Diagnosis of human breast cancer through wavelet transform of polarized fluorescence

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Wavelet transform of polarized fluorescence spectroscopic data of human breast tissues is found to reliably differentiate normal and malignant tissue types and isolate characteristic biochemical signatures of cancerous tissues, which can possibly be used for diagnostic purpose. A number of parameters capturing spectral variations and subtle changes in the diseased tissues in the visible wavelength regime are clearly identifiable in the wavelet domain. These investigations, corroborated with tissue phantoms, indicate that the observed differences between malignant tumor and normal samples are primarily ascribable to the changes in concentration of porphyrin and density of cellular organelles present in tumors.

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Breast cancer has emerged as the most common disease amongst women 1. Although the risk factor for Asian women has been estimated to be one-fifth to one-tenth that of women in North America and Western Europe, it still is the second most malignant condition 2,3. Apart from genetic predisposition, a number of factors like diet, exercise, environment, etc., are being recognized to play major roles in the growth of the disease 4. Early diagnosis is still not possible through conventional diagnostic techniques. If diagnosed early, breast cancer is also one of the most treatable forms of cancer. The requirement of continuous monitoring for breast malignancy of a significant percentage of women population has led to an intense search for safe, reliable and fast diagnostic methods.

Optical diagnosis techniques are now emerging as viable tools for tumor detection. Of these, fluorescence techniques are being increasingly employed to investigate both morphological and biochemical changes in different tissue types, for eventual application in the detection of tumors at an early stage 5. Fluorescence spectroscopy is well suited for the diagnosis of cancerous tissues because of its sensitivity to minute variations in the amount and the local environment of the native fluorophores present in the tissues 6,7,8,9,10. Morphological changes prevalent in tumors, such as enlargement and hyperchromasia of nuclei, overcrowding and irregular cellular arrangement are known to alter light propagation and scattering properties in such media and hence affect the fluorescence spectra 11. A number of fluorophores ranging from structural proteins to various enzymes and coenzymes, some of which participate in the cellular oxidation-reduction processes, are present in the human tissue and can be excited by ultraviolet and visible light 8. The fluorophores, FAD (Flavin Adenine Dinucleotide), its derivatives and porphyrins are particularly useful as fluorescent markers, since they fluorescence in the higher wavelength visible region, when excited by lower wavelength visible light, thereby avoiding the potentially harmful ultraviolet radiation.

The fluorescence emission can differ significantly in normal and cancerous tissues due to the differences in concentrations of absorbers 12,13 and scatterers, as also the scatterer sizes 14. The absorption in the visible range occurs primarily due to the presence of blood, whose amounts vary in various tissue types 15. The presence of scatterers leads to randomization of light, thereby generating a depolarized component in the fluorescence spectra. Polarized fluorescence spectroscopy is useful in isolating the characteristic spectral features from the diffuse background. The parallel component of the fluorescence suffers fewer scattering events. In comparison, the intensity of the perpendicular component is not only affected more by scatterers, but is also quite sensitive to absorption, since the path traversed by the same in the tissue medium is more. Hence, the difference of parallel and perpendicular intensities, apart from being relatively free from the diffuse component 15, can be quite sensitive to microscopic biochemical changes including the effects of absorption in different tissue types.

A number of studies conducted so far have established certain broad morphological and biochemical changes occurring in tumor tissues, which leave characteristic signatures in the spectral domain 16. The analyses of spectral data involve both physical 18,19,20,21 and statistical 16,22 modelling of tissue types, as also statistical methods, e.g., principal component analysis for extracting distinguishing parameters for diagnostic purposes 16. The fact that biological tissues are complex systems, possessing substantial variations among individual patients, depending upon various factors such as age, progress of the disease, etc., makes modelling of the same rather difficult. In using statistical tools, difficulty often arises in relating the statistically significant quantities to physically transparent spectral variables. In recent times, wavelet transform has emerged as a powerful tool for the
analysis of transient data and is particularly useful in disentangling characteristic variations at different scales [24]. This linear transform isolates local features and leads to a convenient dimensional reduction of the data in the form of low-pass (average) coefficients, resembling the data itself. The wavelet or high-pass coefficients, at various levels, encapsulate the variations at corresponding scales. The higher-level coefficients, particularly the global parameters associated with them, like power, are less contaminated by statistical and experimental uncertainties present in the data. An earlier study, of the perpendicular component of the fluorescence spectra, by some of the present authors has indicated the usefulness of wavelet transform in identifying characteristic spectral features [24].

Here, we present the results of a systematic analysis of the wavelet transform of the fluorescence spectra from human breast tissues for malignant and normal tissues. The difference between parallel and perpendicular components of the fluorescence spectra is subjected to this analysis, since the same is comparatively free of the diffusive component. A number of parameters, capturing spectral variations and subtle changes in the intensity profile of the diseased tissues, as compared to their normal counterparts, are identified in the wavelet domain. Based on earlier investigations and the present study of tissue phantoms, the physical origin of these distinguishing parameters can be primarily ascribed to the changes in the concentration of porphyrins and the density of cellular organelles present in tumors [14, 25].

