Dual modal fluorescent colposcope combined with near-infrared fluorescent dye TMTP1-PEG4-ICG to detect cervical lesions

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Abstract: To examine the cervical lesions by using the tumor-targeted near-infrared (NIR) fluorescent dyes TMTP1-PEG4-ICG, a dual modal colposcope with visible reflectance imaging and fluorescence imaging was developed. NIR fluorescence imaging and visible light reflectance imaging were integrated together on a colposcope by designing the specific optics and adopting a dual sensor charge coupled device (CCD) camera. Patients with squamous cell carcinoma, adenocarcinoma, cervical intraepithelial neoplasia (CIN) and cervicitis were examined using this dual modal colposcope to validate its potential of cervical cancer detection. Fluorescent dye TMTP1-PEG4-ICG was applied to the cervix 30 minutes before inspection. The fluorescence images were collected after wiping the unbound fluorescent dye using normal saline. Signal to back ratios (SBR) of the fluorescence images were analyzed and compared with the histological analysis. The results suggest that the fluorescent colposcope combined with tumor-specific near-infrared fluorescent dyes TMTP1-PEG4-ICG could help to evaluate cervical lesions in real time.

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1. Introduction

Cervical cancer is a common gynecological malignancy and ranks the fourth for both incidence and mortality among all cancers in female worldwide. It was estimated that there were about 569847 new cases and 311365 deaths in 2018. Further, cervical cancer was the most commonly diagnosed cancer in 28 countries and was the leading cause of cancer death in 35 countries among women [1]. Compared to the USA and UK, China has lower cancer incidence but higher cancer mortality. The reason may be the low rate of early-stage diagnosis and non-uniformed treatment strategies [2]. The current 5-year survival rates for women with localized (confined to primary site), regional (spread to regional lymph nodes), and distant (metastasized) cervical cancers are 91.8%, 56.3%, and 16%, respectively [3]. Therefore, improving the rate of early diagnosis of cervical cancer is crucial for the reduction of mortality. Cervical cytology and HPV testing are routine screening methods. Colposcopy, along with colposcopically directed biopsies, is indicated for evaluating women with abnormal screening results. A 3% to 5% solution of acetic acid is usually applied to the cervix to direct biopsy [4]. There is a wide variability in the reported sensitivity (ranging 55%-100%) and specificity (ranging 65%-98%) of acetate-induced
visual inspection method [5]. Because this examination relies on the subjective judgement of inspector, the accuracy of the results is highly related to the colposcopists’ experience [6].

A variety of optical techniques are developed for cervical neoplasia detection in recent years, such as the fluorescence spectroscopy [7]. The screening and diagnosing cancer by fluorescence spectroscopy is performed by visualizing some tumor- associated molecules, such as NADH and collagen [8,9]. These molecules produce endogenous fluorescence with different intensities in neoplastic cervical tissues from normal tissues. However, the intensity and contrast of autofluorescence between neoplastic cervical and normal tissues are too low, hindering its applications [7]. Exogenous fluorophores that preferentially accumulate in the lesions may be a better choice [10,11]. Previously, Zhou et al. developed a tumor-targeted near-infrared (NIR) fluorescent probe TMTP1-PEG4-ICG, which showed excellent affinity to cancer cells not only in vitro but also in vivo. Moreover, it is nontoxic and metabolized rapidly [12]. The potential receptor of this tumor targeting polypeptides TMTP1 is aminopeptidase P (XPNPEP2) [13]. It was also found that XPNPEP2 was highly expressed in cervical cancer. Especially, the expression of XPNPEP2 is significantly higher in cervical intraepithelial neoplasia (CIN) than that in normal cervical tissue [14]. Therefore, we proposed to use this probe to detect cervical lesions using the NIR fluorescence imaging.

NIR fluorescence has the advantages of increased tissue penetration and high sensitivity. Various NIR fluorescence imaging systems have been developed for clinical application [15], such as the Flare system designed for intraoperative visualization during open surgery [16–18], fluorescent laparoscopic Pinpoint [19,20], hand-held fluorescent image-guidance system Fluobeam [21,22], etc. However, the above imaging systems are not suitable to observe cervical tissue for the special physiological structure of the cervix.

In this study, we developed a dual mode colposcope with visible reflectance imaging and NIR fluorescence imaging to detect cervical lesions together with the application of probe TMTP1-PEG4-ICG. A visible-NIR dual sensor CCD camera was adopting and the colposcope was designed to achieve simultaneously in vivo reflectance imaging and NIR fluorescence imaging. After the dye TMTP1-PEG4-ICG was topically applied to the cervix, the fluorescent images of cervix were collected and histological analysis were performed to confirm the effectiveness of the dual modal colposcope.

