Detection and localization of the hemoglobin and collagen distribution of the uterine cervix

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Changes of the blood vessels and collagen are associated with the development of abnormal cervical cells. Recently, optical coherence tomography and Mueller polarization images were used to provide information regarding the presence of collagen fibers in the cervical tissue. However, most of these methods need a lot of time for image recording and are expensive. In addition, the general survey on the absorption and distribution characteristics of collagen and blood in the cervical is still lacking. In this study, we developed a colposcopy combining cross-polarized image and image processing algorithm with an efficient analytical model to map the distribution of blood and collagen in the uterine. For this system's proof of concept, we captured and processed the case of cervical ectopy and Nabothian cyst. The results show that the distribution of blood and collagen maps matched with anatomical and physiological when compared with Lugol's iodine images. This technology has some advantages, such as low cost, real time, and can replace the use of acetic acid or Lugol's iodine in the future.

Keywords: Hemoglobin; collagen; cross-polarized image; uterine cervix.

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1. Introduction

Cervical cancer is the second most common cancer among women in the world.^{1–4} In 2012, there were about 528,000 newly diagnosed cases and approximately 266,000 deaths from cervical cancer worldwide.^{5,6} The proportion of cervical cancer is highest in developing countries, which makes up approximately 85%. The main reasons are inadequate prevention policy, lack of infrastructure and resources.^{1,3,7} The incidence of cervical cancer depends on the stage of cancer. If detected early and treated promptly, cervical cancer can be completely cured. The patient's 5-year survival rate is over 90%^{8,9} therefore, early detection of cervical pathology as well as changes in cervical cancer is the most effective method to improve the patients' quality of life and reduce the death rate due to cancer.^{9,10}

Currently, the primary screening tool for cervical cancer prevention is the Pap test, i.e., the visual inspection of cells obtained from the ectocervix.^{11,12} When the Pap test result is abnormal, a colposcopist will examine the cervix of the patient through a low magnification microscope (called a colposcope).^{8,11,13} Colposcopy is often accompanied with the application of acetic acid 3–5% (visual inspection with acetic acid — VIA) and Lugol's iodine (visual inspection with Lugol's iodine — VILI) on the surface of the cervix, which helps to see more clearly the dysplastic regions then biopsied to get a pathology diagnosis.^{14,15} However, this method is quite subjective.^{16–18} Its accuracy depends mainly on the experience and expertise of colposcopist.¹⁹ Thus, approximately, one-third of high grade disease is missed by first colposcopy,²⁰ or patients may be referred for unnecessary biopsies.^{21,22} These avoidable biopsies increase the risk of infection, discomfort in patients, prevent women from attending regular screening and significantly increasing costs.^{23,24} In addition, colposcopy is only effective when detecting superficial lesions around the external cervix orifice, while a significant number of the cervical cancer cases are associated with lesions in the cervical canal.⁸ Therefore, we need to develop new cervical pathology diagnostic technologies with greater accuracy, larger scanning regions, deeper penetration and lower costs.

For past several decades, many optical techniques have been studied to improve the effectiveness of colposcopy, such as fluorescence imaging, spectrally resolved reflectance, *in vivo* confocal microscopy or

optical coherence tomography (OCT).^{2,22,25–30} Another technique is the polarimetric imaging and it is receiving a lot of attention. It is sensitive to morphological changes in tissue structure; it is a noninvasive technique; the image has wide field and cost for light source is cheap.³¹ Polarized images offer tremendous potential for biomedical applications, which distinguish between healthy and pathological regions³² on several types of tissues, such as skin,³³ intestine,³⁴ colon,³⁵ rectum³⁶ and cervix.^{31,37–39} Li *et al.* studied the use of a digital colposcope, which acquires polarized and nonpolarized color cervical images during a clinical exam.³⁷ Several image processing algorithms are used to locate the acetovhite lesion. Pierangelo *et al.* studied the polarized image of cervical samples, both *in vivo* and *ex vivo*.³⁸ The results of this study showed an unexpected optical anisotropy of normal cervical tissue and it was verified by full Mueller imaging of *ex vivo* conizations. Another study by Rehbinder *et al.* obtained maps of the scalar retardance of the uterine cervix from polarized images.³¹ These maps are used to distinguish pre-cancerous zones of high-grade CIN 2-3 (Cervical Intraepithelial Neoplasia 2-3) from healthy zones.

