Enhanced Cerenkov luminescence tomography analysis based on Y$_2$O$_3$:Eu$^{3+}$ rare earth oxide nanoparticles

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Abstract: Cerenkov luminescence imaging offers a new diagnostic alternative to radiation imaging, but lacks intensity and penetration. In this study, a Cerenkov luminescence signal and its image quality were enhanced using rare earth oxide nanoparticles as a basis for Cerenkov luminescence excited fluorescence imaging and Cerenkov luminescence excited fluorescence tomography. The results also provided 3D-imaging and quantitative information. The approach was evaluated using phantom and mice models and 3D reconstruction and quantitative studies were performed in vitro, showing improved optical signal intensity, similarity, accuracy, signal-to-noise ratio, and spatial distribution information. The method offers benefits for both optical imaging research and radiopharmaceutical development.

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1. Introduction

Cerenkov luminescence imaging (CLI) is an emerging approach to optical imaging that can image radiopharmaceuticals in vitro and in vivo in ways that are not feasible with positron emission tomography (PET) or single photon emission computed tomography (SPECT) [1]. As highly sensitive charge-coupled device (CCD) cameras have started to become available, significant interest has focused upon CLI-based studies, such as Cerenkov luminescence tomography (CLT) [2,3] and its potential endoscopic [4–6], or quality assurance [7] applications. CLI has a number of advantages over conventional medical nuclear imaging, including high throughput, low cost and being able to image beta-emitting radiopharmaceuticals [4]. Recent research has identified some specific clinical applications of CLI. Several notable studies, for instance, have been able to use CLI to image clearly things like the accumulation of $^{131}$I in thyroid diseases and the uptake of $^{18}$F-FDG in lymphatic nodes in axillary regions [8–10]. However, although CLI has been applied in a few preclinical and clinical studies, its applications remain limited because of its low intensity and the poor penetration of Cerenkov luminescence (CL), which is mainly distributed in the ultraviolet and blue bands (300-520 nm), leading to mass absorption and scattering in biological tissues [11,12]. Poor imaging quality has added to the difficulty of bringing about effective Cerenkov luminescence tomography (CLT) [2].

Beyond these issues, there is a lot of interest in uncovering characteristic spatial and quantitative information about diseases. This, however, cannot be provided by traditional 2-dimensional (2D) CLI [2]. Three-dimensional (3D) tomography that focuses on spatial and metabolic information can overcome these drawbacks [2,13]. Several research groups have been working on 3D reconstruction methods to improve the sensitivity, accuracy, and
imaging quality of CLT. These include inverse algorithm and diffusion equation (DE) approaches [13], multispectral diffuse CLT [14], hp-finite element methods (hp-FEM) [15] and third-order simplified spherical harmonic (SP3) approximation [16]. Unfortunately, because of the limited CL intensity and penetration, these creative reconstruction approaches still can’t collect enough signal to output a satisfactory 3D CLT image.

In previous research, we have found that rare earth microparticles (REMPs) can convert high-energy photons to low-energy photons, which can transfer CL to the Near-infrared (NIR), thus enhancing signal intensity and penetration [17]. Here we develop this strategy by using new biocompatible rare earth nanoparticles (RENPs) with a much smaller size. These can achieve higher optical signal intensity and even better tissue penetration. We shall also be presenting an optimized 3D reconstruction method based on our previous work that is able to acquire more accurate spatial information in vivo and some quantitative information that has never been obtained by CLI before. We name these new imaging methods as Cerenkov luminescence excited fluorescence imaging (CLFI) and Cerenkov luminescence excited fluorescence tomography (CLFT).

To specifically examine the effectiveness of our new approach, we enhanced Cerenkov Luminescence intensity based on the rare earth nanoparticles (Y_2O_3:Eu^{3+}) and performed CLFI and a 3D reconstruction based on CLFT to monitor spatial distribution and provide quantitative information about radiopharmaceuticals in phantoms and in vivo.

2. Materials and methods

2.1. Preparation of the radiopharmaceuticals and rare earth oxides

The radionuclide ⁶⁸Ga was eluted with 0.05M hydrochloric acid (HCl) from a ⁶⁸Ge-⁶⁸Ga generator (ITG, Germany). The rare earth nanoparticles (Y_2O_3:Eu^{3+} RENPs) were synthesized using the method first reported by Kamimura [18].