2. Materials and methods

2.1. Dual modal fluorescent colposcope

The schematic diagram of the fluorescent colposcope is shown in Fig. 1. The colposcope was designed to integrate the NIR fluorescent imaging with a clinical colposcope (3ML LED, Leisegang, Berlin, Germany) by adopting a custom designed NIR illuminator and a visible-NIR detector. As shown in Fig. 1(b), (a) white light-emitting diode (LED) with electric power of 18W was used for the visible light imaging of cervical tissue. The excitation light source used for fluorescent imaging included four NIR laser diodes (785 nm, L785P090, Thorlabs, New Jersey, the United States) attached with the colposcope, because previous study showed that TMTP1-PEG4-ICG had an absorption peak located near 785nm [12]. The optical power of each laser diode was 90mW. A set of beam shaping lenses coupled with NIR laser was designed to perform a Kohler illumination so that the fluorescent dye within the object plane can be uniformly excited. The illumination power density at the tissue surface was about 19.0 mW/cm², measured by an optical power meter (PM130D, Thorlabs, New Jersey, the United States). The laser power density was lower than the maximum permissible exposure of the skin specified by the International Electro Technical Commission (IEC 60825-1:2014). Furthermore, the optical structure ensured matching the excitation light spot with the light spot produced by the white light LED. A dual sensor CCD camera (AD080GE JAI, Yokohama, Japan) which consisted of a color CCD and a monochrome CCD was utilized to receive both visible light and NIR fluorescent
signal reflected from cervix simultaneously. The NIR fluorescence signals were separated from the visible light by a splitting prism after the excitation light was blocked with a notch filter (NF785-33, Thorlabs, New Jersey, the United States) located in front of the colposcope objective. The transmission of the notch filter was about 0.0001% (OD=6) at the wavelength of 785nm and 90% at the range of 810-1040nm, which had enough attenuation to the excitation light and good transmission to the fluorescence signal. In order to make the size of CCD sensors to be matched with the field of view of the colposcope, a doublet lens with a focal length of 40mm (49-354, Edmund Optics, New Jersey, the United States) was placed between the camera and the colposcope.

![Fig. 1. The schematic diagram of the dual modal fluorescent colposcope.](image)

To avoid the contaminant to the fluorescent signal from the white light LED, a 650 nm short pass filter (FES650 Thorlabs, New Jersey, the United States) was placed in front of the white LED to filter out its NIR component. A short pass filter (FES650 Thorlabs, New Jersey, the United States) was also installed on each eyepiece to protect the operator from the possible laser irradiation. The instrument setup is shown in Fig. 2.

![Fig. 2. The photo of the dual modal fluorescent colposcope.](image)

A control and display software based on Labview (National Instrument, Texas, the United States) was developed. Image enhancement was integrated in the software. The bottom 10% of all pixel values were set to 0 and the top 0.1% of all pixel values were set to 255. Other pixel values were linearly mapped to the grayscale of 0-255. The enhanced fluorescence image was regarded as the green channel of an image, while the red and blue channel of the image were set to 0, so that the fluorescence image was converted to green pseudo-color, which helped the operator to easily recognize the fluorescent signal. Then the fluorescence image was merged with the reflectance image to provide better spatial localization of the fluorescent signal. The
fluorescent image, the reflectance image and the merged image were displayed simultaneously, which provides multiple references for the operator, as shown in the Fig. 2.

2.2. Experimental protocols

The study was conducted at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The study protocols were reviewed and approved by Ethics Committee of Tongji Hospital and had been registered on ClinicalTrials.gov with a number of NCT0332146. Before this study, a series of safety tests, including acute toxicity test, mucosal irritation test, sterility test and endotoxin-free test, were conducted and submitted to Ethics Committee of Tongji Hospital. The main risks of topical application on the surface of the cervix are local irritation of the mucous membrane and allergies caused by the dye or ethanol. Patients were recruited to participate in the application of this fluorescent colposcope. Inclusion criteria were as following: 1) patients were with an abnormal Pap test and/or positive HPV test, or visible cervical morphological abnormalities; 2) were at least 21 years of age; 3) were willing and able to sign the informed consent. Exclusion criteria were as following: 1) patients were during pregnancy or lactation; 2) were allergy to the fluorescent dye TMTP1-PEG4-ICG and/or alcohol; 3) diagnosis of bacterial vaginitis, fungal vaginitis and other vaginitis; 4) were with cardiac dysfunction or hepatic insufficiency or renal insufficiency; 5) participation in another clinical trial with an investigational drug within 3 months.

