

Chapter - 5

Conclusion and Future Work

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5.1 Summary

In this thesis, the spectral properties of polarized fluorescence have been investigated for diagnostic purposes. The investigations have been carried out on biological tissues through the use of Wavelet Transform and Singular Value Decomposition. Conclusions drawn from this study are summarized in this chapter.

5.2 Highlights of investigation and results

Recently, optical diagnosis is emerging as a viable tool for tumor detection and for monitoring the stages of its growth. Probing tissues through light gets complicated due to the fact that it is a turbid medium. A number of fluorophores, starting from structural proteins to various enzymes and coenzymes are present in human tissues which can reflect the bio-chemical and morphological changes taking place in cancerous tissues. Fluorescence emission can differ significantly in normal, benign and cancerous tissues due to the differences in concentration of absorbers and scatterers, as also the scatterer sizes. The absorption in the visible range occurs primarily due to the presence of blood, whose amounts vary in various tissue types. The presence of scatterers leads to randomization of light, thereby generating a depolarized component in the fluorescence spectra, indicating that polarized fluorescence spectroscopy can be useful in isolating the

5.2 Highlights of investigation and results

characteristic spectral features from the diffuse background. The fact that cancerous tissues are much more inhomogeneous with irregular nuclear size distribution and tighter packing indicates that the corresponding spectral fluctuations will be more prone to randomization. The perpendicular component is more sensitive to both absorption and randomization since the path traversed by the same in the tissue medium is more. Hence the parallel and perpendicular components of the tissue auto fluorescence are expected to reveal different aspects of tissue characteristics and help extract information from this turbid medium.

1. Wavelet transform of polarized fluorescence spectra of human breast tissues is found to localize spectral features that can reliably differentiate normal, benign and malignant tissue types. The systematic separation of variations at different wavelength scales from the broad spectral features pinpoints several quantifiable parameters to distinguish cancer, benign and normal tissues. These distinguishable features are related with the biochemical and morphological changes. The spectral profile of diseased and the non-diseased tissues behave very differently, which manifest in the difference of the low pass power profiles. The fact that these characteristic signatures are based on higher level average coefficients makes them robust and less susceptible to experimental and statistical uncertainties. It is found that low level high pass coefficients differ significantly between cancer, benign and normal tissues. It is worth emphasizing that a straightforward averaging with arbitrary window sizes would not allow an independent separation of fluctuations and average behavior at multiple scales. It is observed that, scaling and translation are the key operations, which enable one to carry out a local analysis at desired scale and leads to independent wavelet coefficients, devoid of redundancy. This procedure is common to all discrete wavelets, where suitable form of weighted averaging and differentiation involving more than two points are carried out. The need for the early identification and constant monitoring of breast cancer for a large population makes this method eminently suitable since the same can be automated.

2. The continuous wavelets have pin-pointed significant differences in the tissue autofluorescence of the cancer and normal breast tissues. The multi-resolution

5.2 Highlights of investigation and results

ability and the overcomplete nature of the continuous Morlet wavelets are responsible in finding out these minute differences in the spectral feature, with a high sensitivity of 83%. Interestingly, these features manifested in the differences of the parallel and perpendicular components of the fluorescence intensities, since the same is less affected by the diffusive component, indicating the subtle nature of this effect. The auto-correlation of these periodically modulated wavelet coefficients showed strong differences between normal and cancer tissues. The fact that, these features appeared in the medium scales and in the first half of wavelength regime implies that these are probably due to flavin activity and blood absorption.

3. The study of spectral correlations in the polarized tissue fluorescence reveals information about wavelength ranges showing significant activity in cancer, benign and normal tissues. Very interestingly, the spectral fluctuations in the perpendicular channel showed the randomization effect of the tissue medium for the cancerous samples. This has been indicated through earlier studies involving wavelet transform. It is heartening that the fluctuations match very well with the random matrix prediction. The good fit with the probability distribution clearly indicates the Gaussian random nature of the spectral fluctuations in the perpendicular channel for the cancerous tissue. The parallel and perpendicular components also yield other complementary information. The latter indicates the wavelength domain where absorption takes place, and the former reveals significant fluorophores in the tissue. The correlated domains clearly pinpoint the presence of fluorophores like flavin and its derivatives as well as porphyrin. The range of this domain reveals the degree of correlation in different wavelength regimes. As is evident, the correlations are very different in parallel and perpendicular cases, for cancer, benign and normal tissue fluorescence. It is observed that only a few eigenvalues dominate in the correlation matrix both for parallel and perpendicular tissues. The corresponding eigenvectors clearly show differences between these tissue types.

4. Wavelet Decomposition, Singular Value Decomposition and Principal Component Analysis techniques have potential to reflect the changes in inherent prop-

erties like emission, absorption and scattering of the given tissue. The subtle changes in these properties reflected through these techniques can be used for diagnostic purposes.

5.3 Future Scope

A valuable lesson from the present analysis is that fluctuation characteristics of breast cancer tissues can be quite different from normal tissues, apart from differences in average spectral behavior between these two tissue types. This suggests that in using principal component analysis and other statistical tools like singular value decomposition to study tissue fluorescence, one should also analyze the fluctuations captured by smaller components, as compared to the principal components studied so far in the literature. Furthermore, experimental modelling of the fluctuations can be carried out for possible identification of the cause of randomization. Refractive index fluctuations, which are responsible for spectral randomization, may yield information about the nature and condition of the tissue. We also intend to use statistically robust tools to find out the degree of significance of the extracted features, both in the wavelet and singular value decomposition approach. Extending the study to other forms of cancer and also testing out the robustness of the extracted parameters in in-vitro condition should be quite satisfying.