In vivo detection of cervical intraepithelial neoplasia by multimodal colposcopy

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ABSTRACT

Cervical cancer is the leading cause of cancer death for women in developing countries. Colposcopy plays an important role in early screening and detection of cervical intraepithelial neoplasia (CIN). In this paper, we developed a multimodal colposcopy system that combines multispectral reflectance, autofluorescence, and RGB imaging for in vivo detection of CIN, which is capable of dynamically recording multimodal data of the same region of interest (ROI). We studied the optical properties of cervical tissue to determine multi-wavelengths for different imaging modalities. Advanced algorithms based on the second derivative spectrum and the fluorescence intensity were developed to differentiate cervical tissue into two categories: squamous normal (SN) and high grade (HG) dysplasia. In the results, the kinetics of cervical reflectance and autofluorescence characteristics pre and post acetic acid application were observed and analyzed, and the image segmentation revealed good consistency with the gold standard of histopathology. Our pilot study demonstrated the clinical potential of this multimodal colposcopic system for in vivo detection of cervical cancer.

Keywords: multimodal imaging, multispectral, autofluorescence, colposcopy, cervical intraepithelial neoplasia

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1 INTRODUCTION

Cervical cancer is the second most common type of cancer in women worldwide.[1] It is estimated that nearly 380,000 new cases are diagnosed each year, with more than 80% of the occurrences in developing countries.[2] Early detection and treatment of precancerous lesions will prevent most cases of cervical cancer. Conventional clinical approaches to identify cervical intraepithelial neoplasia (CIN) include examination of Papanicolaou (Pap) smear, colposcopy detection and directed biopsy. A fundamental part of the colposcopic exam is the application of acetic acid, and the transient whitening changes that occur after acetic acid treatment are regarded as an important indicator of dysplastic cervical squamous epithelium. Although this strategy has decreased the incidence of cervical cancer in many developed countries, many developing countries still lack the necessary infrastructure and resources for rapid, extensive and low cost screening.
In the past years, development of optical imaging technologies have opened a new avenue for rapid, wide-field, and noninvasive assessment of cervical pre-cancer.[3-5] A number of imaging technologies have been investigated to enhance the contrast between normal and abnormal area based on the changes in optical properties of neoplastic tissue. Among them, multispectral reflectance imaging acquires a series of images with global spectral information at multiple wavelengths, which can provide insight into tissue characteristics such as scattering particle and absorbing chromophore concentration, particularly hemoglobin concentration and oxygenation levels.[6] Similarly, cervical autofluorescence imaging has shown its promise for effective discrimination between normal and abnormal tissue in vivo. Fluorophores including NADH, FAD, keratin, tryptophan, elastin, and collagen exist in tissue and their concentration changes with various diseases.[7] Simply speaking, over the excitation wavelength range for ultraviolet (UV) (~330-370 nm) and green (~510-550 nm) sources, normal cervical tissue gives rise to brighter fluorescence in stroma than that of cancerous tissue, which can be exploited to determine the presence of pre-cancerous lesions.[8, 9] Combining these two imaging modalities, multimodal imaging can provide a comprehensive assessment of multiple tissue parameters.[10, 11] Chang et al. compared the performance of reflectance and fluorescence spectroscopy alone with that in combination and found that algorithms based on fluorescence spectra alone yield better diagnostic performance than those based on reflectance spectra alone, the addition of fluorescence information to reflectance spectra showed modest improvement of diagnostic performance.[12] However, this study was limited to single point probe-detection and lacked global imaging information of the tissue. Gustafsson et al. acquired the hyperspectral reflectance and fluorescence data cube of the entire cervical tissue and demonstrated the spectral difference between different lesion types.[13] However, such a multimodal imaging system and the image co-registration algorithm are cost ineffective and time consuming, inappropriate for the clinical application of CIN screening, particularly in developing countries that have the greatest need for such technologies.