The patients were invited to join the study and informed consent was obtained before the examination. The examination was mainly conducted by the experienced gynecologist with rich experience in colposcopy operations. All gynecologists participated in the study were trained with the operating skills of the fluorescent colposcope before the start of the study and actively collected the data.

As mentioned in our previous research, the fluorescent dye TMTP1-PEG4-ICG was designed by the authors and synthesized by WuXi AppTec, Shanghai, China [12]. 1 mg dye was dissolved with 100 µl 75% ethanol solution, and then diluted with 900 µl sterile water before it is used. The small amount of ethanol was added to aid solubility.

The procedure of colposcopy was as following: The patient lay on the examination bed in lithotomy position. After opening the vagina with the vaginal expander, the doctor cleaned the mucus from the patient’s cervix with a sterile cotton swab. Then 0.1% fluorescent dye TMTP1-PEG4-ICG was apply evenly on the surface of the cervix with a cotton swab. Afterwards the patient could get up and take a rest. 30 minutes later, open the vagina again with the vaginal expander and wiped the cervix with a sterile cotton ball with normal saline to remove unbound fluorescent dye. Then fluorescence images and reflectance images of the cervix were obtained simultaneously. The exposure time of the fluorescence images was 500ms. The photographing was completed within a few minutes. Subsequent to imaging, the visually observed lesions or areas with obvious fluorescent signal were sampled with biopsy forceps and sent to pathology laboratory for histological analysis. If no visible lesions were observed, then take 3, 6, 9, 12 o’clock area of the cervix for biopsy.

2.3. Immunohistochemistry (IHC)

The tissues were fixed with 4% paraformaldehyde and then paraffin embedded and cut into 4 µm thick sections for immunohistochemical analysis. A goat anti-rabbit Avidin-Biotin Complex (ABC) Vectastain kit (SP-9001, Zsgb-Bio, Beijing, China) was used to detect XPNPEP2 expression level according to the manufacturer’s instructions. Antigen retrieval was performed with sodium citrate at 95°C for 8 min. The primary antibody was rabbit anti-XPNPEP2 (1:200, GTX109995, GeneTex, USA). The staining was developed with a DAB chromogenic substrate kit (G1212, Servicebio, Wuhan, China). We used the IHC profiler plugin in ImageJ 1.52v software to quantitatively analyze IHC staining pictures. This method could minimize the problem of
inter-observer variations across labs [23]. We set the mode of Cytoplasmic Stained Image and vectors of H-DAB. The result was output as four levels of DAB staining intensity and the accurate percentages of the pixels present in each level. The IHC score calculation formula was as following: \( \sum (\text{Score of the level}) \times (\text{percentage of the pixels present in the corresponding level}) \). The score of levels was assigned as: 4 for high positive, 3 for positive, 2 for low positive and 1 for negative. If images containing 66% or more percentage of pixels in a level, the images were assigned a score of that level without using the formula. For each patient, 5 high-powered field of view (200X) pictures were taken for analysis, and the final score was averaged.

2.4. Data analysis

An images analysis program based on Matlab (MathWorks, Massachusetts, the United States) was developed to analyze the fluorescence images. SBR and contrast to background ratio (CBR) of the fluorescence signal were calculated. The selection of regions of interest (ROI) followed the same principle as biopsy. Four ROIs on tissue areas with obvious fluorescence signal were selected. If no fluorescence signal were observed, 3, 6, 9, 12 o’clock areas of the cervix were chosen for analysis. The ROIs on reflectance images were located at the same position with that on fluorescence images and each ROI included 100*100 pixels (~3mm), as shown in Fig. 3(a) and Fig. 3(b).

![Fig. 3. Analysis of the fluorescence images. (a). ROIs on the reflectance images (b). ROIs on the fluorescence images (c). The result of image segment, where blue area was considered as the background.](image)

To avoid nonspecific fluorescence caused by the residual dye at the edges of the speculum, an area that excludes the speculum was manually selected on the fluorescence image. Then, the background was determined by automatic image segmentation with Ostu’s threshold in the selected area. As shown in Fig. 3(c), the image was automatically divided into two regions shown in red and blue, and the blue area was considered as the background.

