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Diffuse reflectance spectroscopy for monitoring physiological and morphological changes in oral cancer

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ABSTRACT

Oral cancer is prevalent in India due to addictive habits such as smoking and tobacco chewing. Simple steady-state diffuse reflectance spectroscopic technique may facilitate routine check up and early diagnosis of oral cancer. In this regard, inverse Monte Carlo algorithm is implemented to estimate the optical properties of tissue from diffuse reflectance spectra. A modified tissue model has been proposed in this algorithm which considers predominant Rayleigh scattering in the visible region and absorption of methemoglobin and melanin in tissue. The proposed tissue model improves the accuracy of estimation of optical properties. The root mean square variance between the measured and modeled diffuse reflectance spectra is found to be less than 5%. Further, lookup table based technique adopted in this algorithm enables real-time estimation of optical parameters of tissue in about 5 s. Significant variations in the scattering coefficient, hemoglobin oxygen saturation and fraction of methemoglobin are identified between normal and cancer tissues. Step-wise linear discriminant analysis have revealed that the tissue parameters were statistically significant and the cancer tissue are discriminated from normal with 90% sensitivity and 90% specificity.

1. Introduction

National Cancer Registry Programme (NCRP) initiated by the Indian Council of Medical Research (ICMR) reports that the estimate of cancer cases in India is 979,786 in the year 2010 and it is likely to go up to 1,148,757 cases in the year 2020. In India oral cancer ranks third next to breast and cervix uteri cancers and it accounts for more than 7% of the cases out of 28 types of cancer reported [1]. This high incidence of oral cancer is primarily due to addictive habits like tobacco chewing and exposure to drugs. Most oral cancer originates as epithelial dysplasia and spreads to underneath stroma only at later stages. If cancerous tumors were found in early stages, tumors can be surgically removed before cancer advances and metastasizes. However vast number of oral cancer cases are diagnosed only at later stages, which leads to increased mortality rate. Histopathological examination of the hematoxylin–eosin stained biopsy tissue samples is considered as the golden standard for identification of cancer, which is rather painful and makes routine check-up for cancer diagnosis impossible. Hence, early detection of cancer with simple, non-invasive and efficient technique is essential to combat oral cancer in India.

In this context, molecular level diagnosis using various optical spectroscopic techniques has been proven as an effective non-invasive tool for characterizing the neoplastic conditions [2]. Among them, diffuse reflectance spectroscopy has the advantage that it has the integrity of both simplicity and vast diagnostic potential. Further diffuse reflectance spectrum from tissue contains a wealth of information about the biochemical and ultrastructural variation. Hence, it has been used to diagnose various diseases including breast cancer [3–5], cervical cancer [6], oral cancer [7–9], colon cancer [10], skin cancer [11], ovarian cancer [12], lung cancer [13], bladder cancer [14], and Barrett's esophagus [15]. Further, it has also been used for dosimetry calculation (during tissue ablation, coagulation and photodynamic therapy) [16], monitoring morphological changes in red blood cells [17], tumor margin analysis before surgery [18], for measurements of photosensitizer uptake [19] and blood glucose measurement for diabetics [20].

It is important to note that, tissue undergoes physiological, biochemical and morphological changes due to altered metabolic, genomic and proteomic level of cancerous cell. Further, during the transformation of normal to malignant condition changes in tissue such as angiogenesis, variation in hemoglobin oxygen

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saturation, increased nuclear size, increased nucleus to cytoplasmic ratio, increased number of mitochondria, lipid peroxidation and alteration in the extracellular matrix of the tissue may affect the tissue optics [21–29]. These biochemical and morphological variations between normal and cancer tissues may affect the optical properties of tissue. These changes in tissue optics may be exploited to discriminate the cancer from normal.

Different empirical methods such as use of spectral ratio DR540/DR575 for the estimation of oxygen saturation, neural network and pattern recognition techniques have been used to discriminate cancer [30–32]. The extraction of tissue optical properties using various analytical (diffusion approximation, and Kubelka Munk theory) and numerical methods (inverse Monte Carlo simulation, inverse adding-doubling) have proven to be an accurate quantitative technique to diagnose various conditions of tissue including cancer [33–40]. In general analytical methods, suffers from the disadvantage that it is limited to predominant scattering and it is not possible to incorporate any experimental condition in the algorithm [41]. In this regard, the numerical methods yield highly accurate result and it is flexible to take into account of any artifacts produced due to experimental condition [41]. The only problem with numerical method is that it requires high computation time which can be rectified by improvising a lookup table based inverse algorithm [42,43]. Conventional methods estimate optical properties of tissue from diffuse reflectance and transmittance measurements, but to estimate optical properties of tissue from diffuse reflectance measurement alone a tissue model has to be assumed [44,45].