To overcome the limitations, we proposed a multimodal imaging device that combines multispectral reflectance imaging, autofluorescence imaging and RGB color imaging for real-time assessment of cervical cancer at low cost. In this device, RGB imaging functions as a conventional colposcopy; multispectral reflectance imaging detects the functional characteristics of cervical tissue; and fluorescence imaging reveals the molecular signature of the lesion. We use only a single camera for all the imaging tasks without the need for co-registration. In this paper, we first optimized the working wavelengths for reflectance and autofluorescence imaging based on optical model for light transportation in cervical tissue and spectral characteristics. Our multimodal device is capable of acquiring RGB, autofluorescence, and multispectral images consequently, which allows dynamic assessment of cervical tissue acetic acid whitening process. Advanced image processing algorithms were developed for effective differentiation between squamous normal (SN) and high grade (HG) types of cervical tissue. The image segmentation revealed good consistency with gold standard of histopathology. Our pilot study demonstrated the clinical potential of using this multimodal colposcopic system for in vivo detection of cervical cancer.

2 MODEL-BASED ANALYSIS AND WORKING WAVELENGTH SELECTION

We established a cervical optical model as shown in Figure 1 to determine the working wavelengths for instrument design. Cervical tissue is comprised of an epithelial layer on the top and a stroma layer at the bottom. The development of cervical pre-cancer leads to changes in the structural and optical properties of both layers[1]. The properties of the
SN and HG tissue are anisotropy $g=0.95$ for the first layer and $g=0.88$ for the second layer, refractive index $n=1.4$ for both layers. The absorption coefficient ($\mu_a$) and the scattering coefficient ($\mu_s$) of SN and HG tissue between 500 nm to 600 nm were available in the literature and were reproduced in Figures 2 (A) and (B) with an assumption of 85% hemoglobin oxygen saturation.[14]

![Diagram](http://proceedings.spiedigitallibrary.org/)  
**Fig. 1** Geometry of the cervical tissue model. The cervical tissue is composed of two layers: epithelium and stroma. Corresponding optical and structural parameters are shown on the figure.

### 2.1 Determination of multispectral reflectance imaging wavelengths

As a flexible approach to study photon propagation, a modified two-layered Monte Carlo (MC) simulation based on Ref. [15] was developed for tissue diffuse reflectance simulation between 500 nm and 600 nm in 2 nm increments.[15] To minimize the number of wavelength used for reflectance imaging, a wide gap second derivative method was employed to analyze the simulated reflectance from MC simulation. This method was capable of removing both baseline offset and linear slope due to the wavelength-dependent scattering.[16] Meanwhile, the measurement error resulted from non-uniform illumination can also be corrected. With a fixed wavelength gap interval $\Delta \lambda$, the second derivative value of wavelength $\lambda$, i.e. $R(\lambda)^\prime\prime$ was calculated by the reflectance intensity $R$ of three wavelengths as follow:

$$R(\lambda)^\prime\prime = \frac{R(\lambda + \Delta \lambda) + R(\lambda - \Delta \lambda) - 2R(\lambda)}{2\Delta \lambda} \tag{1}$$

Then, proper wavelengths set and gap interval $\Delta \lambda$ were derived based on the wide gap second derivative spectrum to extract the difference of reflectance between normal and abnormal cervical tissue. A screening filter as described in Ref. [17] was employed to acquire the best spectral configuration with superior capability of tissue classification.[17]
Figure 2 (A) Absorption coefficients of SN and HG tissue. (B) Scattering coefficients of SN and HG tissue. (C) Cervical reflectance spectra resulted by MC simulation from 500 nm to 600 nm. (D) The second derivative reflectance spectra of normal and abnormal tissue. Three optimal wavelengths are marked on the plot.

