Current Medical Imaging Reviews, 2017, *13*, 50-57

REVIEW ARTICLE

Harnessing the Power of Cerenkov Luminescence Imaging for Gastroenterology: Cerenkov Luminescence Endoscopy

Xin Cao[#], Yonghua Zhan[#], Xu Cao, Jimin Liang and Xueli Chen[*]

Engineering Research Center of Molecular and Neuro Imaging of the Ministry of Education & School of Life Science and Technology, Xidian University, Xi'an, Shaanxi 710126, China

ARTICLE HISTORY

Received: August 05, 2015
Revised: May 17, 2016
Accepted: May 20, 2016

DOI:
10.2174/1573405612666160607094334

Abstract: The Cerenkov luminescence imaging (CLI) has built a bridge between the nuclear and optical imaging, and opened a new direction of optical imaging in clinic translation. However, the wide applications of CLI are limited by the weak intensity and poor penetration capacity. By coupling highly sensitive charged coupled device camera with optical fiber bundle based endoscope, Cerenkov luminescence endoscopy (CLE) has recently been developed to overcome these limitations. Using the CLE, diseases deep buried in the body, such as the gastrointestinal (GI) tract cancer, can be identified by radiotracer, which can hardly be detected by CLI from the surface of the body. Here, we review recent progress on CLI and CLE, including the principle, imaging instrument, as well as applications. In addition, the intensity enhanced strategies for Cerenkov luminescence are presented, which may provide an ideal solution to the obstacle of CLE in the translation studies. In the end, we present possible future directions of the CLE technology.

Keywords: Cerenkov luminescence imaging, Endoscopy, Gastrointestinal disease, Molecular imaging, Optical imaging.

INTRODUCTION

With unique advantages such as the high imaging sensitivity, relatively good tissue specificity, and short acquisition time and low cost, optical imaging (OI) technology has become a hot research topic in the field of molecular imaging in last two decades [1]. Up to now, OI has been widely used in the field of gene expression, tumor detection, drug development as well as therapy evaluation, involving the small animal based preclinical studies [2-5]. In World Molecular Imaging Congress 2009, Roger Yonchien Tsien, who shared the 2008 Nobel Prize in chemistry, reported that the tumor of a mouse could be resected under the guidance of fluorescence microscopy, which opened the door to the surgical navigation using OI [6]. Afterwards, European scientists developed a prototype system of surgery navigation guided by OI, which has been successfully applied to clinical resection of human ovarian cancer and further promotes the clinical translation of OI technology [7, 8]. Although the OI technology has achieved successful applications in clinical surgery navigation, two obstacles also limit its widely clinical applications. The first one is the scarce probes that can be used for human studies. Until now, only the Iodocyanine Green (ICG), Methylene Blue and fluorescein have been approved by Food and Drug Administration (FDA) [9]. The second one is the short penetration depth of optical signal when transmitted in living body. Light would be greatly attenuated because of the absorption of biological tissues, so that the optical signals can hardly be detected at the surface for the deeply buried diseases [10]. Recently developed Cerenkov luminescence endoscopy (CLE) technology may provide an ideal solution to the obstacle of CLI in the translation studies. Firstly, the optical signal detected by CLE is the Cerenkov radiation, which is emitted during the decay of radionuclides. There are many kinds of radionuclides approved by FDA in the clinical use. Secondly, with the help of an endoscope, the CLE can directly arrive the lesion site and collect the luminescence signals, which avoids the attenuation of light caused by long propagation path in living body [11-15].

CERENKOV LUMINESCENCE IMAGING (CLI)

Cerenkov radiation (CR) was first discovered by a Russian scientist of Pavel Alekseyevich Cerenkov in 1934, who shared the 1958 Nobel Prize in physics [16]. The CR is a kind of visible and near-infrared light, which is continuous in its spectrum and emitted when a charged or a high-energy particle moves in a dielectric media with speed larger than the phase velocity of light in the media [16-18]. In 2009, Roberson *et al*. first applied CR to the field of OI using 2-18-fluoro-D-glucose (^{18}F-FDG), and named this new imaging concept as Cerenkov luminescence imaging (CLI) [19]. The

*Address correspondence to this author at the Engineering Research Center of Molecular and Neuro Imaging of the Ministry of Education & School of Life Science and Technology, Xidian University, P.O. Box: 97, 2 South Taibai Road, Xi'an, Shaanxi, 710071, China; Tel: +86-29-88202649; E-mail: xlchen@xidian.edu.cn

[#]These authors contributed equally to this work.

1875-6603/17 $58.00+.00

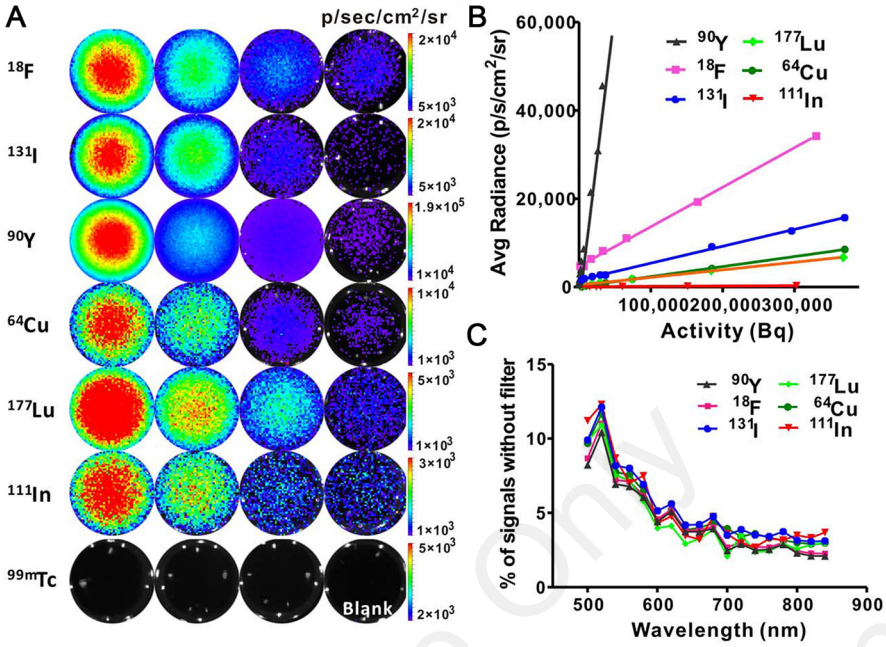

Fig. (1). CLI images of different radionuclides. (**A**) Optical signal images, with the different activities of each holes in each lines (18F: 5, 2, 1, and 0.1 *mCi*; 131I: 10, 5, 1, and 0.1 *mCi*; 90Y: 5, 1, 0.2, and 0.01 *mCi*; 64 Cu:10, 5, 1, and 0.1 *mCi*; 177Lu: 10, 5, 2, and 0.5 *mCi*; 111In: 10, 5, 1, and 0.5 *mCi*; and 99mTc: 20, 10, and 5 *mCi*). (**B**) Relationship between activity and average CLI radiance. (**C**) Spectrum of CR. Adapted with permission from ref [22].