The SBR of the fluorescence images was defined as the ratio of the average intensity of the ROIs to that of the background region, as shown in Eq. (1).

\[
SBR = \frac{<I_{ROI}>}{<I_{back}>}
\]  

(1)

The noises of the camera were considered in CBR, which was defined as Eq. (2) [16].

\[
CBR = \frac{<I_{ROI}> - <I_{back}>}{<I_{noise}>}
\]  

(2)

To estimate the noises of the camera, a dark image was acquired with the lens covered. The exposure time was the same as that used in the experiment, which was 500ms. The average intensity of the dark image was 8.14 counts, which was considered as the camera noises.
The signal to noise ratio (SNR) of the fluorescent colposcope was defined as Eq3.

\[
\text{SNR} = 10 \log\left( \frac{<I_{\text{signal}}>}{<I_{\text{noise}}>} \right)
\]  

(3)

where \(<I_{\text{signal}}>\) represented the maximum unsaturated fluorescence signal that can be detected. The SNR was 15 dB since the depth of the camera was 8 bits.

The histopathological diagnosis of the sampled tissue was given by professional pathologists, which was considered to be the gold standard. Fluorescence imaging results were compared with the gold standard and the receptor (XPNPEP2) expression levels of the fluorescent dye TMTP1-PEG4-ICG.

3. Results

3.1. System performance

To estimate the fluorescence detection sensitivity of the dual mode fluorescent colposcope, phantom experiment was performed. Fluorescence images without image enhancement was shown in Fig. 4(a) and the average fluorescence intensity in the ROIs was shown in Fig. 4(b). The fluorescence intensity increased linearly with the increasing concentration of TMTP1-PEG4-ICG and the correlation was \(y = 3.15x - 0.52\). Considering the camera noise estimated above, the minimum detectable concentration was approximately 2.7 ug/mL.

Fig. 4. Concentration gradient experiment. (a). Fluorescence images of TMTP1-PEG4-ICG solutions with different concentrations. (b). Average fluorescence intensity in ROIs presented in (a).

The properties of the fluorescent colposcope is shown in Table 1. The working distance of the colposcope was 300 mm. The colposcope provided 7.5/15/30 magnification which can be adjusted by a hand wheel, and the corresponding imaging field of view was 35.0/16.4/8.6 mm in diagonal. Images of the whole cervix could be obtained when observed with 7.5× magnification and detail images of the cervix could be acquired with 15× and 30× magnification. The spatial resolution of the colposcope was tested by using a USAF 1951 test target (R3L3S1P, Thorlabs, New Jersey, the United States). At the three magnification, the spatial resolution was 7.13/14.30/20.16 line pairs/mm, respectively.

3.2. Clinical trials

Table 2 summarizes the clinic pathological information of the enrolled patients, including age, histopathology, FIGO stage, fluorescence imaging results and XPNPEP2 expression level. 11 eligible women participated in the trial, including 5 patients with cervical cancer, 4 with CIN and 2 with benign cervicitis. CIN 1 corresponds to mild dysplasia, exhibits a high rate of
Table 1. The properties of the fluorescent colposcope

| Property                              | Value          |
|---------------------------------------|----------------|
| Excitation wavelength (nm)            | 785            |
| Working distance (mm)                 | 300            |
| Magnification                         | 7.5×/15×/30×   |
| Field of view (mm)                    | 35.0/16.4/8.6  |
| Spatial resolution (line pairs/mm)    | 7.13/14.30/20.16 |
| Fluorescence image frame rate (frames/sec) | 2-10          |
| Sensor                                | ICX204AK       |
| Sensor resolution (pixels)            | 1024*768       |
| SNR (dB)                              | 15             |
| Sensitivity (TMTP1-PEG4-ICG, ug/mL)   | 2.7            |

spontaneous regression and requires no clinical intervention [4]. In this study, the fluorescent signal at the lesion of CIN 1 patient was as low as that of patients with cervicitis, both had almost no fluorescent signal display.

