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Polarized spectral features of human breast tissues through wavelet transform and principal component analysis

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Abstract. Fluorescence characteristics of human breast tissues are investigated through wavelet transform and principal component analysis (PCA). Wavelet transform of polarized fluorescence spectra of human breast tissues is found to localize spectral features that can reliably differentiate different tissue types. The emission range in the visible wavelength regime of 500–700 nm is analysed, with the excitation wavelength at 488 nm using laser as an excitation source, where flavin and porphyrin are some of the active fluorophores. A number of global and local parameters from principal component analysis of both high- and low-pass coefficients extracted in the wavelet domain, capturing spectral variations and subtle changes in the diseased tissues are clearly identifiable.

Keywords. Wavelet transform; principal component analysis; fluorescence.

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1. Introduction

Optical diagnostic techniques such as fluorescence, Raman, and light scattering are viable tools for tumor detection. Amongst these methods, fluorescence technique is one of the most widely used method in light-based diagnostic systems. Biological tissue is a turbid medium. The fluorescence from such a medium is affected by scattering and absorption. Due to its sensitivity to minute variations, fluorescence spectroscopy can provide quantitative biochemical information about the state of the tissue, which may not be obtained by using standard pathology. Fluorophores, such as flavin, nucleotides (NADH), tryptophan, tyrosine, elastin, collagen etc.,

having excitation frequencies in UV and visible regimes, have been used as markers. Flavins are intrinsic fluorophores, which fluoresce in the higher wavelength visible region when excited by lower wavelength visible light. Fluorescence emission can differ significantly in normal, benign and cancerous tissues due to the differences in concentration of absorbers and scatterers and also the size of the scatterers. 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. Decrease in fluorescence intensity due to absorption by blood is thus a signature of early tumor growth. Amount of flavin (FAD) increases in cancer tissues compared to normal tissues.

As pre-cancerous tissue develops into cancerous one, porphyrin increases. Porphyrins have drawn immense attention because of their role in the human body, ability to accumulate in many kinds of cancer cells, as well as their magnetic and optical properties. These features make them useful in cancer therapy [1].

The tissue samples were excited with 488 nm wavelength plane polarized light from an Ar-ion laser (Spectra physics 165,5w) and keeping the excitation polarizer horizontal, fluorescence was recorded with the emission polarizer in both the parallel ($\|$) and vertical ($\perp$) positions to obtain the parallel and perpendicular components respectively for 500–700 nm wavelength range. The polarized fluorescence spectra were collected in right angle geometry using triplemate monochromator (SPEX-1877E) and PMT (RCA C-31034). The laser spot was around 1 mm.

2. Wavelet transform

Any finite energy signal $f(t) \in L^2(R)$ can be expanded as

$$f(t) = \sum_{k=-\infty}^{\infty} c_k \phi_k(t) + \sum_{k=-\infty}^{\infty} \sum_{j=0}^{\infty} d_{j,k} \psi_{j,k}(t), \tag{1}$$

where c_k and $d_{j,k}$ are the wavelet transforms of the signal $f(t)$ and known as low-pass and high-pass coefficients respectively. The low-pass coefficients at various levels represent average behaviour of the data over the corresponding window sizes, whereas high-pass coefficients represent differences around the location k, over a window size which depends on level j.

Using multiresolution analysis one can show that

$$c_{j,k} = \sum_n h(n - 2k) c_{j+1,n} \tag{2}$$

and

$$d_{j,k} = \sum_n \widetilde{h}(n - 2k) c_{j+1,n}. \tag{3}$$

Here $h(n)$ is the low-pass filter and the corresponding one for the high-pass coefficients is the high-pass filter.

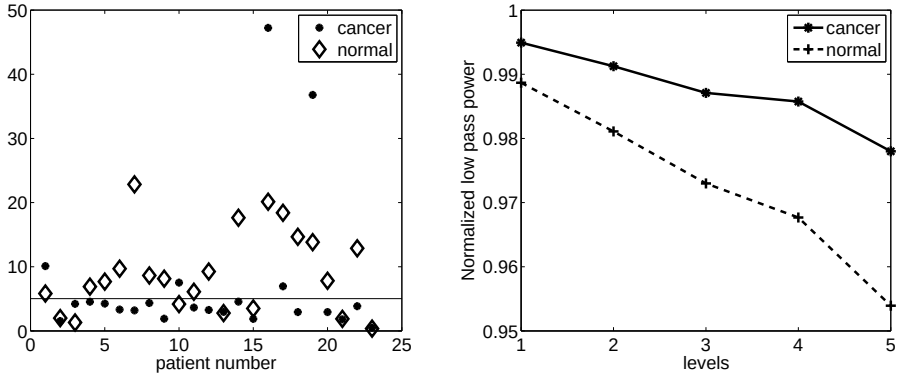

Figure 1. (**a**) Standard deviation of percentage fluctuation (of difference of intensities of parallel and perpendicular components) and (**b**) low-pass power.