radioactive decay of nuclides, such as ^{18}F (110 min half-life) and ^{131}I (8 days half-life) that are popular PET and SPECT isotopes in clinics, is companied by the emission of CR (also called the Cerenkov luminescence, CL). Using an optical detector, such as a CCD camera, the companied CL could be collected to form an optical image. Because of the widely available characteristic of medical isotopes in clinics, the CLI technology bridges the newly OI and traditional nuclear imaging technology, and soon becomes a hot topic in recent years [9, 19-25].

Compared with the common OI technology, CLI has a huge advantage of the widely available radionuclides approved by FDA [9]. Liu *et al*. reported that many kinds of radiotracers, including β^+ (used for PET) radionuclides and β^- (used for SPECT) radionuclides, emitted the CR [22]. They also investigated the relationship between the radioactivity of radionuclide and the CLI intensity, as well as the Cerenkov luminescence spectrum of each radionuclide (Fig. **1**). With such various kinds of radiotracers, CLI enables much wider applicability than the commonly used OI techniques, which would accelerate the clinical translation of the OI technology. Especially, a dual-modality imaging of the CLI and nuclear imaging can be achieved with only one single radiotracer, which allows the cross validation of CLI using the proven nuclear imaging technology.

Compared with the proven nuclear imaging technology, CLI has all of the advantages OI has, such as high throughput, high sensitivity, low cost, easily accessible, as well as reasonable spatial and temporal resolution. Until now, CLI has been successfully applied to tumor detection, drug development and therapy evaluation, gene expression monitoring, as well as clinical studies [26-32]. Ruggiero *et al*. used both the CLI and immune-PET to observe the uptake and localization of ^{89}Zr-DFO-J591 in the dual subcutaneous LNCaP tumors bearing mice (n=3) (Fig. **2A** and **B**). The biodistribution results showed a strong correlation between the CLI and immune-PET (Fig. **2C**) [9]. Xu *et al*. used CLI to screen the optical signal from the H460 xenografts in treated mice (treated with Bevacizumab) from pre-treatment to the third day after treatment (Fig. **2D** and **E**). The results were validated with PET scan which showed a great consistency between them (R^2=0.9309, Fig. **2E**) [33].

Besides the applications in small animal studies, CLI has obtained some successful human trial applications. Spinelli *et al*. reported the first human trial of CLI in 2013 [27]. They acquired the CLI image of the thyroid of a patient who suffered from hyperthyroidism and was treated with a 15 *mCi* ^{131}I. They named this method as the Cerenkography and very encouraging and promising result was obtained [27]. To investigate the feasibility of acquiring CR emissions from the patients undergoing routine diagnostic scans of ^{18}F-FDG, Thorek *et al*. conducted another pilot trial of human studies [26]. In the experiment, four patients (2 lymphoma, 1 lung cancer, and 1 breast cancer) firstly underwent the PET/Computed Tomography (CT) scan and then CLI. Results showed a good relationship between PET/CT scan with CLI images on the site of lymph node, as shown in Fig. (**3C-E** [26].

Although the CR signals could be observed during the decay of many kinds of clinically-used radionuclides, two big obstacles exist to limit the further wide applications of CLI technique. The first one is the weak intensity of the CR emission. As a secondary product, the CR emission is about 9 orders of magnitude lower than the ambient light [26, 34, 35]. To effectively detect the CR emission, a relatively long integration time and a completely dark environment are

