal subjects is 100% and the sensitivity in the discrimination of cancer patients is low except for the discrimination of cervical cancer.
Figure 7.4 Shows the percentage of sensitivity and specificity of the statistical analysis (a) original classification (b) leave one out cross validated classification, for the fluorescence emission spectral data at 450 nm excitation.
7.3 NATIVE FLUORESCENCE EXCITATION SPECTROSCOPIC CHARACTERIZATION OF URINE OF NORMAL SUBJECTS AND CANCER PATIENTS

7.3.1 For Emission at 390 nm

- The fluorescence excitation spectrum of urine samples of cancer subjects exhibit higher fluorescence intensity than that of normal subjects. The normal subjects and cancer patients exhibit a broad spectrum with maximum peak intensity at 351 nm and 355 nm respectively.

- The fluorescence excitation spectrum of early cancer patients is higher than that of advanced cancer patients and normal subjects in the wavelength region above 340 nm and below 340 nm it is in the order of early cancer > normal > advanced cancer. The maximum peak intensity for early cancer patients occurs at 353 nm, 356 nm for advanced cancer patients and for normal subjects at 351 nm. The early and advanced cancer sample shows a 2 nm and 5 nm red shift from that of normal subjects.

- The fluorescence excitation spectrum of oral cancer patients exhibits higher fluorescence intensity than that of normal subjects. The normal subjects shows a maximum peak intensity at 351 nm and oral cancer patients at 355 nm with 4 nm red shift from that of normal subjects.

- The fluorescence excitation spectrum of normal and cervical cancer subjects shows a broad spectrum and the fluorescence intensity of cervical cancer patients is higher than that of normal subjects. The normal subjects show maximum peak intensity at
353 nm and cervical cancer patients at 355 nm with a 2 nm red shift from that of normal subjects.

- The observed peak around 350 nm indicates the contribution of NADH, pteridines and its derivatives. However, the fluorescence emission of NADH, pteridines and its derivatives lays around 450 nm. The variation in the fluorescence intensity around 350 nm may be due to the overwhelming effect of pteridine and/or NADH over indoxyl sulphate which is having absorption maximum around 300 ± 10 nm. The broad fluorescence excitation spectrum indicates the contribution of absorption of many fluorophores.

- In all the cases of classification, the difference spectrum reveals that the fluorescence intensity between 310 nm and 350 nm is more for normal subjects than that of cancer patients. In the wavelength region below 310 nm and above 350 nm, the intensity is maximum for cancer patients than that of normal subjects. However the difference spectrum between normal and cervical patients shows a negative difference throughout the wavelength region except around 350 nm.

- No statistical difference was observed for the fluorescence spectral data at 390 nm emission.
7.3.2 For Emission at 450 nm

- The overall fluorescence intensity for cancer and normal subjects are very close without much variation. The excitation peak of urine for both normal and cancer subjects are centered around 366 nm.

- The fluorescence intensity is of the order of early cancer > normal > advanced cancer and there exists a similar spectral signature with a predominant peak around 366 nm.

- The overall fluorescence intensity for normal subjects is higher than that of oral cancer patients. The excitation peak intensity of urine for both normal and cancer is centered on 366 nm and no markable difference was observed.

- Normal subjects and cervical cancer patients shows a broad spectrum with maximum intensity at 364 nm and 371 nm respectively with a red shift 7 nm from that of normal subjects. The fluorescence intensity of cervical cancer patients is higher than that of normal subjects.

- The peak around 360 nm may be due to the absorption of pteridines and its derivatives and NADH/NADPH. In all the cases of classification, the difference spectrum shows a positive difference in the wavelength region 320 nm to 365 nm and negative at wavelengths 300 nm to 320 nm and above 365 nm. The difference may be attributed to NADH, pteridines and its derivatives. However, the magnitude of difference is very small.

- From the Figure 7.5, it is observed that the cancer patients are discriminated with a sensitivity of 66.2% and normal subjects
with a specificity of 51.5%. Cervical cancer patients are discriminated with a sensitivity of 80.9% from that of normal subjects with a specificity of 90.6%. No statistical difference was observed across other groups.

Figure 7.5 Shows the percentage of sensitivity and specificity of the statistical analysis (a) original classification (b) leave one out cross validated classification, for the fluorescence excitation spectral data at 450 nm emission
7.3.3 For Emission at 520 nm

- The fluorescence excitation spectrum of urine samples of cancer subjects exhibit higher fluorescence intensity than that of normal subjects. The normal subjects show an excitation maximum at 396 nm and cancer patients at 450 nm. Apart from the maxima, both normal subjects and cancer patients exhibits secondary peak around 450 nm and humps around 400 nm, 420 nm and 440 nm.

