Extracting fluorescence efficiency and emission spectra of cervical tissue

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Abstract: This study aims to extract layer-resolved fluorescence efficiency and emission spectral shape of in-vivo cervical mucosa tissue by a two-layered fluorescence Monte Carlo model. The emission spectra are modeled to have skew-normal distributions, and genetic algorithm is used to find parameters that optimize the fit to the measured spectra. Excellent model fits suggest that the proposed method provide more accurate evaluation of intrinsic fluorescence properties of the cervical mucosa, which has the potential to aid the non-invasive diagnosis of cervical precancers.

1. Introduction

Fluorescence spectroscopy has been widely used in the diagnosis of superficial malignancy., Quantifying the fluorescence generated from the reduced nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), and collagen cross-links provides potential biomarkers. It is advantageous to extract the intrinsic fluorescence from tissue, removing effects of absorption and scattering in the tissue. Methods such as adjoint reflectance and fluorescence Monte Carlo models [1] and polarization-gated detection [2] have been proposed to detect the intrinsic fluorescence. However, NADH and FAD predominantly exist in the epithelium and collagen only exists in the stroma. Moreover, fluorescence emission spectra may be affected by the local environment and the collagen fluorescence is highly dependent on its specific composition and structure. To address these issues, we utilized a two-layered fluorescence Monte Carlo model which has been proposed by our group in a previous tissue-phantom study [4]. The two-layered structure models the epithelium and stroma adequately, and the layer-resolved model is capable to distinguish the fluorescence contribution of each fluorophore. In this study we modified the model for analyzing in-vivo data from normal and precancerous cervical tissue, quantifying the fluorescence efficiency and extracting the emission spectral shape of each fluorophore.

2. Materials and Methods

We employed a multi-LED light source (Bluloop, Ocean Optics, 12W, 355-750nm) and made a fiber bundle consisting of seven fibers to acquire reflectance spectra and fluorescence spectra from the uterine cervix in vivo. One of the fibers served as the light source, and the rest fibers were detectors. The spectra were dispersed by an imaging spectrograph (ACTON, SpectraPro SP-2150i) and captured by a CMOS camera (PointGrey, GS3-U3-23S6M-C). Details of the experimental setup are described in [5]. The fluorescence spectra of tissue were acquired in vivo from four subjects who all signed informed consent. The spectra were calibrated by comparing with spectra measured from phantoms made with known scattering, absorption and fluorescence properties. 0.5 μm diameter polybeads (Polysciences, Inc., Polybead ® Microspheres) and India Ink (NENN YIH CO.) were the scatterers and absorbers, respectively. Fluorescein and stilbene are the fluorophores [6].

The model presented in this study is based on a two-layered diffuse reflectance spectroscopy Monte Carlo (DRSMC) [5], and a two-layered fluorescence Monte Carlo (FMC) [4]. By processing the two models sequentially, scattering and absorption coefficients and epithelial thickness can be retrieved by DRSMC. These parameters are then input into FMC, to estimate the fluorescence efficiency and emission spectral shape. For the ease of computation, the fluorescence efficiency μ_{eff}, and the probability of emission P is set to 1.

$$\mu_{eff} = \mu_f(\lambda_{ex}) \cdot \phi \tag{1}$$

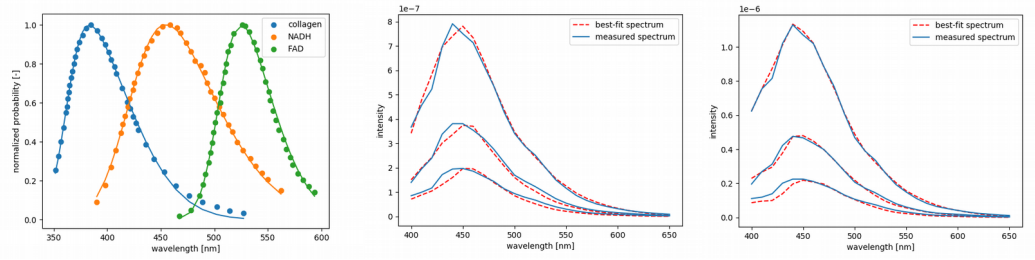
$$F_{measured}(\lambda_i) = \sum_{j=1}^{m} F_{simulated}(\lambda_i) \cdot \frac{\mu_f(\lambda_{ex})}{\mu_a(\lambda_{ex})} \cdot \phi \cdot P_j(\lambda_i) \tag{2}$$

Equation (2) shows the idea of how the fluorescence efficiency and emission spectral shape can be estimated, where m is the number of fluorophores, which is 3 in this case, including collagen, NADH and FAD, μ_f, μ_a are the absorption coefficient of the fluorophore and total absorber in tissue, respectively, φ is the quantum yield and P is the emission probability. After the Monte Carlo simulation, fluorescence efficiency and emission spectral shape are

set as parameters to be determined by minimizing the root-mean-square error between the measured spectrum and the model predicted spectrum. The emission spectral shape P is modeled by skew-normal distributions by tuning the mean, standard deviation, and skewness of the fluorophore. The optimization process is based on the genetic algorithm [7] which sets each parameters as an 1D array, namely a gene, picks the relatively low-error genes, randomly operates pairwise linear combinations to generate a new gene, and iterates the process to minimize the root-mean-square error.

3. Results and Discussions

For validating the feasibility of the proposed fitting method, we fit the emission spectra measured in a previous study [8]. The result in Fig. 1(a) shows that the emission spectra can be represented adequately by skew-normal distributions. Moreover, compared to other numerical method such as polynomial fits, the proposed model requires fewer parameters, which means that the calculation complexity is lower. The average root-mean-square error over 52 spectra is 9.46%.



(a) (b) (c)

Fig. 1. experiment result

One example result of fitting fluorescence spectra are shown in Fig. 1(b) and 1(c), which are from cervical intraepithelial neoplasia grade 2 (CIN2) and normal sites on the same subject, respectively. In average, when the tissue developed from normal to cancerous, the fluorescence efficiency of collagen decreases by 62% in average, the peak wavelength of the collagen emission spectra is red-shifted by 9nm, and the ratio of fluorescence signals between NADH and collagen increases by 0.29. The results agree with findings in previous studies [9, 10] and shows that the extracted parameters is capable of distinguishing the normal and dysplasia tissue.

4. Conclusions

The two-layered FMC model can fit the measured fluorescence spectra with a small average error over all spectrum (9.46%). By using skew normal distribution to reconstruct the emission spectra, only three free parameters are needed, which helps speed up calculations. The fluorescence efficiency and emission spectral shape are significant biomarker. Based on the parameters acquired from FMC and DRSMC, it is possible to make existing dysplasia grade classifier more robust, and provide more information for professionals to make decision.

5. References

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