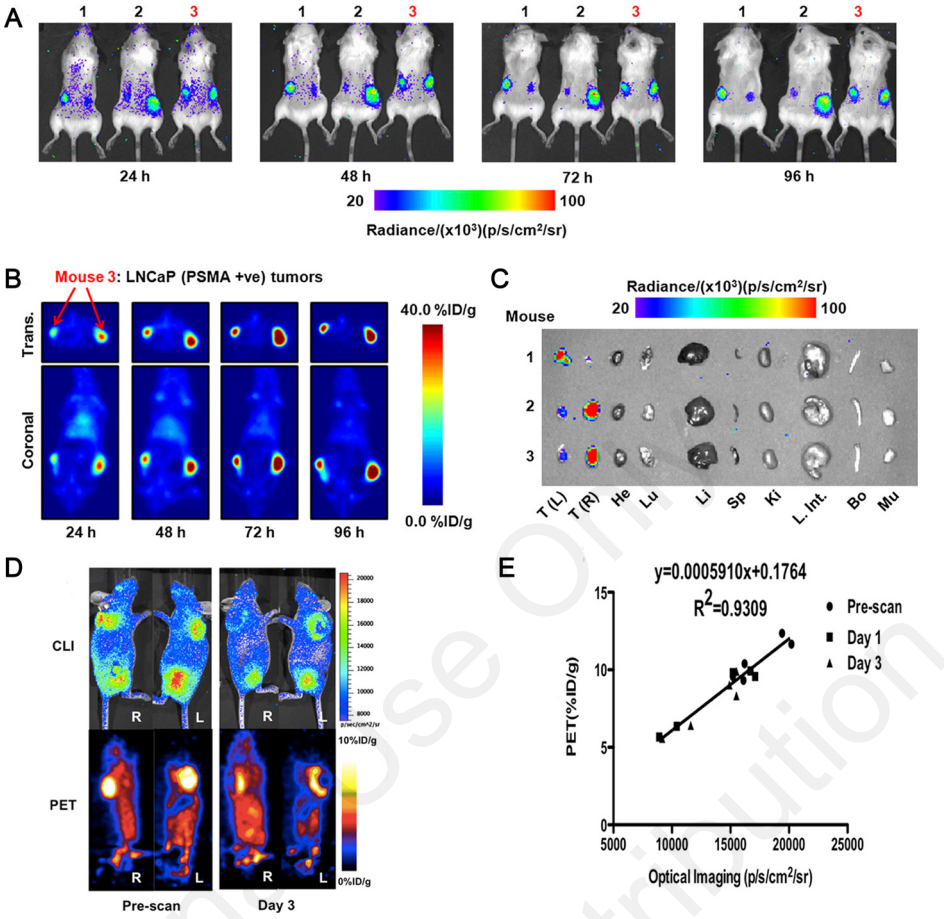

Fig. (2). Representative applications of CLI in small animal studies. (**A-C**) Application of CLI in tumor detection; Adapted with permission from ref [9]. (**D-E**) Application of CLI in drug therapy monitoring; Adapted with permission from ref [33]. (**A**) *In vivo* CLI image of 89Zr-DFO-J591 tumor in 3 mice between 24 and 96 h after administration; (**B**) Corresponding coronal and transverses immune-PET images of mouse 3; (**C**) CLI images of organs after acute *ex vivo* biodistribution at 96 h. (**D**) *In vivo* CLI and PET images of mice bearing H460 xenografts before and after treatment with Bevacizumab; (**E**) Quantitative correlation analysis of CLI and PET results (n=3).

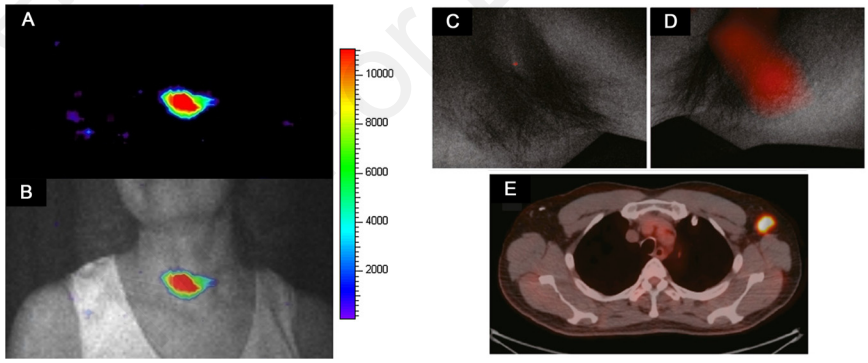

Fig. (3). Human trial of CLI. (**A**) The CLI image of thyroid gland. (**B**) Overlay image between CLI image (colorful) and photograph (gray-scale color) of the patient thyroid. (**C**) Negative CLI scan of right axilla without 18F-FDG positive lymph node. (**D**) Image of left axillar, overlaid with significant CR signal. (**E**) Result of PET/CT scan. (A) and (B) were adapted with permission from ref [27]; (C)-(E) were adapted with permission from ref [26].

needed. The another obstacle is the great attenuation of the CR light during the propagation in a living body [36, 37]. According to the biological window of biological tissues, the shorter of the wavelength of light, the great of the attenuation would be [10]. From Fig. (**1B**), it can be obviously observed that the major components of the CR emission are located in the range of short wavelength, indicating that the CR light encounters a large attenuation rate. Because of these two limitations, most of the CLI applications are limited to *in vitro* studies, small animal imaging or surface imaging of human body [26, 27, 37, 38]. For the radionuclide deeply buried in the living body, it become very hard to detect from the surface of the body [12]. Even though the recently developed Cerenkov luminescence tomography (CLT)

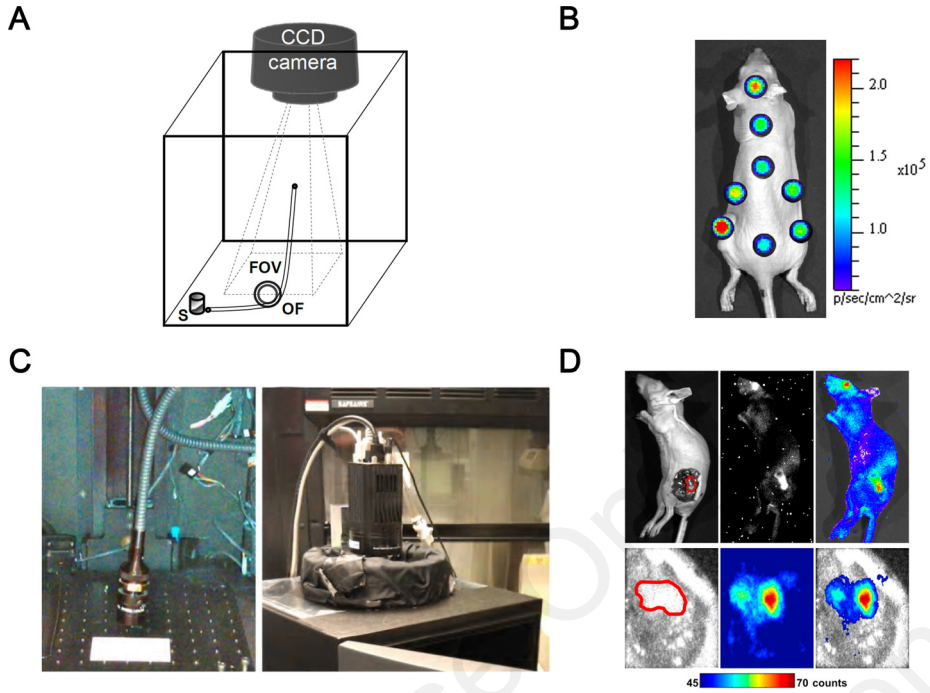

Fig. (4). (**A**) Schematic of the CLE system proposed by Kothapalli *et al*.; (**B**) Results of *in vivo* mice experiment imaged using the system of (**A**); (**C**) Result of system sensitivity evaluation for Liu's system; (**D**) Results of the small animal *in vivo* experiment in Liu's paper; the above is the CLI images while the below is the images acquired by using the CLE system. (**A**) and (**B**) were adapted with permission from ref [11]; (**C**) and (**D**) were adapted with permission from ref [14].

technique could resolve the three-dimensional distribution of radiotracers inside the small animals [39-43], it still cannot solve the problem of detecting deeply buried lesions, because the primary hypothesis of CLT was the CR emission could be detected from the surface of living body [12].

