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Conference Report

Fluorescence spectroscopy as a diagnostic tool for detecting cervical pre-cancer☆

Introduction

Cervical cancer is the second most common cancer in women worldwide; 80% of cases arise in the developing world. Cervical cancer remains the leading cause of cancer deaths in women worldwide. The survival of cervical cancer overall is 40%, but survival is greatly increased when it is treated at early stage of the disease [1]. The prevention of cervical cancer could be improved by devices that automate the screening and detection of cervical cancer and decrease cost. In particular, real-time diagnosis could allow patients to be seen, diagnosed, and treated in a single visit.

Biophysical changes that accompany dysplastic progression often lead to alterations in the optical characteristics of tissue. Optical technologies sensitive to these alterations can lead to the development of quantitative, noninvasive, real-time diagnostic tools. In particular, a number of clinical studies based on fluorescence spectroscopy have demonstrated high sensitivity and specificity in diagnosing cervical precancer [2–4]. Fluorescence spectroscopy measures a wide range of tissue optical characteristics that are sensitive to molecular and architectural changes that accompany dysplastic progression. For example, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) are fluorescent molecules in the mitochondria that have an important role in cellular metabolism. An increase in NADH and FAD fluorescence in dysplastic cells [5,6] and tissue [7] has been correlated with increased cellular metabolic activity. Other optical interactions such as light scattering and absorption are affected by dysplastic progression. Nuclear atypia in abnormal cells lead to increased light scattering by the cellular nuclei. Hemoglobin in the vasculature is an important source of light absorption in tissue, and higher level of light absorption is observed with angiogenic developments in abnormal tissue.

Fig. 1 shows the overall strategy for applying fluorescence spectroscopy in the detection of epithelial precancers including cervical precancer. Excitation light with a narrow bandwidth is generated by placing an optical bandpass filter in front of the light source. The excitation light will be absorbed by various endogenous fluorescent molecules in tissue, resulting in emission of fluorescent light. The excitation light as well as the fluorescent light will also interact with molecular, subcellular, and cellular components in tissue that will scatter and absorb light. The fluorescent light remitted from tissue is then detected using an optical detector.

Methods

In order to evaluate the diagnostic potential of fluorescence spectroscopy in cervical precancer detection, we have developed a clinical device to acquire fluorescence spectra from cervical tissue in real-time and in vivo. The device is equipped with a broadband light source coupled to a set of bandpass filters to deliver 16 different excitation wavelengths in the visible spectrum. The excitation light is guided to the measurement site using a fiber-optic based probe, and the resulting fluorescence emission collected through a different channel in the probe. Fluorescence spectrum is recorded using a charge coupled device (CCD) camera coupled to an imaging spectrograph.

Using this device, we have collected optical data from 850 patients with abnormal Papanicolaou smears and 1000 patients with a history of normal Papanicolaou smears. Each patient was evaluated by colposcopy prior to the spectroscopic measurement. Fluorescence spectra were acquired from normal and abnormal areas, and colposcopically directed biopsies were taken from these sites as a gold standard. These biopsies were read blinded three to six times by study pathologists. Fig. 2 shows an example of fluorescence measurements from a squamous normal site and *carcinoma in situ*.

Diagnosis is derived by extracting and analyzing diagnostically significant information from the fluorescence measurements. Statistical features that exhibit strong separation between the normal and abnormal samples can be used as a diagnostic discriminator. Although statistical features have lead to the development of sensitive and specific diagnostic algorithms [2,3,8], it is generally difficult to correlate them with the underlying biophysical implications. Mathematical models can be developed based on light interaction in tissue, and these models can be applied to the fluorescence measurements to estimate the various optical characteristics of measured tissue such as light scattering and absorption properties and relative concentration of the different fluo-

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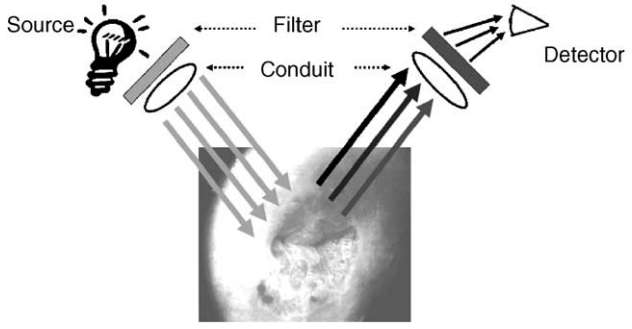

Fig. 1. Overall strategy for applying fluorescence spectroscopy in the detection of epithelial precancers.

rescent molecules. Since the tissue optical characteristics are sensitive to the biophysical changes in dysplasia, they can serve as diagnostically significant features.