Wavelet transform satisfies the Parseval theorem

$$P \equiv \sum I_i^2 = \sum c_k^2 + \sum_{j,k} d_{j,k}^2, \tag{4}$$

where I_i are the intensity values.

We used wavelet transform to isolate the variations at different scales in different tissue types. Because of its multiresolution and localization properties, this linear transform was ideally suited for disentangling variations at different scales.

First we employed discrete wavelet transform on the signal and then computed the standard deviation of the percentage fluctuations, i.e., the high-pass coefficients divided by their corresponding low-pass coefficients for statistical analysis shown in the scatter plot (figure 1a). Standard deviation of normal tissue is dominantly greater than that of cancer tissues. This confirms that randomization of fluctuations is more in malignant tumor due to larger number of cells, and the spectrum is more densely packed compared to the normal tissue spectra.

Then we computed low-pass powers (figure 1b) at different levels which consistently differed between tissue types. The power at different levels captured the variance at that level. Normalized low-pass powers of cancerous tissues decreased more slowly as a function of levels.

Earlier, it was found that perpendicular component was the best discriminator, so we have concentrated on the parameters of this component [2].

3. Correlation matrix

We have computed $\delta I_i(k)$ through mean subtraction of the low-pass coefficients I_i to construct the correlation matrix C [3]

$$C = (A^T A)/N, \tag{5}$$

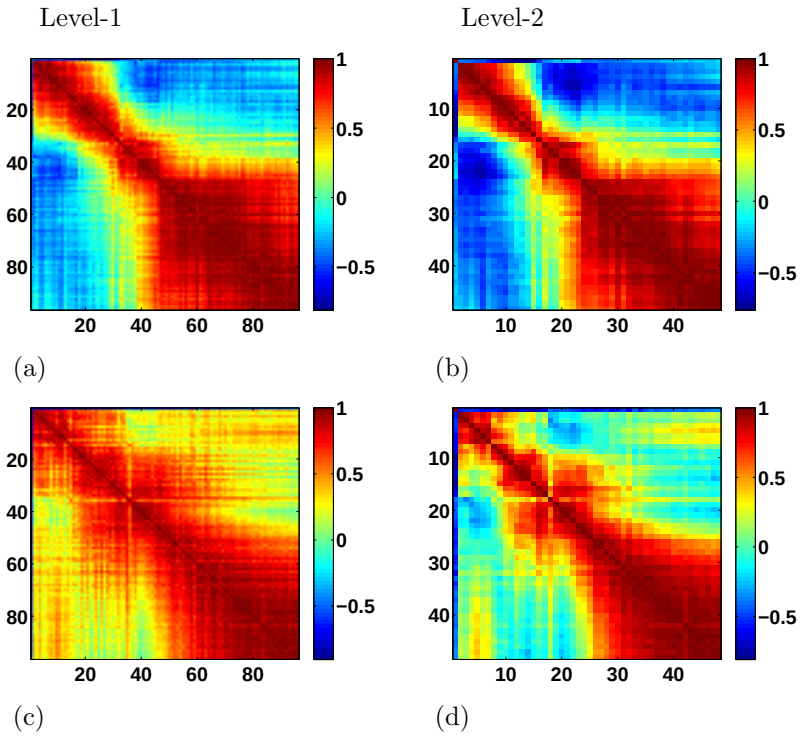

Figure 2. Correlation matrices of low-pass level-1 and level-2 wavelet coefficients for the (**a**), (**b**) cancer and (**c**), (**d**) normal tissues showing in the case of cancer highly correlated domains to higher wavelength.

where $A_{ik}^{T} = \delta I_i(k)$, is a $m \times n$ rectangular matrix. The δIs have been normalized to have unit variance. N is the normalization factor.

Here $i = 1$–96 low-pass coefficients of level-1 correspond to the original intensity values 192 (for the wavelength range 500–691 nm), $i = 1$–48 low-pass coefficients for level-2 and $k = 23$ corresponds to tissue sample number.

The nature of correlations, as seen through different sized domains, are clearly different for cancer and normal low-pass coefficients of level-2 (figure 2). It is also clear from the entries of eigenvalues corresponding to second dominant eigenvector that both the tissues show opposite activities (figure 3).