CERENKOV LUMINESCENCE ENDOSCOPY (CLE)

Endoscopic imaging technique can obtain an accurate image of lesion target deep inside living body by taking the detector close to the lesion surface via an organ cavity with a minimally invasive manner, which would provide a powerful technical support for the limitation of penetration depth encountered in the clinical translation of OI technology. Because of this feature, it has obtained wide applications in clinical medicine, such as the gastroscopy [44], colonoscopy [45], bronchoscopy [46], and confocal laser endomicroscopy [47]. By integrating the advantages of endoscopic imaging technique and CLI, a novel technique named Cerenkov luminescence endoscopy (CLE) has recently proposed, providing with an ideal solution to the limitations of scarce probes and poor penetration depths of OI in translation studies. In this scenario, the probe end of the CLE, an optical fiber based endoscope, is inserted into a living body with a slightly invasive manner and reach the location where the radiotracers accumulated. The CR signals emitted from the surface of lesion were then collected with the endoscope and propagated in the optical fibers, and finally detected by the CCD camera to form an image [11-15]. By constructing a mathematical model to accurately describe the CL propagation in the CLE system and incorporating some tomographic techniques, the location and distribution of radiotracers can be three-dimensionally estimated.

Kothapalli *et al*. developed the first prototype system for CLE in 2012 [11]. The system consisted of two parts, the optical fiber based endoscope and CCD camera (IVIS-200, Xenogen) [11]. Fig. (**4A**) shows the schematic of the system. To reveal the feasibility of the CLE system, they performed *in vivo* small animal experiments by using an optical bundle with a diameter of 6 mm. Mice (n=4) bearing subcutaneous C6 glioma were firstly administrated with 940-970 μCi ^{18}F-FDG via tail vein injection, and then the CLE system was utilized to acquire the CR signal with the distal end of fiber bundle placed close to the region of interest. The encouraging results are shown in Fig. (**4B**). However, because the optical fiber based endoscope was not tightly coupled with the CCD camera, this CLE system cannot acquire the photograph of the imaging object, and hardly avoids the leakage of ambient light. Afterwards, Liu *et al*. improved the CLE system by building another one, in which the optical fiber bundle based endoscope was tightly coupled with the highly sensitive intensified CCD camera by a micro-imaging lens [14]. The minimum activity which can be detected by this system is about 1.21 μCi (as shown in Fig. **4C**). By using this improved CLE system, they performed a feasibility experimental study of intraoperative imaging in guiding minimally invasive surgical resection of tumor [14]. In the experiment, mice (n=5) bearing subcutaneous C6 glioma were received about 1 mCi of ^{18}F-FDG via the tail vein and kept warm for 60-70 min. Tumors were then imaged with the IVIS and the CLE systems before and after excision of the tumor. Corresponding results showed that the CLE system worked well explored the feasibility of using the CLE for the detection of tumor *in vivo* for guided surgery, as shown in Fig. (**4D**) [14].

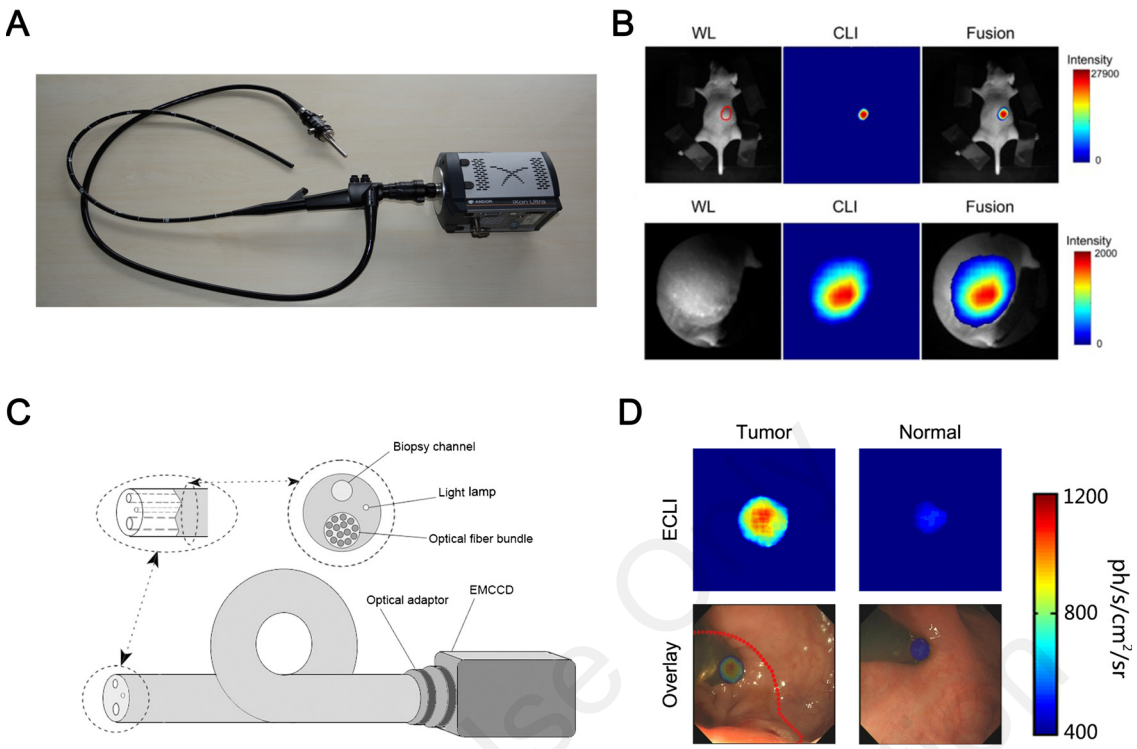

Fig. (5). (**A**) Photograph of the CLE system proposed by Cao *et al*.; (**B**) Result of the spatial resolution evaluation experiment using system of (**A**); (**C**) Results of the pseudotumor based small animal imaging; the above is CLI images while the below is the CLE images using the system of (**A**). (**D**) Schematic of the CLE system proposed by Hu *et al*.; (**E**) Human trial results using the system of (**D**). (**A**)-(**C**) were adapted with permission from ref [12]; (**C**)-(**D**) were adapted with permission from ref [15].

Different from the CLE system developed by Kothapalli and Liu, Cao *et al*. proposed another CLE system by seamlessly connecting the clinically-used fibergastroscope with a highly sensitive electron multiplying CCD (EMCCD) camera *via* an optical adapter, as shown in Fig. (**5A**) [12]. The sensitivity of the system was measured approximately 32.92×10^{-3} $\mu Ci/\mu L$ for *in vivo* studies, and the luminescent lateral resolution was better than 1 mm (as shown in Fig. **5B**), which was comparable with that of PET [12]. The final *in vivo* pseudotumor experiment revealed the great potential of the CLE system in the living organisms (Fig. **5C**). Based on this fibergastroscope-based CLE system, Hu *et al*. set up another CLE system by replacing the fibergastroscope with the clinically-used bronchofiberscope (the schematic shown in Fig. **5D**), and conducted human trial studies for the first time [15]. In the human trials, patients suffered from rectal cancer were first undergone the PET/CT scan and then under the CLE examination. Results illustrated the feasibility and potential of the CLE system in distinguishing and quantifying cancerous lesions in clinics (Fig. **5E**), and this pilot study can be recognized as a great breakthrough in accelerating the clinical translation of CLE technology.