The tissue model considered in this study makes an assumption, that the tissue absorption is due to chromophores – deoxy hemoglobin, oxy hemoglobin, methemoglobin, β-carotene and melanin present in the tissue. The addition of one of hemoglobin derivative methemoglobin in tissue model was carried out, since a significant concentration of methemoglobin has been noted in the reflectance spectra from cancer tissues. Melanin is included due to excess melanin content in oral tissues from people of Indian ethnicity. Bashkatov et al. reported on the optical properties of mucous tissue on wide spectral region from 400 to 2000 nm, which revealed three important aspects: (1) Mie scattering is predominant in IR region, (2) both Mie and Rayleigh scattering is present in the visible region, (3) scattering coefficient deviates from monotonic dependence on certain wavelengths in strong absorption bands [29]. Hence the tissue scattering in visible region is mathematically modeled as a combination of both Rayleigh ($\sim\lambda^{-4}$) and Mie contribution ($\sim\lambda^{-b}$, where b is Mie scattering power) and the wavelength region for tissue modeling is chosen such that there is no deviation in monotonic dependence (excluded 415 nm from tissue model). A modified tissue model proposed in this work enables to extract the tissue optical properties with improved accuracy. Further the implementation of Monte Carlo lookup table (MCLUT) based inverse algorithm reduced computation time and facilitated real-time diagnosis by extracting the entire tissue parameters from diffuse reflectance measurements within few seconds.

2. Materials and methods

2.1. Biopsy tissue samples

Tissue biopsies of 20 oral squamous cell carcinoma and 20 normal buccal mucosa were obtained from the Aringar Anna Cancer Hospital and Research Center (Kancheepuram, India) with previous consent of the patient and ethical clearance from the Hospital. The samples were placed in normal saline to avoid dehydration and the freshly excised tissues were all measured within 5 h of excision. The dimension of the biopsy tissue varied between 3 cm^2 and 4 cm^2.

Table 1
Input parameters for Monte Carlo simulation.

Physical quantity	Parameter
Refractive index of surrounding	1
Refractive index of tissue	1.4
Coefficient of anisotropy	0.9
Number of photons	10^6

2.2. UV-Visible Spectrometer

Reflectance spectrum of tissue was measured using a UV-Visible Spectrometer (Perkin Elmer Lamda-35) with Integrating Sphere setup (RSA-PE-20). The diameter of the integrating sphere is 50 mm and coated with spectralon. The scan speed was 120 nm/min with a spectral bandwidth of 1 nm. Halogen lamp was used to irradiate visible light on tissue. The baseline correction for the reflectance measurement were made with spectralon diffuse reflectance standard SRS-99.

2.3. Monte Carlo simulation based lookup table

A homogeneous tissue model with bulk optical properties was considered in this study. The tissue geometry was taken in the shape of cylinder with a height and radius of 3 cm. Since the penetration depth of visible light in tissue is much less than 3 cm, a semi infinite tissue geometry is indirectly assumed. The optical properties of tissue are parameters that characterize light propagation in tissue, which include absorption coefficient μ_a, scattering coefficient μ_s, anisotropy coefficient g and refractive index of tissue n. Light scattering in tissue is anisotropic and it is mostly forward scattered. By using similarity principle a reduced scattering coefficient given by, $\mu_s' = \mu_s \times (1 - g)$ corresponding to isotropic scattering is used instead of scattering coefficient of tissue [46].

A homemade program written in *C* was used to generate a Monte Carlo simulation based diffuse reflectance lookup table. In Monte Carlo simulation a random number generator is used to simulate absorption and scattering events in tissue. The probability of absorption and scattering events of photons per unit path length is specified by absorption and scattering coefficient respectively. The angle at which photon is scattered during a scattering event is dictated by Henyey–Greenstein phase function [46]. Anisotropy coefficient of human tissue varies from 0.7 to 0.95 and a typical value for most of the biological tissue is 0.9 [47]. Hence, *g* for soft tissue was assumed to be 0.9. Finally, the refractive index of tissue was fixed as 1.4 for soft tissue, in accordance with the report by Bolin et al. that the refractive index of homogenized mammalian tissue spans a similar value [48,49].

The absorption and reduced scattering coefficient of tissue vary a lot under different physical and pathological condition [47]. For a range of absorption and reduced scattering coefficient encountered in our study, a diffuse reflectance lookup table using Monte Carlo simulation is generated. The absorption and reduced scattering coefficient is varied between 0.1 and 50 with 21 increments. Number of photons in Monte Carlo simulation was set as 10^6 and it reduced the standard deviation of 3 independently generated lookup table to less than 0.1% of the mean. The Generated Monte Carlo lookup table can be used to compute diffuse reflectance for the given absorption and reduced scattering coefficient. Diffuse reflectance was computed in the units of percentage of incident photons and assumed it is equal to percentage of incident intensity. A slight variation between the same has been neglected. The input parameter used in Monte Carlo simulation to generate the lookup table is listed in Table 1 and the resulting Lookup table is shown in Fig. 1.