In total, 28 breast cancer tissue samples were studied; out of these, 23 samples came with their normal counterparts. The tissue samples were excited by 488nm wavelength polarized light and the parallel and perpendicularly polarized fluorescence light were measured from 500 to 700 nm. Differences of parallel and perpendicular components of fluorescence intensity \((I_\| - I_\perp)\) versus wavelength profiles for all the tissue samples were analyzed by Haar wavelets [26].

We have identified three independent parameters, derived from the coefficients in the transform domain, which differentiate cancer and normal tissues quite accurately. The first parameter is the local maxima in the third quarter of the fourth level low-pass coefficients. As will be elaborated later, this feature owes its origin to porphyrin emission [17, 25]. The other two parameters are based on wavelet high-pass coefficients, representing both global and characteristic local variations of the fluorescence spectra. In the domain of these three parameters, all the malignant and normal tissues studied here could be accurately differentiated. The five unpaired samples were used as checks for the consistency of two of the chosen parameters, since one of the parameters is a ratio, which involves both tissue types. Studies on tissue phantoms, corroborating the above choice of parameters and the inferences about the aforementioned biochemical changes in the tissues are presented below for comparison.

In the fourth level low-pass coefficients, the one originating from the fluorescence signals around 630 nm of the original data is found to be considerably higher in cancer tissues as compared to the corresponding normal ones. This is possibly due to the presence of more porphyrin as well as scattering agents. A particularly noisy fluorescence data of \((I_\| - I_\perp)\) from cancer and normal human breast tissues does not reveal significant differences (Fig. 1). However, the low-pass wavelet coefficients of the same data (Fig. 1, inset) capture these differences quite remarkably, highlighting the usefulness of wavelet analysis.

The local maxima at third quarter of fourth level low-pass coefficients of cancer samples are more than 0.1 while those of normal tissues are less than 0.1, with a sensitivity of 100% and specificity of 83% (Fig. 2). It should be noted here that the values for normal tissues which are more than 0.1 still show lower values than the corresponding tumors, consistent with all the other samples. Thus intra-patient diagnosis gives a clear distinction between cancer and normal tissues. Variations in inter-patient diagnosis may be due to the fact that, the growth of tumor depends on genetic (major genes, modifier genes) and non-genetic factors (birth, age, weight/diet, exercise, environmental exposures, etc). [4]

An important observation here is that the 630 nm band gets emphasized only in the fourth level low-pass coefficients. This band is masked by other noisy signals at the third level and is averaged out at the fifth level (Fig. 3). In cases where the fourth level does not highlight this band, the previous level does.

FAD and porphyrins are the major fluorophores that fluoresce in the visible wavelength regime, with peak intensities at 530 and 630 nm respectively. These fluorophores are considered as contrast agents for cancer de-

FIG. 1: Plot of the difference of parallel and perpendicular components of the fluorescence spectra for tumor and normal tissues. Inset shows the corresponding fourth level low-pass coefficients.
Local maxima at third quarter of fourth level low-pass coefficients of cancer and normal breast tissues

FIG. 4: (a). Fourth level low-pass coefficients of phantoms with fixed FAD (20 M) and porphyrin (10 M) concentrations and varying scatterer concentrations, (i) s = 10 mm\(^{-1}\), (ii) s = 20 mm\(^{-1}\), and (iii) s = 30 mm\(^{-1}\). Inset shows the values of the 7th to 10th low-pass wavelet coefficients of the same phantoms, highlighting the variations of a local peak. (b) Fourth level low-pass coefficients of phantoms, with fixed FAD (20 M) and scatterer (10 M) concentrations and varying porphyrin concentrations (i) 10 M, and (ii) 20 M.

It has been suggested that deficiency in ferrochelatase, the enzyme required for conversion of protoporphyrin IX (PpIX) to heme, in tumors results in accumulation of PpIX in these tissues relative to the normal ones (10). Such accumulation changes the relative concentration of these fluorophores thus altering the fluorescence spectra significantly, which in turn changes the peak heights of the emission bands of the two fluorophores. The scattering centers are known to enhance the fluorescence intensity (27). Thus the large size of cell suspensions, higher density of cells and accumulation of more porphyrin in tumors all contribute to a small peak at 630 nm wavelength region.

Studies of tissue models show that the 630 nm band gets enhanced at the fourth level low-pass coefficients of phantoms, with an increase in the scatterer concentrations (Fig. 4a) as well as with increase in porphyrin concentrations (Fig. 4b). A small peak around 630 nm is clearly visible at suitable concentrations. Significantly, in these tissue phantoms too, the third and fifth level low-passes do not highlight the 630 nm band, as observed in tissue samples.

The power spectra at different levels are defined as the sum of the square of high-pass coefficients at those levels. Normalization of the power spectra is done by dividing it by the sum of the square of intensities at all the wavelengths. In twenty two paired samples, it was found that the tumors have lower power at the third level as compared...
pared to their normal counterparts with a sensitivity of 96%.