The emergence of the CLE technique provides a possibility to overcome the obstacles of OI technology in clinic translation, and the published results indeed revealed the potential of the CLE system in clinical applications. However, a great attenuation exists in the developed CLE system, compared with the CLI results, which results in a relatively low detection sensitivity of radiotracers and affects the effective applications in clinic. According to the observed result of Cao *et al*. [12], the attenuation rate can be as large as 93%, which is mainly caused by the small aperture of endoscope (the diameter is no more than 2 mm for clinically-used endoscope) and the weak CR emission of radiotracers. Thus, to improve the detection sensitivity of the CLE system, which could be achieved by increasing the intensity of the acquired CL, would be of significance for the CLE technique in the wide applications in clinics.

INTENSITY ENHANCED CERENKOV LUMINESCENCE ENDOSCOPY

The intensity of the CR signal is very weak, which further aggravates poor detection sensitivity of the CLE system, indicating that the detection of low dose radionuclides is very difficult. In the clinical studies, the dosage of the radionuclides gathered around the tumor is very low. For example, the mean activity of the radiotracer that gathered around the GI tract tumors in a clinical diagnosis is about 1.5×10^{-3} $\mu Ci/\mu L$ [48]. Thus, to enhance the intensity of the CR emission from radiotracers is one of possible ways to improve the detection sensitivity of the CLE system, even though the system itself remains unchanged, which would facilitate the clinical applications of the CLE system.

Up to now, there are two possible ways to enhance the intensity of the CR signals. One is to shift the peak wavelength of the CR spectrum from an ultraviolet/blue to a near-infrared/visible one, because the near-infrared light has better penetration ability in biological tissues[10]. This method is usually implemented using the CR luminescence to excite other particles, such as fluorephore [49, 50], quantum dots

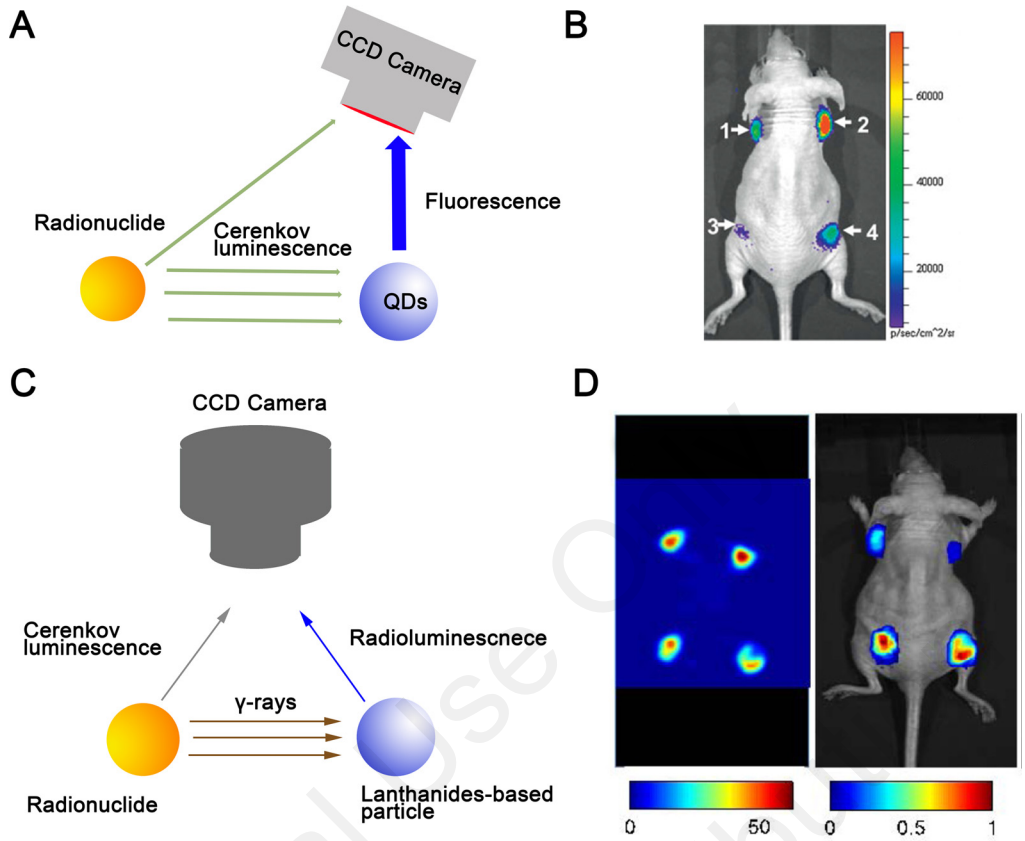

Fig. (6). (**A**) Schematic of enhancing the CR intensity by using the peak wavelength shifting strategy; (**B**) Representative result of (A). In the result, nude mouse with four pseudotumors was used. Each pseudotumor contains different mixtures and the injection method is also different. 1: $Na^{131}I$ with subcutaneous injection; 2: QD655+$Na^{131}I$ mixture with subcutaneous injection; 3: $Na^{131}I$ with intramuscular injection; 4: QD655+$Na^{131}I$ with intramuscular injection. Adapted with permission from ref [54]. (**C**) Schematic of enhancing the CR intensity based on the RLI technology; (**D**) One of the representative results of (C), left was PET image and right was optical fused image. In the result, a nude mouse bearing four pseudotumors was employed. Each pseudotumor contains the mixture of ^{18}F and lanthanides-based materials with different categories. The lanthanides-based materials contained in the pseudotumors are different: the left foreleg is $Ba_{0.55}Y_{0.3}F_2$:Eu3+, the left flank is $Ba_{0.55}Y_{0.3}F_2:Tb^{3+}$, the right flank is the mixture of equal concentration of $Ba_{0.55}Y_{0.3}F_2:Tb^{3+}$ and $Ba_{0.55}Y_{0.3}F_2:Eu^{3+}$, and the right foreleg is an inactive undoped nanophosphor. Adapted with permission from ref [58]; (**E**) Result of *in vivo* sensitivity experiment after using Gd_2S_2O:Tb to enhance the luminescent intensity, specially, the ratio of signal to background (SBR) was set as a function of the activity of ^{68}Ga. Adapted with permission from ref [59]; (**F**) Sensitivity comparison between ^{18}F and ^{90}Y. Adapted with permission from Ref [13].