A number of recent studies have shown that to improve real-time *in vivo* diagnosis of epithelial precancers and cancers, researchers used optical spectroscopic and imaging techniques.^{40–43} Optical properties of tissues such as absorption, scattering and fluorescence properties are considered to investigate morphological and cytological changes.² Indeed, changes in the absorption characteristics of oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) will alter the diffusely reflected component of the incident light from cancerous cervical tissues.⁴⁴ Thus, multi-spectral reflectance images at the absorption peaks of oxyHb and deoxyHb were investigated to analyze the region of interest (ROI), where tissue transitions to malignant state.⁴⁵ This provides important information for biopsy location and early detection of cervical cancer.

In addition, most abnormalities for the onset of cervical disease as well as pre-cancerous epithelial growth are characterized by structural degradation of collagen fibers in nearby connective tissue.^{31,44,46} Light scattering also decreases near the dysplastic epithelium because collagen structure is degraded and its mass decreases.¹⁰ Another cause associated with the development of cervical lesions is blood vessel changes in the cervical tissue, which is usually

the generation of abnormal new blood vessels.^{44,47} Therefore, collagen and blood vessels are two important components in cervical tissue because their changes are associated with the development of abnormal cervical cells. However, the general survey on the absorption and distribution characteristics of collagen and blood is still lacking. Bancelin *et al.* determined the orientation of collagen fibers in histological slide by using the Mueller polarized image (it is enhanced by Picrosirius red staining) and confirmed by second harmonic generation imaging.⁴⁸ Gan *et al.* proposed an automated registration method to stitch multiple OCT volumes.⁴⁹ It allows the study of the ultrastructure and collagen fiber dispersion of human cervical samples. Vizet and his coworkers fabricated the first wide field Mueller Polarimetric Colposcope (MPC) for *in vivo* analysis of the uterine cervix.³⁹ They proved that the Mueller polarization image can clearly distinguish two major types of epithelia (malpighia and glandular) on the healthy uterine cervix. On the other hand, Mueller polarization images also provide information regarding the presence of collagen fibers in cervical tissue. The above researches are helpful in examining the malignant cervical epithelium through the orientation or distribution of collagen fibers. Nevertheless, most of these methods need a lot of time for image recording and are expensive. It is not suitable for deployment in developing countries and rural regions of limited resources.

In this paper, we exploit the unique optical properties of cervical tissues including the absorption and distribution of blood vessels and collagen in cervical tissue. A digital colposcope is designed to acquire high-resolution images with cross-polarization

technique. During a clinical exam, cross-polarized images of cervix before and after applying Lugol's iodine were recorded. Color-reflective images with white light can be separated into color-specific channels that provide detailed information on the absorption and scattering properties of oxyHb, deoxyHb and collagen fibers. A series of image processing algorithms have been developed and applied to enhance morphological features that may exist, such as Blood Contrast and Collagen Contrast. Specific images for blood vessel morphology and collagen distribution are generated. This is really significant in detecting lesions in the cervical canal, more reliable diagnosis, minimization of the variability among colposcopists and the false positive rates leading to unnecessary biopsies. In the results section, we will give examples of experiments on human cervical samples with cervical ectopy and Nabothian cyst. Finally, the results are verified by the cervical image with Lugol's iodine. This method brings a new approach, which results in real-time, is low cost and can replace the use of acetic acid or Lugol's iodine in the future.

2. Methods

2.1. Device design

The device shown in Fig. 1 is based on classic colposcope which enables the illumination and observation of the uterine cervix easily. The components of the device include two main parts: imaging system and light source.