Figure 2(C) shows the relative reflectance intensity of MC simulation from 500 nm to 600 nm. The second derivative reflectance spectra are plotted in Figure 2(D). Based on the screening filter described in Ref. [10], the combination of wavelength 545 nm, 560 nm, and 575 nm will make a significant discrimination between normal and abnormal cervical tissue, which means only five wavelengths ranging from 530 nm to 590 nm with an interval of 15 nm were selected for multispectral reflectance imaging.
2.2 Determination of cervical autofluorescence imaging wavelengths

Fig 3 (A) Fluorescence excitation emission matrix (EEM) with the black box on the left-bottom of the matrix indicating the potential Excitation-Emission arrangement to distinguish normal and abnormal cervical tissue. (B) Fluorescence spectra at 365 nm excitation, showing the fluorescence intensity disparity between normal and CIN2 tissue from 420 nm to 480 nm.

We investigated previous studies to determine the fluorescence excitation and emission arrangement in our system. Intuitively, cervical autofluorescence data can be represented as an excitation-emission matrix (EEM), where the emission spectra at the various excitation wavelengths are concatenated into a 2-D matrix so that the calibrated fluorescence intensity can be expressed as a function of excitation and emission wavelengths. Figure 3(A) shows a typical cervical fluorescence EEM reproduced from Ref.[18] Furthermore, to determine the specific excitation wavelength and collection wavelength range for our colposcopy designing, we referred to the work reported by Parker in 2002, where an excitation wavelength at 365 nm and effective emission range from 445 to 475 nm were used for tissue anomaly differentiation. [19] As shown in Figure 3(B), the fluorescence spectra of normal and abnormal tissue at 365 nm excitation are compared. Quantitatively, the SN tissue has a 61.17 % larger envelope area in the fluorescence emission curve than that of the abnormal tissue, corresponding to the brighter region in the acquired autofluorescence images. Based on the above analysis and practical optical components availability, we determined the excitation wavelength to be 365 nm and the fluorescence receiving range from 420 nm to 480 nm.

3 MATERIALS AND METHODS

3.1 Instrumentation

A multimodal colposcopy system as depicted in Figure 4(A) has been developed for comprehensive assessment of cervical tissue lesions. The system is composed of three modules: (1) a colposcopy consists of a multispectral narrow band LED light source, a bandpass fluorescence filter with rotatable holder, and a monochromatic CCD camera; (2) a multi-channel light source controller used for power supply and channel selection; (3) a laptop used for system control and data acquisition.
The multispectral light source contained eight groups of specific narrow band ultra-bright LEDs (Xilan photoelectricity group, China) including 365nm, 475nm, 530nm, 545nm, 560nm, 575nm, 590nm, and 635nm wavelengths. Each group consisted of eight same LEDs, which were distributed equably on a ring-form aluminum substrate as shown in Figure 4(B). Among them, the 365 nm LEDs were used for autofluorescence excitation, the 475nm, 545nm, 635nm LEDs were used for RGB image illumination, and the rest LEDs were used for multispectral illumination. The band pass filter (420-480 nm band pass, Rayan Technology CO., LTD. China) with a rotatable mount was used for cervical tissue autofluorescence collection. When the excitation light (365nm) was on, the filter would be rotated and block the optical path, otherwise the filter would be off the optical path. The monochromatic CCD camera (Microvision Digital Imaging Technology Co. Ltd, China) with a 50 mm focal prime lens was used for imaging with a maximum resolution of 1392×1040 pixels. The light source controller (OPT Tech Co. Ltd, China) was connected to the laptop via RS232 serial port for intensity adjustment and channel selection, which was capable of providing 24 Volt power supplies for 8 channels separately.

For the sake of exposure control and image transmission, the camera was connected to an image grabber through a 1394A port. The operation of the whole system was controlled using a LabVIEW (National Instruments, TX, USA) based program running on the laptop. Figure 3(C) shows the photography of the multimodal colposcopy system.