- The normal subjects show an excitation maximum occurred at 396 nm, whereas for early and advanced cancer subjects, the excitation maximum occurred at 450 nm. Apart from the maxima, normal subjects, early and advanced cancer patients exhibits secondary peak around 450 nm and humps around 400 nm, 420 nm and 440 nm. Fluorescence intensity varies in the order of advanced cancer > early cancer > Normal subjects.

- The fluorescence excitation spectrum of oral cancer subjects exhibits higher fluorescence intensity than that of normal subjects. The normal subjects show an excitation maximum at 396 nm, whereas for the oral cancer subjects the excitation maximum occurred at 450 nm. Apart from the prominent maxima, normal subjects and oral cancer patients exhibits secondary peak around 450 nm and humps around 400 nm, 420 nm and 440 nm.

- The fluorescence excitation spectra of cervical cancer subjects exhibit higher fluorescence intensity than that of normal subjects. The normal subjects show an excitation maximum occurred at 396 nm and cervical cancer subjects at 450 nm. Apart from the prominent maxima, urine samples of normal subjects and
cervical cancer patients exhibits secondary peak around 450 nm and humps around 400 nm, 420 nm and 440 nm.

- The broad region around 350 nm may be due to the contribution of pteridines and NADH/NADPH and other secondary peaks and humps observed may be due to the absorption of porphyrins, flavins and its derivatives.

- In all the cases of classification, the difference spectrum shows a positive difference below 420 nm and negative in the wavelength region above 420 nm. The positive difference in the region 310 nm to 420 nm may be due to the increased contribution of pteridines and its derivatives and NADH/NADPH in the urine of normal subjects than that of cancer patients. At wavelengths higher than 420 nm, the contribution of flavins is relatively higher in the case of urine of cancer patients than normal subjects.

- The fluorescence excitation spectral data at 520 nm emission was subjected to statistical analysis. From the Figure 7.6, it is observed that the discrimination of cervical cancer from normal subjects yields 90.9% sensitivity followed by overall cancer with a sensitivity of 80%. However, except for the discrimination across normal subjects and early stage cancer patients, the specificity to detect normal subjects is minimum.
Figure 7.6  Shows the percentage of sensitivity and specificity of the statistical analysis (a) original classification (b) leave one out cross validated classification, for the fluorescence excitation spectral data at 520 nm emission.
7.4 SYNCHRONOUS LUMINESCENCE SPECTROSCOPIC CHARACTERIZATION OF URINE OF NORMAL SUBJECTS AND CANCER PATIENTS

- The Synchronous Luminescence spectral intensity of cancer subjects is higher than that of normal subjects and vice versa around 325 nm. The normal subjects show a prominent maximum at 369 nm and cancer patients at 483 nm. Normal subjects exhibit secondary peaks at 420 nm, 440 nm, 450 nm, 470 nm, 483 nm, 492 nm and cancer patients at 369 nm. The other peaks around 450 nm are not clear and this may be due to the overwhelming effect of the peak around 480 nm. Also, normal subjects exhibits a shoulder around 515 nm and the same was absent in the case of cancer patients.

- The normal subjects shows a prominent maximum at 369 nm and secondary peaks at 420 nm, 440 nm, 450 nm, 470 nm, 483 nm, 492 nm and a shoulder around 515 nm whereas early and advanced cancer patients shows maximum at 483 nm and secondary peak at 369 nm. SL intensity at 369 nm varies in the order of normal subjects > early cancer > advanced cancer and at 480 nm, it varies in the order of early cancer > advanced cancer > normal subjects.

- The normal subjects, oral cancer and cervical cancer shows the same set of peaks that are observed in the case of normal subjects vs cancer patients. However, for normal vs oral cancer, the SL intensity at 369 nm varies in the order of normal subjects > oral cancer and around 480 nm, it in the order of oral cancer > normal subjects.
- For normal vs cervical cancer, the SL intensity at 369 nm varies in the order of normal subjects > cervical cancer and around 480 nm, it is in the order of cervical cancer > normal subjects.

- From the peaks observed in the urine of normal and cancer subjects, it is evident that there will be some contribution of pteridines, enzymes NADH/NADPH, FAD, bilirubin, riboflavin and porphyrins in the urine of normal subjects. However, some of the peaks seen in normal subjects were not clear in the case of cancer patients and this may be due to the overwhelming effect of riboflavin over others fluorophores.

- In all the cases of classification, the difference spectrum shows a broad positive peak in the wavelength region between 300 nm and 460 nm and another positive peak around 520 nm which may be due to the increased contribution of NADH / NADPH, pteridine and its derivatives, and riboflavin are more in the urine of normal subjects than cancer patients. Further, sharp negative peak at 483 nm which may be due to higher concentration of porphyrin in the urine of cancer patients than that of normal subjects.