For the imaging system, the used lens has the focal length range 18–35 mm and lens focus distance of 150–350 mm which helps us have a deeper and

Fig. 1. Polarized light source for colposcope: (1) Region of interest; (2) The first polarizer; (3) LEDs, (4) The body of light source and (5) Analyzer.

better cervical tissue observation. In addition, the utilizing of the auto-focusing full HD camera with the auto-focusing within 1 s leads to a quick imaging acquisition to help volunteers feel more comfortable. The image sensors for this camera is complementary metal oxide semiconductor (CMOS), hence the imaging system are not offered by numerous original equipment. Besides, this camera provides smooth color images with the size of 1080×1920 pixels allowing us to have clear cervical tissue observation. In order to produce the color images, the camera separates the entering white light into red, green, and blue channels. The most obvious solution is to filter the entering white light into those channels to capture red, green and blue information and combine them into a full-color image. Each channel provides specificity for studies because of the different absorption of the cervical tissue constituents corresponding to disparate wavelength regions.

For the light source, we use three green LEDs and three white LEDs, shown in Fig. 2, placed symmetrically and alternately to make a concentric illumination ring having diameter of 40 mm on the cervix ROI. We use Cree XLamp® XP-G2 LEDs 5W for white light and Cree® XLamp® XP-E LEDs 3W for green light that provide not only high lumen output in a small package but also wide viewing angle up to 115° . As a result, these LEDs create a small optical source for excellent optical control and bring high performance and quality of light. The combination of three LEDs generates more than enough light intensity with a 30° opening angle to fully illuminate cervical tissue observation and its surroundings, reaching 10,000 Lux measured

by Lutron LX-101 Lux Meter at distance of 300 mm equal to the working distance. In order to generate the uniform light distribution on the ROI, the first polarizer is placed in the front of the LEDs meanwhile the analyzer is placed in the front of the lens, which leads to cross-polarized light passing the image acquisition system. In addition, this polarizer system minimizes specular reflection significantly in comparison with images captured by traditional colposcope. It can use green light source in order to get green cross-polarized images for red blood cells detection by eyes, or white light source to get white ones for image processing. The whole LED system is placed near the focal plane of the lens at working distance of 300 mm which is the mean distance between the colposcope and the cervix in practice.

2.2. Light and tissue interaction

The epithelium and the stroma have a reciprocal relationship resulting in the cervical image.⁵⁰ The colorless epithelium on the surface of the cervix acts like a filter through which both incident and reflected light passes.⁵⁰ The stroma under the epithelium contains collagen and the capillaries supplying the tissue with the blood. If the light is illuminated to the tissue, only a small fraction will be directly reflected while most of the light will enter the epithelium and be either absorbed or scattered. In this study, linearly polarized incident light illuminated by the light source firstly interacts with the liquid layer — the most significant factor causing the glare on the cervical, where the incident light is partly directly reflected and partly passes through (Fig. 2). The reflected light preserves its state of polarization and is eliminated as mentioned above. The light passing through the liquid layer will then reach the epithelium layers on which the light divides two parts: directly reflected light remains its state of polarization like reflected light in the liquid layer and is eliminated by the same way and light transmits the epithelium layers and reach the stroma below having the presence of hemoglobin and collagen. In stroma, the light is either absorbed or diffusely scattered, thus backscattered light will be randomly polarized and then partly passes through the filter and reaches the detector.

The intensity of backscattered light depends on the structure of layers of the epithelium, the optical density of the epithelium (i.e., the morphology and organization of the epithelium), and the vascularity

Fig. 2. Propagation of polarized light in the uterine cervix.

Fig. 3. Anatomy of the uterine cervix and squamocolumnar junction.

and nature of the underlying stroma.⁵⁰ Squamous epithelium and columnar epithelium, shown in Fig. 3, are two kinds of epithelium on the surface of the cervix having different structures which leads to different optical properties. The squamous epithelium has 15–20 layers divided into four layers of cell types — basal cells, the parabasal layers, the intermediate layers and the superficial layers. Much of the incident light is reflected to the detector before reaching the stromal vessels because these layers function as a stratified barrier between light and stromal vessels. In comparison with the squamous epithelium, the columnar epithelium has only a transparent single layer in where the large portion of incident light can easily pass through and be absorbed by the stromal vessels. Furthermore, we can see that the surface of squamous epithelium is smoother than the surface of the columnar one which leads to significant optical difference between these epithelia. The junction between squamous epithelium region and columnar epithelium region is called squamocolumnar junction (SCJ). In this study, we consider the absorption spectrum of cervical tissue constituents (oxyHb, deoxyHb, collagen, and lipid) including water from 400–700 nm. This absorption spectrum according to data from Refs. 51–57 is shown in Fig. 4.^{51–57} However, we dismiss the influence of lipid and water because the absorption coefficients of water and lipid from 400–700 nm are many times as low as those of oxyHb, deoxyHb, and collagen. Three wavelength regions include wavelengths from 400–500 nm, wavelengths from 500–600 nm, and wavelengths from 600–700 nm (blue, green, and red, respectively). With the blue and green wavelength regions,