### 3.2 Clinical protocol

The clinical protocol was reviewed and approved by the Institutional Review Board (IRB) of the Second Affiliated Hospital of Chongqing Medical University (IRB No: 2013KLS002). Eligible patients included those over the age of 18 who were not pregnant and who were referred to the colposcopy clinic with an abnormal liquid based cytology result. All patients signed an informed consent form and underwent a loop electrosurgical excision procedure (LEEP) after images acquisition. Before formal acquisition, a 4 mm × 4 mm white Teflon board with black “+” form marker, which
was used for assisting focusing and spectrum correction, was placed on the cleaned cervical surface. The spectral characteristics of Teflon board were calibrated in advance by a NIST traceable white diffuser (NIST, Gaithersburg, MD). After fine-focused and pre-exposure procedure, the Teflon board was removed. The clinical collection protocol followed consecutive steps as described below.

1) Acquiring the background images with the environmental lights on and the colposcopy light off.

2) Acquiring multispectral reflectance images, autofluorescence images, RGB images, and green channel enhanced images sequentially. Each imaging session was triplicated in order to reduce the measurement error.

3) Applying acetic acid (5%) on the cervix for 50 seconds and capturing RGB images at 60s, 90s, 120s, 150s, and 180s respectively. Considering the practical operation situation, cervical autofluorescence images was captured only at 180s.

4) Recording the RGB images as Lugol’s iodine was applied.

After the above multimodal imaging session, the ectocervix was removed by a LEEP procedure for pathologic analysis to differentiate between normal (normal epithelium, inflammation, and CIN 1) and HG (high grade, including CIN 2 and CIN 3) tissue types.
3.3 Image processing

3.3.1 Image preprocessing

For the multispectral reflectance images, the first step of image preprocessing was to remove noise interference by image average and background subtraction as Equation (2), where \( R_{\text{raw}}(\lambda) \) denotes the reflectance intensity of raw images collected by our system, \( R_{\text{bg}}(\lambda) \) denotes the background images acquired with constant room lights. \( \bar{R}(\lambda) \) was averaged from three continuous acquired images for denoising.

\[
\bar{R}(\lambda) = \frac{1}{3} \sum_{n=1}^{3} \frac{R_{\text{raw}}(\lambda) - R_{\text{bg}}(\lambda)}{3}
\]

Then, the multispectral reflectance images were corrected using a Teflon board to remove the influence of inconsistent reflectivity at different wavelengths. The corrected reflectance \( R(\lambda) \) is expressed as Equation (4), where \( R_{\text{board}}(\lambda) \) is the mean intensity of a ROI selected on the surface of the Teflon board.

\[
R(\lambda) = \frac{\bar{R}(\lambda)}{R_{\text{board}}}
\]

For autofluorescence images, background subtraction and image average were also performed for denoising. Then the histogram was equalized for better contrast.

For RGB images, one color image was generated by recoding three 8-bit monochrome images acquired at red, green, and blue illumination into a 24-bit true color image, which realized visualize the color and morphological characteristics of cervical surface.

3.3.2 Image classification algorithm

In order to identify the abnormal areas on cervix, each pixel of a cervical image was classified to be either SN or HG according to a classification algorithm. Our approach for image segmentation consists of two steps. First, a supervised classification algorithm was performed utilizing the second derivative multispectral reflectance images. Second, a simple correcting process was further performed using autofluorescence images.

In the first classification step, the classifier was trained using leave-one-patient-out cross-validation. The training sets including background (BG), squamous normal (SN), and high grade (HG) were selected on a patient with local CIN 2/3 lesion and then applied to the held-out patient. Then three 2nd derivative images were deduced from the raw reflectance images at five specific wavelengths according to Equation (2). The absolute values of three derived intensities were summed and a minimum Euclidean distance (MD) segmentation algorithm was adopted for classification, which can be expressed as:
where $R_{si}$ is the summed second derivative value of each pixel on the image and $R_{tx}^i$ is the training data of three tissue types, here $x = (1, 2, 3)$ indicates the index of the three different tissue categories. $D_{Ex}$ is the Euclidean distance and $C_x$ indicates that the $R_{si}$ is classified to the corresponding category by evaluating the minimum value $D_{Ex}$. The classifier designed with training set data was then used to classify entire images into corresponding categories. While in the second step, the autofluorescence images was binaryzation processed to create a mask for first-step results correction, where the threshold was determined by the disparity between normal tissue intensity and abnormal tissue intensity. As a comparison, we show the segmentation results with and without autofluorescence correction in the results.