It was also found that, in case of the cancer tissues, the third wavelet coefficient at the fifth level (originating from the fluorescence emission at 580 to 596 nm region in the original spectrum), is less negative than those of the normal ones. This implies that the normal tissue fluorescence spectra fall more sharply than those of the cancer tissues. Out of 28 cancer samples, which includes 23 paired and 5 unpaired tissues, 21 samples have third coefficients less than -0.31; out of 23 normal tissue samples, 14 samples have third coefficients more than -0.31. However, intra-patient diagnosis by high-pass coefficients shows that the third coefficient, for 17 out of 23 paired samples of normal tissues, is more than that of cancerous ones. Hence, for this coefficient, the cancer to normal ratio is less than one, with a sensitivity of 74%.

It may be noted that the above-mentioned three parameters also distinguish tumors of different grades. It is found that for grades I and II cancerous tissues the values of the local low-pass maxima at the third quadrant are less than 0.2, but more than 0.2 in the grade III cancers, with a sensitivity of 75%. At third level, the power ratio is less than 0.3 for grade I and grade II cancers and is between 0.3 to 0.8 for grade III cancers.

In conclusion, the systematic separation of variations at different wavelength scales and the broad spectral features in the wavelet domain pinpoints several quantifiable parameters to distinguish cancer and normal tissues. These distinguishable features are related with the biochemical and morphological changes, as is also evident from the phantom study. The fact that these characteristic signatures are based on higher level wavelet coefficients make them robust and less susceptible to experimental and statistical uncertainties. 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.

[1] I. Wang et al., In-vitro laser-induced fluorescence studies of malignant breast tumors following low-dose injection of Photofrin (LUMLC Progress Report, 28, 1993-1995; [http://www-lmlc.fysik.lth.se/Prog9395/p28.htm].
[2] M.E. Lippman, *Harrison's Principles of Internal Medicine* (Mc Graw Hill, 1998) 562.
[3] P.H. Pestonjamasp, I. Mittra and J. Biosc. 25, 19 (2000).
[4] E. Levy-Lahad and S.E. Plon, Science 302, 574 (2003).
[5] Recent references on various applications of lasers in biology and medicine can be found in IEEE Journal of Selected Topics in Quantum Electronics 9, 140 (2003).
[6] R.R. Alfano et al., IEEE Journal of Quantum Electronics 23, 1806 (1987).
[7] G.C. Tang et al., Appl. Opt. 28, 2337 (1989).
[8] G.C. Tang, A. Pradhan and R.R. Alfano, Lasers Surg. Med. 9, 290 (1989).
[9] R.R Kortum and E. Sevick Muraca, Ann. Rev. Phys. Chem. 47, 556 (1996).
[10] G.A. Wagniers, W.M. Star and B.C. Wilson, Photochem. Photobiol. 68, 603 (1998).
[11] V. Backman et al., Nature 406, 35 (2000).
[12] M. Keizer, R.R. Kortum, S.L. Jacques and M. S. Feld, Appl. Opt. 28, 4286 (1989).
[13] A.J. Durkin, S. Jaiikumar, N. Ramanujam and R.R. Kortum, Appl. Opt. 33, 414 (1994).
[14] L.T. Perelman et al., Phys. Rev. Lett. 80, 627 (1998).
[15] N. Ramanujam et al., Photochem. Photobiol. 64, 720 (1996).
[16] V. Backman et al., IEEE Journal of Selected Topics in Quantum Electronics 5, 1019 (1999).
[17] N. Ramanujam, *Encyclopedia of Analytical Chemistry* (Wiley, New York, 2000) 20.
[18] C.M. Gardner, S.L. Jacques and A.J. Welch, Appl. Opt. 35, 1780 (1992).
[19] J. Wu, M.S. Feld, R.P. Rava, Appl. Opt. 32, 3585 (1993).
[20] A.J. Durkin and R.R. Kortum, Laser Surg. Med. 19, 75 (1996).
[21] B.V. Laxmi et al., Lasers Life Sci. 9, 229 (2001).
[22] R.W. Dillon and M. Goldstein, *Multivariate Interpretation of Clinical Laboratory Data* (Marcel Dekker, New York, 1987).
[23] I. Daubechies, *Ten Lectures on Wavelets* (CBMS-NSF regional conference series in applied mathematics, Philadelphia, PA, 1992, Vol. 64).
[24] N. Agarwal et al., IEEE Journal of Selected Topics in Quantum Electronics 9, 154 (2003).
[25] M.S. Nair, N. Ghosh, N.S. Raju and A. Pradhan, Appl Opt. 41, 4024 (2002).
[26] C. Chui, *An Introduction to Wavelets* (Academic press, New York, 1992).
[27] N. C. Biswal, S. Gupta, N. Ghosh and A. Pradhan, Opt. Exp. 11, 3320 (2003).