2.2. The characteristics of Y_2O_3:Eu^{3+} RENPs and the in vitro assay for CLFI of Y_2O_3:Eu^{3+} nanoparticles

Scanning electron microscopy (SEM) (FEI Quanta 200, Philips) was used to identify the size and morphology of the Y_2O_3:Eu^{3+} nanoparticles. The absorption spectra of the RENPs at a concentration of 5 mg/mL were recorded with a UV-VIS-NIR absorption spectrophotometer (Cary 500, Varian). The emission spectrum of the Y_2O_3:Eu^{3+} nanoparticles was measured by a fluorescence spectrophotometer (Edinburgh Instruments) and a 300 nm excitation source was generated using the optical grating of the spectrophotometer. All of the experiments were repeated 3 times (n = 3) and performed at room temperature.

100 μL ⁶⁸GaCl_₃ (⁶⁸Ga) solutions (3.7 MBq) both with and without Y_2O_3:Eu^{3+} RENPs (10 mg/mL) were added to a 96-well plate for measuring the CLI and CLFI intensities. The CLI and CLFI measures were acquired using an IVIS Spectrum System (Caliper Life Science). This experiment was repeated 3 times, then quantitative analysis was performed.

After this, we studied the relationship between both the CLI and CLFI optical intensity and the radioactivity of ⁶⁸Ga, followed by the relationship between the CLFI optical intensity and the concentration of the Y_2O_3:Eu^{3+} nanoparticles. In the first of the above experiments, Cerenkov luminescence imaging (CLI) was performed of various activities of ⁶⁸Ga (2.960 MBq, 1.480 MBq, 0.740 MBq, 0.370 MBq, 0.185 MBq, and 0 MBq). For the next set of experiments, Y_2O_3:Eu^{3+} nanoparticles (10 mg/mL) were excited by ⁶⁸Ga with various activities and CLFI was performed. For the final set of experiments, CLFI was performed of various masses of Y_2O_3:Eu^{3+} that had been excited by ⁶⁸Ga (2.96 MBq). The mass gradients of the Y_2O_3:Eu^{3+} nanoparticles were 10.0 mg/mL, 5.0 mg/mL, 2.5 mg/mL, 1.3 mg/mL, 0.6 mg/mL and 0 mg/mL. The CLI and CLFI images were all acquired simultaneously with a binning value of 8, an integration time of 60s and an aperture number (f_num) of 1. Each experiment was repeated 3 times (n = 3).
2.3. The penetration ability of CLFI and CLI

Phantoms based on tissue simulated liquid (TSL) were prepared according to the method proposed in [19] and were used to simulate biological tissue for the experiments. Basically, the TSL phantom consisted of 500 mL of Intralipid (10%) and 0.5 mL of India Ink. Two glass tubules (diameter = 3.00 mm) were placed in parallel in the bottom of a black lidless container. The experimental tubules were loaded with 370 kBq of ⁶⁸Ga and 10 mg/mL of Y₂O₃:Eu³⁺ nanoparticles. Control tubules, meanwhile, were loaded with 370 kBq ⁶⁸Ga only. The tubules were then covered with TSL to a depth of 0mm, 2mm, 4mm, 6mm and 8mm. CLI and CLFI images were acquired simultaneously using a CCD camera with a binning value of 8, an integration time of 60s and an aperture number (f_num) of 1. The experiment was repeated 3 times (n = 3).

Similar to the former step, the experimental( ⁶⁸Ga + RENPs)/control( ⁶⁸Ga) tubes were covered with 4 mm TSL. The activities of ⁶⁸Ga vary from 2960 to 185 k Bq (2960, 1480, 740, 370, 185 k Bq). CLI and CLFI images were acquired simultaneously using a CCD camera with a binning value of 8, an integration time of 60s and an aperture number (f_num) of 1. The experiment was repeated 3 times (n = 3).

2.4. CLFT and CLT of the phantoms

All subsequent optical images were acquired using a Xenogen In-Vivo Imaging System (IVIS Kinetic, Caliper Life Sciences). Regions of interest (ROI) in the same area were drawn over the sources on the optical images and the average radiances were measured using Living Image 3.2 software (IVIS Kinetic, Caliper Life Sciences), which provided the intensities of the optical signals.