## 4 RESULTS AND DISCUSSION

### 4.1 Patients characteristics

Total 48 patients were measured at Second Affiliated Hospital of Chongqing Medical University. However, 11 patients were excluded from the study because of the lack of biopsy results or the poor imaging quality due to motion artifacts. The other 37 data sets consist of a series of multimodal images dataset that were deemed adequate for both reflectance and fluorescence images analysis by independent reviewers. Among them, 5 patients (13.5%) with acute/chronic inflammation, metaplasia or CIN 1 were included in the normal category, and 32 patients (86.5%) with any CIN 2/3 diagnostic were included in the corresponding abnormal category.
4.2 Imaging characteristics

4.2.1 Multimodal images dataset

A typical raw multimodal images dataset is shown in Figure 5. The figure is arranged by the acquisition steps from top to bottom. The top row displays the cervical images without applying any agent, including background image without light illumination, RGB image of cervix, green channel enhanced color image, multispectral reflectance images, and autofluorescence image. The second row illustrates the post-acetic acid images of cervix at 60s, 90s, 120s, 150s, and 180s respectively, which will be used for dynamic analysis. The third row show a RGB image post-Lugol’s iodine.

One advantage of acquiring images in multiple modalities using an integrated imaging system is the elimination of the time-consuming image co-registration process. Besides, since all the images were acquired by a single imaging module at preset operating parameters, the potential mismatch between different imaging modalities can be eliminated and the testing time for each patient can be further reduced.
4.2.2 Dynamic imaging of acetic acid-induced whitening phenomenon

Dynamic acetic acid-induced whitening phenomenon was witnessed on examined patients with CIN 2/3 diagnosis. To quantitatively analyze the temporal kinetic of cervical reflectance characteristic during acetowhitenining process, the multispectral reflectance intensity was averaged at every wavelength and compared between normal and CIN 2/3 sites.

Figure 6 illustrates mean of relative reflectance intensity versus time curves, obtained from all 32 positive cases with both normal and CIN 2/3 sites according to biopsy results. In normal sites, the reflectance intensity shows a moderate increase at 60 s relative to pre-acetic acid, followed by an almost constant value over the last time. However, the CIN 2/3 sites are characterized by a dramatic increase in the first 60 s, followed by a continuous decrease. It can be seen that the standard error of each data is large because of individual patient tissues vary considerably. Physiologically speaking, this reflectance intensity change is thought to be result from scattering property changes in the cell nucleus.[20, 21] The application of acetic acid enhances the difference between normal and abnormal tissue by altering the index of refraction in the cell nucleus. This feature may be further researched to distinguish cervical lesion.
From the view of autofluorescence characteristic, the fluorescence intensities pre and post application of acetic acid (AA) are plotted in Figure 7, where the intensities are normalized and averaged from ROIs on normal and CIN 2/3 cases. One can see that the fluorescence intensities of both two types of tissue show slight decrease after application of acetic acid, although it is not easy to visualize from the multimodal images dataset. The explanation of this decrease presumably due to increased scattering interfere the penetration of fluorescence, and changes in pH environment may also influence fluorescence signal as the intensity from normal tissue also decrease.[22]