Table 2. Demographics and individual data for patients.

| Patient No. | Age (years) | Histopathology      | FIGO stagea | Fluorescence SBR | XPNPEP2 IHC score |
|-------------|-------------|---------------------|-------------|------------------|-------------------|
| 1           | 44          | squamous cell carcinoma | IB2        | 1.23 ± 0.16      | 1.86 ± 0.16       |
| 2           | 47          | squamous cell carcinoma | IIA1       | 1.14 ± 0.02      | 1.36 ± 0.10       |
| 3           | 47          | squamous cell carcinoma | IIA2       | 1.68 ± 0.24      | 1.48 ± 0.04       |
| 4           | 57          | adenocarcinoma       | IB1         | 1.16 ± 0.02      | 1.86 ± 0.06       |
| 5           | 30          | adenocarcinoma       | IB3         | 1.33 ± 0.05      | 1.50 ± 0.13       |
| 6           | 56          | CIN 3                | not applicable | 1.15 ± 0.02 | 1.59 ± 0.07       |
| 7           | 51          | CIN 2-3              | not applicable | 1.15 ± 0.03 | 2.10 ± 0.17       |
| 8           | 28          | CIN 2-3              | not applicable | 1.26 ± 0.11 | 1.59 ± 0.05       |
| 9           | 38          | CIN 1                | not applicable | 0.99 ± 0.01 | 1.00 ± 0.00       |
| 10          | 56          | cervicitis           | not applicable | 1.00 ± 0.04 | 1.00 ± 0.00       |
| 11          | 30          | cervicitis           | not applicable | 1.10 ± 0.04 | 1.00 ± 0.00       |

aInternational Federation of Gynecology and Obstetrics.

The fluorescence SBRs of cervical lesions were calculated with the original images before image enhancement. Overall patients with cancerous or precancerous lesions showed higher fluorescence SBRs than cervicitis or CIN 1 patients. We used IHC assay to detect the expression level of TMTP1 receptor XPNPEP2 in cervical lesions. The level of IHC score represented the expression level of XPNPEP2 protein. Analogously, patients with malignant pathological results and precancerous lesions of CIN 2-3 had higher XPNPEP2 expression than patients with benign pathological results and CIN 1.

Figure 5(a) provides fluorescent colposcope images along with the corresponding tissue H&E staining pictures and XPNPEP2 immunohistochemistry results of patients in Table 2. The bar graphs of SBRs and IHC scores were shown in Fig. 5(b).

To investigate the influence of the image enhancement on SBR calculation, SBRs with and without image enhancement were presented in Fig. 6(a). After image enhancement, SBRs was significantly increased due to the increasing of image contrast.

To further study the fluorescence images acquired by the dual mode colposcope, CBRs of the fluorescence images were calculated, as shown in Fig. 6(b). CBRs showed a similar distribution...
Fig. 5. Results of the clinical trials. (a). Fluorescent colposcope images and corresponding H&E, IHC stained pictures. (b). Bar graphs of SBRs and IHC scores.
to SBRs. Patients with malignant pathological results and precancerous lesions CIN 2~3 had higher CBR than patients with benign pathological results and CIN 1. For patient 8 and 9, the CBRs were close to 0 because there was almost no fluorescence signal detected.

4. Discussion

Intraoperative NIR fluorescence imaging is widely applied in clinical practices such as sentinel lymph node mapping, tumor imaging and angiography [15]. Since the light penetrates deeply in tissue and the tissue exhibits almost no autofluorescence in NIR range, fluorescent images with low background noise can be obtained [24,25]. In this study, it was shown that this fluorescent colposcope combined with a tumor-targeting NIR fluorescent dye TMTP1-PEG4-ICG had the potential to display positive fluorescent signal at the lesions for squamous cell carcinoma, adenocarcinoma and CIN. Immunohistochemical staining revealed TMTP1 receptor XPNPEP2 protein was also overexpressed at the lesions, further proving the effectiveness of this dye. None of the 11 patients participating in the study found any adverse reactions, which further illustrates the safety of this dye.

The biopsy sampling tissues were usually 3-5mm, we uniformly set the size of the ROIs to be about 3mm. Since biopsies and ROIs were manually selected, we tried to make the ROI and biopsies come from the same area, but we could not guarantee that they are exactly the same location. Overall, patients with cancerous or CIN2+ precancerous lesions showed stronger fluorescent signals at the lesions than those with low-grade lesions or normal cervix, as well as XPNPEP2 IHC staining intensity. However, when we analyzed the correlation between the IHC score and the SBR of the fluorescent signal in the 11 samples, no significant correlation was found. There were several explanations for this. Firstly, in addition to the expression level of XPNPEP2 in cervical epithelium, there may be other factors that affect the fluorescence signal of TMTP1-PEG4-ICG during the operation of colposcopy. For example, cervical mucus was abundant and continuously secreted among some patients, which may affect the staining effect of fluorescent dyes. Before applying the dye, we cleaned up the mucus as much as possible, and we found new mucus secretion before capturing the image. Besides, bleeding from the lesion in patients with cervical cancer may also affect the staining effect. Secondly, the design of this fluorescent dye was based on TMTP1 as a tumor-targeting polypeptide. In the previous study, TMTP1 was filtrated by using the FliTrx bacterial peptide display system to perform four rounds of positive and negative screening in high-invasive metastatic cancer cells and low-invasive cancer cells, but not filtrated by XPNPEP2 protein. XPNPEP2 was identified in vitro by affinity chromatography (ProFound Pull-Down) and time-of-flight delayed extraction MALDI mass spectrometer (Bruker Autoflex) [13]. TMTP1 may also have other potential receptors.