A number of mathematical models were developed to estimate the tissue optical characteristics from fluorescence measurements [9–11]. These models describe light interactions in single layered tissue. In contrast, we have developed a more realistic mathematical model in which the light interaction takes place in a two layered medium composed of the epithelium and the stroma. Subsequently, this model is applied to fluorescence measurements to estimate the epithelial and stromal optical characteristics. This is particularly important considering that the epithelium and the stroma undergo different changes in biophysical and optical characteristics with dysplastic progression.

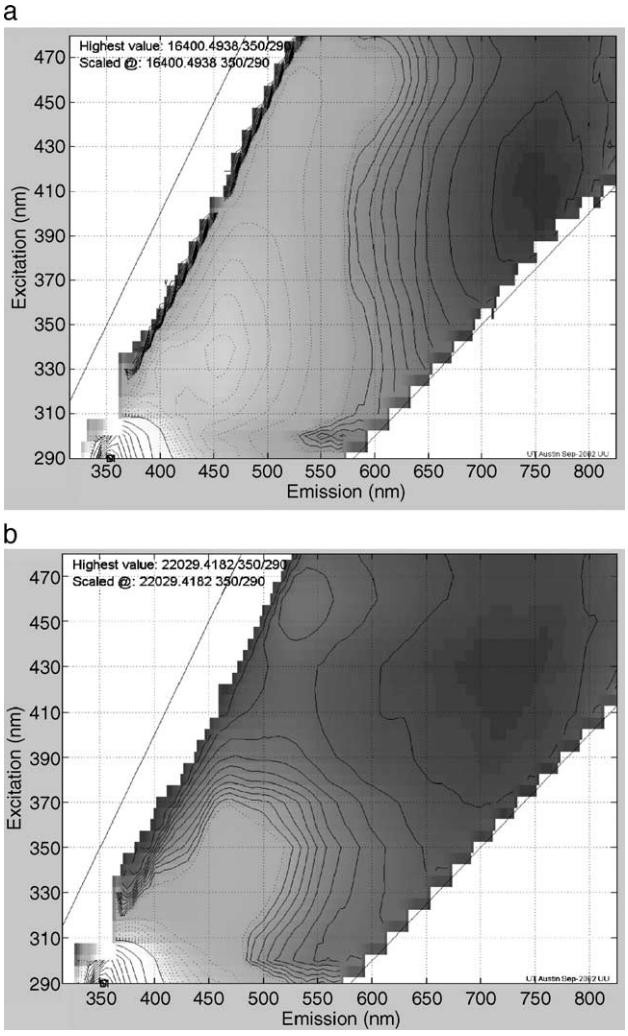

Fig. 2. Fluorescence excitation–emission matrices measured from (a) a squamous normal site and (b) *carcinoma in situ* of a single patient. The *y*-axis and *x*-axis show the excitation and emission wavelengths, respectively.

Table 1
Number of clinical measurement in three diagnostic categories by age of patients (SN = squamous normal, LGSIL = low grade squamous intraepithelial lesion, HGSIL = high grade squamous intraepithelial lesion)

	SN	LGSIL	HGSIL	Total
Premenopausal < 40 years old	205	64	121	390
Premenopausal > 40 years old	29	9	14	52
Postmenopausal	41	6	4	51
Total	275	79	139	493

Using the mathematical model, we estimated the tissue optical characteristics of a subset of 493 clinical measurements from 292 patients as described in Table 1. We evaluated the diagnostic significance of each of the estimated optical properties.

Results

As has been demonstrated in other studies, we found that increased epithelial light scattering and stromal light absorption due to hemoglobin are statistically significant indicators of dysplasia. Decrease in epithelial NADH fluorescence and increase in epithelial FAD fluorescence were weakly correlated with dysplasia. Decrease in stromal fluorescence from the collagen crosslinks was observed in abnormal tissue compared to normal tissue. Matrix metalloproteinase (MMP) expressed by dysplastic cells may have a potential role in degradation of extracellular matrix and the subsequent reduction in stromal fluorescence [7].

Interestingly, epithelial light scattering and stromal light absorption by hemoglobin showed statistically significant differences with age. Collagen fluorescence in the stroma also increased with age, as has been demonstrated in previous studies. Although the biological causes behind these age-related changes need to be studied further, it emphasizes the need to understand changes in tissue optical characteristics related with various biographical variables in order to identify those changes that are directly relevant to diagnosis.

Conclusions

Fluorescence spectroscopy can provide real-time, cost-effective, quantitative, and noninvasive diagnosis. Optical characteristics of tissue such as light scattering and absorption properties and the relative concentration of

endogenous fluorescent molecules measured with optical technologies can be used to monitor the tumorigenesis process. In order to estimate these optical characteristics from clinical measurements, we have developed a mathematical model of light interaction in the tissue epithelium and stroma. A number of optical characteristics estimated from a subset of our clinical data show statistically significant differences between diagnostic classes. However, other biographical variables such as age can also lead to changes in tissue optical characteristics. Further identification of diagnostically relevant changes in optical characteristics can lead to the development of robust diagnostic algorithms.

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