The phantoms (n = 3) were made of lidless cylinders which were loaded with TSL. The experimental optical sources were made from 185 kBq ⁶⁸Ga and Y₂O₃:Eu³⁺ (10 mg/mL). The control sources were filled with 185 kBq ⁶⁸Ga only. All of the sources were placed at the bottom of each phantom and the depth of the TSL was 3 mm. After acquiring optical imaging, the phantoms were immediately prepared for PET/CT imaging.

3D distribution images of the optical sources were then reconstructed using the optimized 3D reconstruction method we had developed.

2.5. CLFT and CLT of the mice models in vivo

All animal procedures were conducted in accordance with the Fourth Military Medical University (FMMU) approved animal protocol. The experimental sources were made of 185 kBq ⁶⁸Ga and Y₂O₃:Eu³⁺ (10 mg/mL). The control sources were made of just ⁶⁸Ga (185 kBq). Implantation models (n = 3) were established by implanting the optical sources into the abdomen of athymic mice (body weight 20 ± 2 g). The mice underwent aseptic celiotomy and the embedded experimental/control sources were positioned close to the right dorsal abdomen of the mice. During the experimental procedure, the mice were anesthetized using isoflurane (2%). After acquiring the CLI and CLFI images, the phantoms immediately underwent PET/CT imaging for further CLT or CLFT acquisition.

A further set of CLI/CLT and CLFI/CLFT results were obtained after injecting the mice intravenously with 7.4 MBq of ⁶⁸Ga-NGR or 7.4 MBq of ⁶⁸Ga-NGR with 1 mg of RENPs.

2.6. Reconstruction of the CLT and CLFT

As with CLT, there is no external excited light for CLFT, so the same reconstruction method can be used for both CLT and CLFT [3,13]. In order to establish a forward problem for CLFT, the following diffusion equation was used to model the propagation of radioluminescent photons in biological tissues with high scatter and low absorption characteristics [20]:
where $\Phi (r)$ is the flux density, $S(r)$ is the unknown radioluminescent source, $\mu_a$ is the absorption coefficient, $D = 1/(3(\mu_a + \mu_s))$ is the diffusion coefficient, and $\mu_s$ is the reduced scatter coefficient. $\Omega$ and $\partial \Omega$ represent a region of the object and its boundary, respectively. $A$ is a boundary constant, representing the refractive index mismatch between the tissue and the surrounding medium.

A tangential planar approach called Kirchhoff approximation was used to find an analytical formulation for the Green’s functions of the diffusion equation [21]. After an imaged medium has been discretized, a linear equation can be established to describe the relationship between the flux of the radioluminescent source at the internal voxels of the grid and the boundary measurements of the radioluminescent photons. To overcome any possible weaknesses in this optical inverse problem, a regularized L1 normalized method (Regularized Orthogonal Matching Pursuit, ROMP) was used to solve the linear equation [22].

2.7. Relative quantitative assay

A relative quantitative study was performed in vitro. Four transparent tubes containing a $^{68}$Ga solution (14.8 MBq or 7.4 MBq) or a RENPs-$^{68}$Ga solution (10 mg/mL-14.8 MBq or 10 mg/mL-7.4 MBq) were placed on the bottom of 4 lidless containers (diameter = 50.0 mm, height = 10.0 mm). The tubes were then covered with TSL (depth = 5mm) in order to simulate the optical absorption and scatter characteristics of biological tissue. The CLFI and CLI were acquired using the IVIS System and the CLFT and CLT were reconstructed. After this, the relative quantitative analysis then was carried out according to the following steps:

1. $^{68}$Ga (7.4 MBq) calculated = $^{68}$Ga (14.8 MBq) realistic × ($^{68}$Ga (7.4 MBq) reconstructed ÷ $^{68}$Ga (14.8 MBq) reconstructed)

2. RENPs-$^{68}$Ga (7.4 MBq) calculated = RENPs-$^{68}$Ga (10 mg/mL-14.8 MBq) realistic × (RENPs-$^{68}$Ga (10 mg/mL-7.4 MBq) reconstructed ÷ RENPs-$^{68}$Ga (10 mg/mL-14.8 MBq) reconstructed)

3. Activity quantitative error (CLT) = ($^{68}$Ga (7.4 MBq) calculated – $^{68}$Ga (7.4 MBq) realistic) ÷ $^{68}$Ga (7.4 MBq) realistic