(QDs) [51-54], and lanthanides [55], with the diagram shown in Fig. (**6A**). Liu *et al*. validated this enhancement strategy using QDs [54]. In their study, the CR luminescence is regarded as the excitation source and the QDs are excited by the CR to emit the luminescence with longer peak wavelength. Results of pseudotumors based *in vivo* experiment demonstrated the feasibility and effectiveness of this intensity enhancement strategy, as shown in Fig. (**6B**) [54]. The other way to enhance the emitted luminescence intensity is based on the emerged radioluminescence imaging (RLI) technology. RLI is based on the radioluminescence phenomenon by which the luminescence would be generated in the lanthanides-based materials when it they are bombarded with the ionizing radiation of high energy rays, such as X-rays, γ-rays, and beta particles [56-58]. In this strategy, the lanthanides-based materials are excited by γ-rays, which accompany the decay of radionuclides, and then emit luminescence. The emitted luminescence contains not only the CR emission but also the radioluminescence, as shown in Fig. (**6C**), so that the emitted luminescence intensity from target is enhanced. Carpenter *et al*. described this phenomenon in 2012, and validated the feasibility and potential of this strategy with both phantom and *in vivo* experiments, as shown in Fig. (**6D**) [58].

Utilizing the RLI technology, Cao *et al*. improved the detection sensitivity of their developed CLE system by mixing the radiotracer with a kind of lanthanides-based nanoparticles [59, 60]. With the *in vitro* and *in vivo* pseudotumor experiments, they showed that using the mixture of the lanthanides-based nanoparticles and the radiotracer enabled a superior sensitivity compared with the radiotracer only (about 50-fold improvement, as shown in Fig. **6E**), which guaranteed meeting the demands of the clinical diagnosis of GI tract tumors [59]. On the other hand, Carpenter *et al*. employed β– emitting radiotracer of ^{90}Y to improve the detection sensitivity of their developed CLE system [13]. Because ^{90}Y is one kind of higher energy β^- radiotracer that will bring about more CR light output and lower γ noise, the signal-to-noise ratio of CLE image using ^{90}Y is much better than that using ^{18}F (as shown in Fig. **6F**) [13].

CHALLENGES AND OPPORTUNITIES

We present above an account of recent progresses on the CLI and CLE technologies. With the development of highly sensitive signal detection technique and clinically-used probes, we believe that the CLI technology would be a powerful tool with a great potential in clinical translation. By integrating the endoscopic imaging technique, the recently emerged CLE technology further expand these applications from surface imaging to endoscopic detection, accelerating the translation of the CLI technology into the clinics. We anticipate some new directions in the future development of the CLE technology, which provides both big challenges and great opportunities for researches:

1. Instrumentation

Current progresses show that great attenuation exists in the available CLE system, which gives rise to a relatively low detection sensitivity of radiotracers. To improve the detection sensitivity by developing intensity enhanced CLE technology may be a great challenge and big opportunity for the researchers in the near future. The most direct way is to improve the CLE system by building a new fiber endoscope. In this fiber endoscope, the number of optical fibers and the field of view need to be enlarged enough to increase the luminescence collection capacity; the transmission efficiency of the fibers around the CR spectrum should been improved to decrease the luminescence attenuation quantity.

2. Imaging Agents

Referencing to the study of Zavaleta *et al*. [61], except for the optimization of CLE system described above, another way to improve the sensitivity is to increase the intensity of the emission luminescence. The RLI technology may be a good option. The problem is the cytotoxicity of the lanthanides-based nanoparticles. To synthesize the clinically applicable multimodal imaging probe based on the combination of lanthanides nanoparticles and radionuclides, which has a very good biocompatibility and extremely low cytotoxicity, is the direction of the long term hard work for biochemistry researchers.

3. Imaging Processing Algorithms

The existing studies on the CLE technology are all based on two-dimensional planar images, which cannot provide accurate location and depth information of the interested lesion or accurate quantification information. Some researchers, such as Piao *et al*., Chen *et al*., and Qin *et al*. [62-65], have done some algorithmic studies on the endoscopic optical tomography, which provides three-dimensional information of the imaging target. However, these algorithms cannot directly apply to the applications of GI tumor detection using the CLE. For the case of GI tumor detection, the tumor where the luminescence signals emit is only several millimeters away from the surface of GI tract. Such a small thickness is hardly discretized by finite element mesh. Thus, a more specific imaging model and more usable inversion algorithms should be developed for the CLE system in application of GI tumor detection, which is a challenge task in front of algorithmic researchers.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China under Grant Nos. 81227901, 81230033, 81571725, the Natural Science Basic Research Plan in Shaanxi Province of China under Grant Nos. 2015JQ6249, 2015JZ019, and the Fundamental Research Funds for Central Universities (NSIZ021402, NSIY061406).

REFERENCES

[1] Ntziachristos V, Ripoll J, Wang LHV, Weissleder R. Looking and listening to light: the evolution of whole-body photonic imaging. Nat Biotechnol 2005; 23(3): 313-20.

[2] Dragulescu-Andrasi A, Chan CT, De A, Massoud TF, Gambhir SS. Bioluminescence resonance energy transfer (BRET) imaging of protein-protein interactions within deep tissues of living subjects. Proc Natl Acad Sci USA 2011; 108(29): 12060-5.

[3] Ale A, Ermolayev V, Herzog E, Cohrs C, de Angelis MH, Ntziachristos V. FMT-XCT: *in vivo* animal studies with hybrid fluorescence molecular tomography-X-ray computed tomography. Nat Meth 2012; 9(6): 615-+.

[4] McCormack E, Silden E, West RM, *et al*. Nitroreductase, a Near-Infrared Reporter Platform for *In vivo* Time-Domain Optical Imaging of Metastatic Cancer. Cancer Res 2013; 73(4): 1276-86.

[5] Ma X, Cheng Z, Jin Y, *et al*. SM5-1-Conjugated PLA nanoparticles loaded with 5-fluorouracil for targeted hepatocellular carcinoma imaging and therapy. Biomaterials 2014; 35(9): 2878-89.