Fig. 6. Analysis of the fluorescence images. (a). SBRs with and without the image enhancement (b). CBRs of the 11 patients
In this study, normal regions in patients with lesions were not taken into consideration. In subsequent studies, we will enroll patients with no visible lesions (mainly screening for precancerous lesions of cervical cancer) and conduct a larger sample size study. For the biopsy sampling, regardless of whether there is an obvious fluorescence signal or the number of fluorescence signal areas, multi-point sampling will be performed in the future.

The t-tests were performed to compare the differences of SBRs and IHC scores between patients with cancerous or precancerous lesions and patients with cervicitis or CIN 1. IHC score and SBRs calculated with enhanced images showed significant difference between the two groups (p<0.001 and p=0.003). But the difference of SBR calculated with images before image enhancement between the two groups was not significant (p=0.06). Image enhancement can greatly increase the image contrast. At present, it is not clear whether SBRs calculated with or without image enhancement is more clinically instructive, which should be further investigated in the future with more enrolled subjects.

Compared with the endoscopes, colposcopes have smaller apertures due to their long working distance and high magnification, which limits the intensity of the collected fluorescent signals. Moreover, the quantum yield of NIR fluorescent dye is about 10%-25% [15], which further hinder the increase of brightness of the fluorescent images obtained by colposcopes. To obtain fluorescent images with acceptable signal-to-noise ratio (SNR), a long exposure time (100-500 ms) and image enhancement was adopted to achieve an imaging rate of 2-10 frames per second. Besides, the frame rates of the color CCD and the monochrome CCD could be set respectively. The frame rate of the color CCD was 30 frames per second, which ensured the images of the cervix could be displayed in real time. The fluorescence images were acquired after focusing with the visible light reflectance images. Therefore, the procedure time would not increase due to the low fluorescence image frame rate.

There are several methods to realize intraoperative visible-NIR fluorescence imaging. The fluorescent laparoscope Pinpoint switches between white light and NIR excitation light on time sequence, and then trigger the camera to synchronize with the switching of light sources [19,20]. This method is simple in structure, but the temporal resolution is relatively lower. The Flare system utilizes an optical relay system that allow multiple cameras to work with the same objective lens [26]. In this study, we chose to use the camera integrated with two CCD sensors so that the visible and NIR images can be recorded simultaneously through a beam splitter prism. This method avoids the switching of the light sources, and has the advantages of compact structure and convenient operation in clinical applications. Besides, the dual CCD camera ensures alignment of the two sensors. Therefore, the matching of ROIs on the reflectance images and the fluorescence images was guaranteed by selecting the same pixel location on the images, which avoided complicated registration algorithm.

Nevertheless, using beam splitter prism has some disadvantages, such as the loss of image intensity and the limitation of sensor size, which leads to the loss of fluorescence detection sensitivity and field of view. The doublet lens before the camera reduced the focal length of the colposcope and increased the field of view, thus increasing the brightness of the acquired images and the detection sensitivity. Besides, the high excitation power used in the system could also increase the fluorescence intensity. Image sensors with higher quantum efficiency can further improve the frame rate and image quality of this fluorescent colposcope in future development. Besides, there is also a possible strategy of improvement by introducing optical relay system and multiple cameras [27].

Recently, fluorescence imaging in the second near-infrared window (NIR-II, 1000-1700nm) has been widely studied because of the advantages of deep penetration and high contrast, which benefits from the low photon scattering and diminished tissue autofluorescence [28]. It has been reported that the fluorescence spectrum of indocyanine green extends to the NIR-II region.
[29, 30]. We will further investigate the optical properties of TMTP1-PEG4-ICG in the NIR-II region and the feasibility of its clinical application.

The dye TMTP1-PEG4-ICG is safe and non-toxic, with stable NIR fluorescent properties. More importantly, it showed good tumor targeting ability [12]. We will further increase the number of patients enrolled in the trial to explore the possibility of using this dual mode fluorescent colposcope for detecting precancerous lesions of cervical cancer.

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