the spectrum suggests that oxyHb and deoxyHb exhibit a major absorption peak. With the red wavelength region, the absorption coefficient of deoxyHb reaches the highest figure while collagen has the secondary absorption peak.

2.3. Image processing

A spectral image can be considered as a three-dimensional image cube, where the two-dimensional image records the spatial information of a sample, and the third dimension represents spectral wavelength.⁵⁸ Digital cameras produce images in the RGB color space. An image from a standard digital camera will have a red, green and blue channels. Spectral information from the same image is seen

Fig. 4. The absorption spectrum of hemoglobin and collagen from 400–700 nm (reproduced from Refs. 51–57).

through a conventional three spectral images where the red channel can be roughly attributed to the 600–700 nm spectral range, green to 500–600 nm, and blue to 400–500 nm. In this study, cervical images acquired by the digital camera are separated into their three color matrices, red, green and blue channels by image processing software.

As mentioned above, the primary absorption spectra of constituents in the cervical come from hemoglobin and collagen in the stroma layer. As illustrated in Fig. 4, the hemoglobin absorption spectrum exhibits a major peak at around 430 nm, and a minor one at around 550 nm, which are close to the Blue channel and Green channel of white image. Collagen has a steadily linearly decreasing absorption trend in the spectral range from 500–700 nm. Light absorption by collagen is less than hemoglobin at wavelengths between 500–600 nm but higher at 600–700 nm. By subtracting the Red plane from the Green plane or the Blue plane with a factor, respectively, it is possible to subtract the influence of collagen absorption while maintaining the effect of hemoglobin absorption. Therefore, the spatial distribution image of hemoglobin can be estimated as Eqs. (1) and (2):

$$I_{\text{Blood1}} = \frac{K_1(\alpha_1 \times I_G - I_R)}{I_R + I_G + I_B}, \quad (1)$$

$$I_{\text{Blood2}} = \frac{K_2(\alpha_2 \times I_B - I_R)}{I_R + I_G + I_B}, \quad (2)$$

in which I_{Blood1} and I_{Blood2} are representatives of the spatial distribution image of hemoglobin in the cervical uterine. After reconstructing the image, we converted the image sum from matrix to uint8 which presented the properties of image with Jet color map.

I_R , I_G and I_B are representatives of red, green and blue channel images, respectively. We tried to increase or decrease the absorption value of hemoglobin and collagen by using weight factor $(\alpha_1, \alpha_2, \alpha_3)$. K_1, K_2, K_3 is an empirical factor fit to produce best distribution and contrast of hemoglobin and collagen in the surface of uterine.

As illustrated in Fig. 4, the absorption of collagen is higher than hemoglobin in the spectrum range 600–700 nm which is suitable with the red plane image. For this reason, the red image is used to produce the distribution collagen image in cervix uterine. By subtracting the minimum values intensity from the values intensity of the red image, it is possible to decrease the influence of hemoglobin absorption while maintaining the effect of collagen

absorption. Therefore, an image with enhanced contrast for collagen is created by performing the operation in Eq. (3):

$$I_{\text{collagen}} = \frac{K_3(\alpha_3 \times I_R - \min I_R)}{I_R + I_G + I_B}. \quad (3)$$

3. Results

3.1. Cervical ectopy

In this section, experimental results on cervical cross-polarized images of a 30 year old woman (performed at a local obstetrician's clinic) are presented and analyzed for some interesting points. The study was approved by the Institutional Review Board of the Vietnam National University, Ho Chi Minh City "135/DHQG-KHCN", and conducted according to the tenets of the Declaration of Helsinki.