[6] Nguyen QT, Olson ES, Aguilera TA, *et al*. Surgery with molecular fluorescence imaging using activatable cell-penetrating peptides decreases residual cancer and improves survival. Proc Natl Acad Sci USA 2010; 107(9): 4317-22.

[7] Crane LMA, Themelis G, Pleijhuis RG, *et al*. Intraoperative Multispectral Fluorescence Imaging for the Detection of the Sentinel Lymph Node in Cervical Cancer: A Novel Concept. Mol Imaging Biol 2011; 13(5): 1043-9.

[8] van Dam GM, Themelis G, Crane LMA, *et al*. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: first in-human results. Nat Med 2011; 17(10): 1315-U202.

[9] Ruggiero A, Holland JP, Lewis JS, Grimm J. Cerenkov Luminescence Imaging of Medical Isotopes. J Nucl Med 2010; 51(7): 1123-30.

[10] Smith AM, Mancini MC, Nie S. BIOIMAGING Second window for *in vivo* imaging. Nat Nanotechnol 2009; 4(11): 710-1.

[11] Kothapalli S-R, Liu H, Liao JC, Cheng Z, Gambhir SS. Endoscopic imaging of Cerenkov luminescence. Biomed Opt Express 2012; 3(6): 1215-25.

[12] Cao X, Chen X, Kang F, *et al*. Performance evaluation of endoscopic Cerenkov luminescence imaging system: *in vitro* and pseudotumor studies. Biomed Opt Express 2014; 5(10): 3660-70.

[13] Carpenter CM, Ma X, Liu H, *et al*. Cerenkov Luminescence Endoscopy: Improved Molecular Sensitivity with beta(-)-Emitting Radiotracers. J Nucl Med 2014; 55(11): 1905-9.

[14] Liu H, Carpenter CM, Jiang H, *et al*. Intraoperative Imaging of Tumors Using Cerenkov Luminescence Endoscopy: A Feasibility Experimental Study. J Nucl Med 2012; 53(10): 1579-84.

[15] Hu H, Cao X, Kang F, *et al*. Feasibility study of novel endoscopic Cerenkov luminescence imaging system in detecting and quantifying gastrointestinal disease: first human results. Eur Radiol 2015: 1-9.

[16] Cherenkov PA. Visible emission of clean liquids by action of γ radiation. Dok Akad Nauk SSSR 1934; 2: 451.

[17] Frank I, Tamm I. Coherent visible radiation from fast electrons passing through matter. CR Acad Sci USSR 1937; 14: 109-14.

[18] Jelley JV. Cherenkov radiation and its applications 1958.

[19] Robertson R, Germanos MS, Li C, Mitchell GS, Cherry SR, Silva MD. Optical imaging of Cerenkov light generation from positron-emitting radiotracers. Phys Med Biol 2009; 54(16): N355-N65.

[20] Spinelli AE, D'Ambrosio D, Calderan L, Marengo M, Sbarbati A, Boschi F. Cerenkov radiation allows *in vivo* optical imaging of positron emitting radiotracers. Phys Med Biol 2010; 55(2): 483-95.

[21] Xu Y, Liu H, Cheng Z. Harnessing the Power of Radionuclides for Optical Imaging: Cerenkov Luminescence Imaging. J Nucl Med 2011; 52(12): 2009-18.

[22] Liu HG, Ren G, Miao Z, *et al*. Molecular Optical Imaging with Radioactive Probes. Plos One 2010; 5(3): 9.

[23] Chin PTK, Welling MM, Meskers SCJ, Olmos RAV, Tanke H, van Leeuwen FWB. Optical imaging as an expansion of nuclear medicine: Cerenkov-based luminescence vs fluorescence-based luminescence. Eur J Nucl Med Mol Imaging 2013; 40(8): 1283-91.

[24] Boschi F, Lo Meo S, Rossi PL, Calandrino R, Sbarbati A, Spinelli AE. Optical imaging of alpha emitters: simulations, phantom, and *in vivo* results. J Biomed Opt 2011; 16(12).

[25] Glaser AK, Davis SC, McClatchy DM, Zhang R, Pogue BW, Gladstone DJ. Projection imaging of photon beams by the Cerenkov effect. Med Phys 2013; 40(1).

[26] Thorek DLJ, Riedl CC, Grimm J. Clinical Cerenkov Luminescence Imaging of F-18-FDG. J Nucl Med 2014; 55(1): 95-8.

[27] Spinelli AE, Ferdeghini M, Cavedon C, *et al*. First human Cerenkography. J Biomed Opt 2013; 18(2).

[28] Boschi F, Calderan L, D'Ambrosio D, *et al*. *In vivo* F-18-FDG tumour uptake measurements in small animals using Cerenkov radiation. Eur J Nucl Med Mol Imaging 2011; 38(1): 120-7.

[29] Robertson R, Germanos MS, Manfredi MG, Smith PG, Silva MD. Multimodal Imaging with F-18-FDG PET and Cerenkov Luminescence Imaging After MLN4924 Treatment in a Human Lymphoma Xenograft Model. J Nucl Med 2011; 52(11): 1764-9.

[30] Liu H, Ren G, Liu S, *et al*. Optical imaging of reporter gene expression using a positron-emission-tomography probe. J Biomed Opt 2010; 15(6).

[31] Xu Y, Chang E, Liu H, Jiang H, Gambhir SS, Cheng Z. Proof-of-Concept Study of Monitoring Cancer Drug Therapy with Cerenkov Luminescence Imaging. J Nucl Med 2012; 53(2): 312-7.

[32] Boschi F, Pagliazzi M, Rossi B, *et al*. Small-animal radionuclide luminescence imaging of thyroid and salivary glands with Tc-99m-pertechnetate. J Biomed Opt 2013; 18(7).

[33] Xu YD, Liu HG, Chang E, Jiang H, Cheng Z. Cerenkov Luminescence Imaging (CLI) for Cancer Therapy Monitoring. Jove-Journal of Visualized Experiments 2012(69): 4.

[34] Beattie BJ, Thorek DLJ, Schmidtlein CR, Pentlow KS, Humm JL, Hielscher AH. Quantitative Modeling of Cerenkov Light Production Efficiency from Medical Radionuclides. PLoS ONE 2012; 7(2).

[35] Mitchell GS, Gill RK, Boucher DL, Li C, Cherry SR. *In vivo* Cerenkov luminescence imaging: a new tool for molecular imaging. Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences 2011; 369(1955): 4605-19.