The synchronous luminescence spectral data for $\Delta \lambda = 20$ nm interval was subjected to statistical analysis as discussed earlier. From the Figure 7.7, it is observed that the discrimination of cervical cancer from normal subjects shows a better sensitivity than others. Whereas, the specificity is above 80% for all the groups compared.
Figure 7.7 Shows the percentage of sensitivity and specificity of the statistical analysis (a) original classification (b) leave one out cross validated classification, for the synchronous luminescence spectral data of urine samples

7.5 STEADY STATE AND TIME RESOLVED FLUORESCENCE SPECTROSCOPIC CHARACTERIZATION OF URINE

- The normal and oral cancer subjects exhibit a broad spectrum in the wavelength region 500 nm to 600 nm with emission maximum at 525 nm and the fluorescence emission intensity of
oral cancer patients is greater than normal subjects. The emission around 520 nm may be attributed to the contribution of riboflavin and its derivatives.

- The full width at half maximum (FWHM) of normal subjects is 89 nm and for cancer subjects it is 88 nm. From the difference spectrum, it is observed that the normal subjects shows positive difference around 480 nm which may be due to increased contribution of pteridines/NADH for normal subjects than cancer patients and negative difference in the region 530 nm to 650 nm may be due to the increased contribution of flavins for oral cancer patients than that of normal subjects.

- The flavin standard exhibits a bi exponential decay with an average lifetime of 4.7 ns. Whereas, the average lifetime of both the bi and tri exponential decay of urine samples of normal subjects is 4.6 ns. The cancer samples with bi and tri exponential decay shows an average lifetime of 3.9 ns and 4.9 ns respectively.

- Not much difference was observed between the lifetime of normal subjects and riboflavin standard. However, the average lifetime of oral cancer patients with bi exponential decay is less than that of normal subjects and cancer patients with tri exponential decay. The variation in the lifetime may be due to the complex nature of urine and the influence of other fluorophores present in urine and has to be probed further.

- For the fluorescence emission spectral data, the intensity parameter at 530 nm which is mainly due to the contribution of riboflavin discriminates the oral cancer subjects from the normal
subjects with a sensitivity and specificity of 44% and 82% respectively.

- No statistical difference was observed between the lifetime values of normal subjects and oral cancer patients.

7.6 CONCLUSION

The photophysical characteristics of urine of normal and cancer subjects were carried out by steady state and time resolved fluorescence spectroscopic technique. The spectra were analyzed statistically to find the potential of the fluorescence technique in the discrimination of cancer patients from normal subjects.

From the results of the statistical analysis, it was concluded that the cervical cancer was well discriminated from normal subjects when compared to other classifications. Also, the fluorescence emission spectra at 405 nm excitation yields good sensitivity and specificity followed by 450 nm excitation and synchronous luminescence spectra. Pteridine/NADH and flavins are responsible for the fluorescence emission at 405 nm and 450 nm excitations respectively. Hence, Pteridine, NADH and flavin may be considered as suitable markers for cancer diagnosis. Excitation wavelengths 405 nm, 450 nm and synchronous luminescence spectra with a Stokes shift of 20 nm may be considered as suitable technique for the discrimination of cancer subjects from normal subjects.

Further studies are to be carried out to collect more data on various cancer patients and to analyse the possibility of discriminating the different aetiologies and stages of cancer. In order to elucidate the possible reason for the altered spectral signatures due to the native fluorescence from pteridine, flavin
and its derivatives and their relation connecting to energy metabolism, and to study variation in the spectral signatures due to the influence of one fluorophore over the other, further combined studies by the biochemist, oncologist and physicians are required.

7.7 FUTURE DIRECTIONS

Urine is highly heterogeneous and it consists of many fluorophores. These fluorophores may be effectively utilized as tumour markers in the discrimination of cancer patients from normal subjects, if suitable wavelength of excitation and emission are selected. Hence, the study demonstrated the following important conclusion that warranted future clinical investigation:

i) The urine could be further investigated by other physicochemical, in particular by Raman spectroscopy.

ii) As the spectral signature is differing with respect to stage and different aetiological condition of patients, the study could also be extended to time resolved fluorescence spectroscopy of all the fluorophores to understand the changes occurred when the normal cell transformed into malignant state.

iii) Further, studies are to be carried out with more number of samples to accurately discriminate the different stages and histopathological conditions of the cancer patients from normal subjects.

iv) Urine samples are to be collected from smokers and patients with urinary tract infection and to analyse the variations and connections with that of cancer patients.