The uterine cervix shown in Fig. 5 is characterized by the normal cervix with cervical ectopy visible to the naked eye and easily recognized by the doctor as a benign finding. Figure 5(a) is the image before the Lugol's iodine application, and Fig. 5(b) is the image after the Lugol's iodine application. The main symptom of cervical ectopy is when the columnar epithelium (1) inside the cervical canal (3) starts to spread outside the cervical surface and covers the squamous epithelium (2). The columnar epithelium appears red, which contrasts with the squamous epithelium that is pink. The transitional boundary between these two types of epithelial cells is called SCJ (4). The white spots on the images are corresponding to the camera's saturation pixels and the reflection of light on the surface of the cervix, are called glare (5). Both the original and mature squamous metaplastic epithelium stain mahogany brown or nearly black with Lugol's iodine solution because these cells contain abundant glycogen. In contrast, columnar epithelium does not produce glycogen. It does not change color after the application of Lugol's iodine or remains slightly discolored with a thin film of iodine solution. Thus, colposcopist can easily and quickly mark the boundary between the region of ectopy and the SCJ from color changes in the cervix after application of Lugol's iodine solution. In order to evaluate the truth information for the processed images, the SCJ was annotated using a computer-based drawing program (Adobe Photoshop CC 2017, Adobe Systems Inc., San Jose, California) on the image after

Fig. 5. *In vivo* cross-polarized RGB pictures of cervical ectopy: (a) Image before applying Lugol's iodine, (b) Image after applying Lugol's iodine, (c) SCJ is characterized dotted black lines, (d) Blood 1 Contrast, (e) Blood 2 Contrast, (f) Collagen Contrast, in which hottest color: columnar area (color map: jet). In which: (1) Columnar epithelium, (2) Squamous epithelium, (3) Cervical canal, (4) SCJ and (5) Glare.

applying Lugol's iodine by an expert colposcopist (Fig. 5(c)).

In the whole cervix, below both columnar and squamous epithelia regions, the distribution of hemoglobin and collagen is seen. However, each region has its own distribution depending on the structure of epithelium. The squamous epithelium is composed by many flatted cells arranged in about 20 layers which act as a barrier. The columnar epithelium is composed of a mono-layer of column-shaped cells. The structure of layers makes outstanding contribution to absorption of hemoglobin and collagen in stroma. The absorption characterization of squamous and columnar epithelia is the start point to enhance columnar epithelium contrast, detect SCJ, and determine the collagen and hemoglobin distribution. Firstly, using Eqs. (1) and (2), the distribution of hemoglobin is calculated, matches with the contrast enhancement of columnar epithelium region which is presented in Figs. 5(d) and 5(e). The columnar epithelium region characterized by hotter color corresponds to the higher concentration of hemoglobin. In comparison with the Lugol image (Fig. 5(c)), the hotter color region is almost similar to columnar epithelium region. The change of absorption can be a relevant

parameter to distinguish columnar and squamous epithelia. With the combination of blue and red channels (Fig. 5(d)), the columnar epithelium region is displayed sharper than the combination of green and red channels (Fig. 5(e)). Secondly, the collagen will easily absorb photon in the columnar epithelium region. Using Eq. (3), we calculate the distribution of collagen in cervical ectopy. As illustrated in Fig. 5(f), collagen is characterized by hotter color region on the whole cervix.

3.2. Nabothian cyst

In this result, *in vivo* measurement was performed on a 35 year old woman hospitalized for Nabothian cyst. As illustrated in Fig. 6(a), bleeding on the cervical os (1) and blood vessel region below are characterized by high-concentration hemoglobin which leads to the redness of this region. The Nabothian cyst region (2) is identified with some cysts that are observed by the naked eye. These cysts are smooth, appear white, and form on the surface of the uterine cervix.