[36] Ma X, Wang J, Cheng Z. Cerenkov radiation: a multi-functional approach for biological sciences. Frontiers in Physics 2014; 2: 4.

[37] Spinelli AE, Boschi F. Novel biomedical applications of Cerenkov radiation and radioluminescence imaging. Physica Medica-Eur J Med Phys 2015; 31(2): 120-9.

[38] Zhang X, Kuo C, Moore A, Ran C. Cerenkov Luminescence Imaging of Interscapular Brown Adipose Tissue. Jove-Journal of Visualized Experiments 2014(92).

[39] Li C, Mitchell GS, Cherry SR. Cerenkov luminescence tomography for small-animal imaging. Opt Lett 2010; 35(7): 1109-11.

[40] Hu Z, Yang W, Ma X, *et al*. Cerenkov luminescence tomography of aminopeptidase N (APN/CD13) expression in mice bearing HT1080 tumors. Mol Imaging 2013; 12(3): 173-81.

[41] Hu Z, Liang J, Yang W, *et al*. Experimental Cerenkov luminescence tomography of the mouse model with SPECT imaging validation. Opt Express 2010; 18(24): 24441-50.

[42] Hu Z, Chen X, Liang J, *et al*. Single photon emission computed tomography-guided Cerenkov luminescence tomography. J Appl Phys 2012; 112(2).

[43] Zhong J, Tian J, Yang X, Qin C. Whole-Body Cerenkov Luminescence Tomography with the Finite Element SP3 Method. Ann Biomed Eng 2011; 39(6): 1728-35.

[44] Gasparovic S, Rustemovic N, Opacic M, *et al*. Clinical analysis of propofol deep sedation for 1,104 patients undergoing gastrointestinal endoscopic procedures: A three year prospective study. World J Gastroenterol 2006; 12(2): 327-30.

[45] Baxter NN, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, Rabeneck L. Association of Colonoscopy and Death From Colorectal Cancer. Ann Intern Med 2009; 150(1): 1-W.

[46] Stover DE, White DA, Romano PA, Gellene RA. Diagnosis of pulmonary disease in acquired immune deficiency syndrome (AIDS). Role of bronchoscopy and bronchoalveolar lavage. Am Rev Respir Dis 1984; 130(4): 659-62.

[47] Kiesslich R, Duckworth CA, Moussata D, *et al*. Local barrier dysfunction identified by confocal laser endomicroscopy predicts relapse in inflammatory bowel disease. Gut 2012; 61(8): 1146-53.

[48] Kameyama R, Yamamoto Y, Izuishi K, *et al*. Detection of gastric cancer using F-18-FLT PET: comparison with F-18-FDG PET. Eur J Nucl Med Mol Imaging 2009; 36(3): 382-8.

[49] Bernhard Y, Collin B, Decreau RA. Inter/intramolecular Cherenkov radiation energy transfer (CRET) from a fluorophore with a built-in radionuclide. Chem Commun (Camb) 2014; 50(51): 6711-3.

[50] Lewis MA, Kodibagkar VD, Oez OK, Mason RP. On the potential for molecular imaging with Cerenkov luminescence. Opt Lett 2010; 35(23): 3889-91.

[51] Kotagiri N, Niedzwiedzki DM, Ohara K, Achilefu S. Activatable Probes Based on Distance-Dependent Luminescence Associated with Cerenkov Radiation. Angewandte Chemie-International Edition 2013; 52(30): 7756-60.

[52] Dothager RS, Goiffon RJ, Jackson E, Harpstrite S, Piwnica-Worms D. Cerenkov Radiation Energy Transfer (CRET) Imaging: A Novel Method for Optical Imaging of PET Isotopes in Biological Systems. PLoS ONE 2010; 5(10).

[53] Thorek DLJ, Ogirala A, Beattie BJ, Grimm J. Quantitative imaging of disease signatures through radioactive decay signal conversion. Nat Med 2013; 19(10): 1345-+.

[54] Liu HG, Zhang XF, Xing BG, Han PZ, Gambhir SS, Cheng Z. Radiation-Luminescence-Excited Quantum Dots for *in vivo* Multiplexed Optical Imaging. Small 2010; 6(10): 1087-91.

[55] Ma X, Kang F, Xu F, *et al*. Enhancement of Cerenkov Luminescence Imaging by Dual Excitation of Er3+, Yb3+-Doped Rare-Earth Microparticles. PLoS ONE 2013; 8(10).

[56] Chen D, Zhu S, Yi H, *et al*. Cone beam x-ray luminescence computed tomography: a feasibility study. Med Phys 2013; 40(3): 031111.

[57] Chen H, Moore T, Qi B, *et al*. Monitoring pH-Triggered Drug Release from Radioluminescent Nanocapsules with X-ray Excited Optical Luminescence. ACS Nano 2013; 7(2): 1178-87.

[58] Carpenter CM, Sun C, Pratx G, Liu H, Cheng Z, Xing L. Radioluminescent nanophosphors enable multiplexed small-animal imaging. Opt Express 2012; 20(11): 11598-604.

[59] Cao X, Chen XL, Kang F, *et al*. Sensitivity improvement of Cerenkov luminescence endoscope with terbium doped Gd2O2S nanoparticles. Appl Phys Lett 2015; 106(21): 4.

[60] Cao X, Chen X, Kang F, *et al*. Intensity Enhanced Cerenkov Luminescence Imaging Using Terbium-Doped Gd2O2S Microparticles. ACS Appl Mater Interfaces 2015; 7(22): 11775-82.

[61] Zavaleta CL, Garai E, Liu JTC, *et al*. A Raman-based endoscopic strategy for multiplexed molecular imaging. Proc Natl Acad Sci USA 2013; 110(25): E2288-E97.

[62] Chen X, Liang J, Cao X, *et al*. Feasibility study of endoscopic x-ray luminescence computed tomography: Simulation demonstration and phantom application. J Appl Phys 2013; 114(8).

[63] Chen X, Liang J, Zhao H, *et al*. Modeling and reconstruction of optical tomography for endoscopic applications: Simulation demonstration. Appl Phys Lett 2011; 99(7).

[64] Qin Z, Cui S, Zhao H, *et al*. An endoscopic diffuse optical tomographic method based on the effective detection range. Journal of X-Ray Science and Technology. 2013; 21(4): 527.

[65] Piao D, Xie H, Zhang W, *et al*. Endoscopic, rapid near-infrared optical tomography. Opt Lett 2006; 31(19): 2876.