Figure 4 depicts high-pass coefficients which differ significantly in cancer and normal tissues, at low level.

4. Conclusion

In conclusion, the systematic separation of variations at different wavelength scales from the broad spectral features pinpoints several quantifiable parameters to distinguish cancer and normal tissues. These distinguishable features are related to biochemical and morphological changes. The spectral profiles of the diseased and

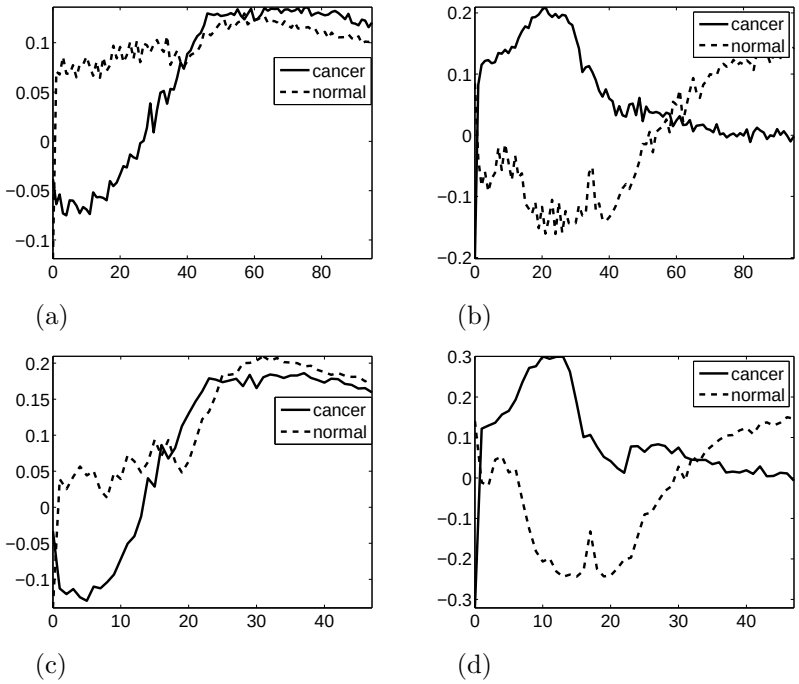

Figure 3. Entries of eigenvectors corresponding to the first two highest eigenvalues for (**a**), (**b**) level-1 and (**c**), (**d**) level-2 low-pass coefficients of cancer and normal tissues.

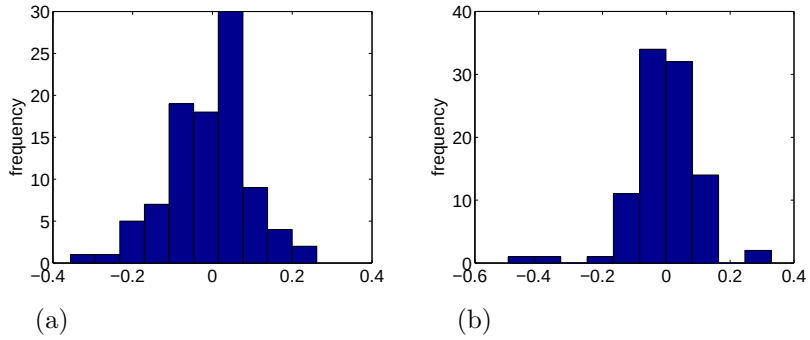

Figure 4. Histogram of the normalized high-pass coefficient for (**a**) cancer and (**b**) normal tissues at level-1.

the non-diseased tissues behave very differently, which manifest in the difference of the low-pass power profiles.

Low level high-pass coefficients differ significantly between cancer and normal tissues. Fluctuation characteristics of breast cancer tissues are quite different from normal tissues captured by smaller principal component.

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References

[1] M I Gaiduk, V V Grigryants and I V Rodstat, Rare-Earth Porphyrin as New Fluorescent Markers for Malignant Tumors Diagnostics: Spectroscopic and Clinic-Physiological Aspects, Report, USSR, *Acad. Sci., Inst. Radio Engineer. Electronics, Moskwa* (1991)

[2] Sharad Gupta, Maya S Nair, Asima Pradhan, Nrusingh C Biswal, Nidhi Agrawal, Asha Agrawal and Prasanta K Panigrahi, *J. Biomed. Opt.* **10(5)**, 054012 (2005)

[3] Anita H Gharekhan, Siddharth Arora, K B K Mayya, Prasanta K Panigrahi, M B Sureshkumar and Asima Pradhan, *J. Biomed. Opt.* **13(5)**, 054063 (2008)