With the blood contrast shown in Fig. 6(b), the bleeding region on the cervical os is characterized by the hottest color because the incident light is

Fig. 6. *In vivo* cross-polarized RGB pictures of cervical ectopy: (a) Image before applying Lugol's iodine, (b) Blood 1 contrast, (c) Collagen contrast (color map: jet). In which: (1) Bleeding region and (2) Nabothian Cyst region.

directly absorbed by hemoglobin on the surface while the blood vessel region is characterized by a little bit colder color due to the hemoglobin under the surface in this region which leads to the weaker hemoglobin absorption. On the contrary, the coldest color region indicates the Nabothian cyst region which means that hemoglobin concentration in this region is very weak. With the collagen contrast shown in Fig. 6(c), the bleeding region and blood vessel region below with high collagen concentration are characterized by the coldest color. In contrast, the Nabothian cyst region is defined with the hottest region especially the cysts observed by naked eye.

As illustrated in Figs. 6(b) and 6(c), the Nabothian cyst region is characterized by the lack of collagen and hemoglobin absorption because the volume of cysts is many times as big as that of the epithelia which act as a barrier reflecting almost incident light before they can reach the hemoglobin and collagen below. The hottest bleeding region in Fig. 6(b) is similar to the coldest bleeding region in Fig. 6(c) which shows the presence of hemoglobin and collagen in this region. Furthermore, there is a similar pattern of the Nabothian cysts between blood contrast and collagen contrast and the highest contrast enhancement of cysts is shown in Fig. 6(c) due to the lack of collagen.

4. Discussion and Conclusions

In this study, the combination of classical colposcope analysis and a series of image processing algorithms for *in vivo* analysis of the uterine cervix are presented. We have relied on the absorption and distribution of oxyHb, deoxyHb and collagen to detect and mark the location of healthy and diseased cervical tissue. Hemoglobin and collagen

are the two main factors that we exploit in this study because most abnormalities for the onset of cervical disease are the development of abnormal new blood vessels and structural degradation of collagen fibers in nearby connective tissue.

Cross-polarized images of the uterine cervix with cervical ectopy and Nabothian cyst were recorded by a digital colposcope. Datasets include both pre- and post-images applying Lugol's iodine. Then, a set of image processing algorithms were developed to enhance the contrast of blood and collagen, it is shown in Eqs. (1)–(3). Figures 5(d) and 5(e) are the results after enhancing the contrast of blood, which helps to indicate the extent of hemoglobin distribution in the uterine cervix. The area of cervical ectopy has more hemoglobin absorption than the normal region. Based on the post-processing image, the expert colposcopist can easily identify columnar epithelium, squamous epithelium and SCJ regions without the need for conventional methods such as applying acetic acid or Lugol's iodine.

The collagen map, illustrated in Figs. 5(f) and 6(c), shows that the distribution of collagen concentration in the cervix is similar to the distribution of hemoglobin concentration. Figure 5(f) confirms that the columnar epithelium is characterized by strong collagen absorption. In contrast, squamous epithelium was observed with weak collagen absorption. Furthermore, our results *in vivo* with Nabothian cyst have shown that the cysts were observed with very weak absorption of hemoglobin (Fig. 6(b)) and collagen (Fig. 6(c)). These observations can be used to detect some kinds of cervical diseases. The image after applying Lugol's iodine is used as a reference to confirm the accuracy of the data obtained. The similarity between the results in this study and the results from the VILI method have reassured the credibility of this approach.

We have presented a new approach with some notable features, such as noninvasiveness, real-time results, less expensive and easier to deploy in developing countries and rural regions with limited resources. This method is particularly useful for detecting lesions in the cervical canal, more reliable diagnosis, minimization of the variability among colposcopists and the false positive rates leading to unnecessary biopsies. The optical contrast between normal cervical tissue and precancerous lesions has been significantly improved. It is a promising future tool for early detection of cervical cancer. However, these results are preliminary, the sensitivity and specificity need to be evaluated on a large-scale clinical examination. In addition, three channels — red, green, and blue — were extracted from the polarized RGB images by the image processing software which leads to the pixel errors in these channels. Therefore, the absorption information for some pixels was missed. Future work will be dedicated to acquiring three channels — red, green, and blue — by using a multispectral approach that provides full absorption information in each channel.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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