4.2.3 Image segmentation results

Based on the multispectral reflectance data, the image classification algorithm was applied for image segmentation between normal and abnormal cervical tissue types. In addition, autofluorescence images were also analyzed for supplementary correction of the segmentation results. Furthermore, the classification results of each patient’s images were compared with that of the gold standard histopathology. The training sample was from a patient with local CIN2/3 lesion on 5 and 8 o’clock. For each classification category, three 20×20 pixels windows on corresponding areas were selected as training data set.
Figure 8 shows the images and analyzed results of a patient with a CIN2/3 lesion at 7 o’clock and 8 o’clock according to the histopathologic result. The upper row images are RGB images of cervix without applying any agent, with green channel enhanced, with acetic acid, and with Lugol’s iodine, respectively. The lesions can be visualized empirically on Figures 8(C) and 8(D), where a whitening area post-acetic acid and a lightly dyed area post-Lugol’s iodine were revealed. Figure 8(E) shows the second derivative image of the multispectral dataset. Based on this data, the segmentation results of the classification algorithm when classifying squamous normal (SN), high grade (HG) tissue and background (BG) can be seen in Figure 8(F). The green areas correspond to SN, the red areas to HG and the blue areas to BG. According to Figure 8(F), one can see tolerable correlation between the classification results and the pathology results despite of some false positive area at 5, 6, 11, and 12, o’clock. Furthermore, Figure 8(G) shows the autofluorescence image at 365 nm excitation, when take the diagnostic effect of autofluorescence into account, the segmentation results were modified as shown in Figure 8(H), which reveals a better segmentation image corresponding to the pathology results.

According to the above analysis, information provided by RGB imaging, multispectral imaging, and autofluorescence imaging is complementary. RGB imaging is an empirical procedure that reveals some but not all information about tissue physiopathologic condition. Particularly, the unrealistic color pattern of the RGB images as shown in Figures 8(A-D) are caused by the unbalance illumination and detection at different wavelengths and can be further fixed by optimizing the LED and the detector designs in the future. Multispectral imaging reveals tissue functional properties while autofluorescence imaging reveals tissue molecular characteristics. In Figure 8(F) multimodal imaging yields a region of tissue anomaly greater than that of pathology, indicating that classification strategy is over-trained that may lead to lower specificity but higher sensitivity. By enhancing the autofluorescence image in Figure 8(G), one is able to
obtain appropriate classification of the lesion location coincident with pathology, as shown in Figure 8(H). In addition to classification, we have also explored several other image processing approaches for effective interpretation of multimodal colposcopic images.[23-27] As a comparison, the classification algorithm implemented in this system shows its diagnostic potential by combining cervical reflectance and auto fluorescence characteristics together. However, the quantitative discrimination algorithm especially based on the autofluorescence characteristic should be studied in future.

5 CONCLUSIONS

In this paper, we presented a multimodal colposcopy system for \textit{in vivo} CIN detection, which was capable of consecutive multispectral reflectance, autofluorescence, and RGB imaging of cervical tissue. This study shows preliminary but promising results by combining multispectral reflectance, autofluorescence and RGB imaging together for \textit{in vivo} detection of CIN. Cervical reflectance and autofluorescence spectra were studied for wavelengths optimization. An approved clinical protocol was performed and a diagnostic algorithm based on cluster segmentation was developed for results evaluation. In the results, a typical data collection including RGB images, autofluorescence image, and multispectral segmentation image was used to identify cervical tissue as squamous normal (SN) and high grade (HG) type. The kinetics of acetic acid-induced whitening phenomenon was compared and analyzed between SN and HG tissue. The pilot study demonstrated the potential of this multimodal colposcopy system in cervical cancer detection. The low-cost, and portable characteristics of the device, the simple operating process, and the automatic image segmentation algorithm of the developed multimodal colposcopy enhance the possibility to address the needs for CIN early screening in a rapid, cost-effective, and non-invasive fashion in the developing countries. The future work will focus on testing large quantity of patients for statistical analysis, enhancing the accuracy of classification algorithm and improving the system construction and design towards a low-cost, rugged, and portable device.

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