4. Activity quantitative error (CLFT) = (RENPs-$^{68}$Ga (10 mg/mL-7.4 MBq) calculated – RENPs-$^{68}$Ga (10 mg/mL-7.4 MBq) realistic) ÷ RENPs-$^{68}$Ga (10 mg/mL-7.4 MBq) realistic

2.8. Statistical analysis

All the data obtained was analyzed through the GraphPad Prism 5.0 and presented as means ± SD. The differences between the two groups were determined by Student’s t-test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. $Y_2O_3:Eu^{3+}$ RENPs characteristics and the in vitro CLFI study

A representative SEM image of the $Y_2O_3:Eu^{3+}$ RENPs is shown in Fig. 1(a), which shows that they were basically globular in form. Their diameter was $51.5 \pm 7.5\mu m$. The UV-NIR spectrum (Fig. 1(b)) shows that the characteristic absorption peaks of the $Y_2O_3:Eu^{3+}$ RENPs distributed at 300 nm. The emission spectrum when excited by a 300 nm laser shows that
their emission distributed at the near-infrared range (600nm - 800nm) (Fig. 1(c)), which provides for a good penetration.

The optical signal intensity for the CLFI was 3.28-fold that of the CLI (3.81 ± 0.23 × 10^7 vs. 1.16 ± 0.21 × 10^7, p = 0.0010 < 0.01), which indicates that the Y_2O_3:Eu^{3+} RENPs significantly enhanced the CL intensity. Figure 1(f) shows that Y_2O_3:Eu^{3+} is excited by Cerenkov luminescence rather than γ rays. After blocking Cerenkov radiation, Y_2O_3:Eu^{3+} RENPs can’t be excited and emit fluorescence. The luminescence spectrum of ^{68}Ga and the fluorescence spectrum of the Y_2O_3:Eu^{3+} RENPs excited by ^{68}Ga (Fig. 1(h)) show that the CLFI signal had an emission peak at 660 nm. The results of the relative quantification indicate that there was a strongly positive linear relationship between the CLFI signal intensity and the concentration of the Y_2O_3:Eu^{3+} RENPs (Fig. 1(i)). Similarly, the intensities of both the CLFI and CLI were positively correlated with ^{68}Ga radioactivity.

Fig. 1. SEM image of the Y_2O_3:Eu^{3+} RENPs (a). The absorption spectrum of the Y_2O_3:Eu^{3+} RENPs (b) and their emission spectra when excited by a 300 nm laser (c). CLFI of the Y_2O_3:Eu^{3+} RENPs excited by ^{68}Ga and CLI of ^{68}Ga (d). Quantitative analysis of the CLFI and CLI results (e). CLFI of the Y_2O_3:Eu^{3+} RENPs excited by ^{68}Ga or ^{68}Ga (blocked) (f). CLI of ^{68}Ga with various activities (the first row), CLFI of the Y_2O_3:Eu^{3+} RENPs excited by ^{68}Ga with various activities (the second row), CLFI of the Y_2O_3:Eu^{3+} RENPs at various concentrations excited by ^{68}Ga (the third row) (g). Fluorescence spectrum of the Y_2O_3:Eu^{3+} RENPs excited by ^{68}Ga and Cerenkov spectrum of ^{68}Ga (h). The quantified relationship between the CLFI intensity and the concentration of the Y_2O_3:Eu^{3+} RENPs (i). The quantified relationship between ^{68}Ga activities and the CLFI intensity or CLI intensity (j).
3.2. Penetration of the CLFI and CLI assay

As the depth of the TSL increased from 0 mm to 8 mm, there was a notable decrease in the detected intensity for both the CLFI and the CLI. When the activity of $^{68}$Ga was 370 kBq and the TSL depth achieved 8 mm, the CLI image could be hardly captured by CCD camera but CLFI image was still obvious (Fig. 2(b)). The results of the quantitative analysis indicate that as the depth increases, the enhancement ability of the $\text{Y}_2\text{O}_3$:$\text{Eu}^{3+}$ RENPs remains steady, which contributes to a better penetration of Cerenkov luminescence excited fluorescence, especially in the deep tissues. As for the influence of intensity on the penetration of CLFI or CLI, the results (Fig. 2(e) and 2(f)) indicated that with the decreasing of activity of $^{68}$Ga, the penetration depth of CL and CLF were both declining and CLF equipped a better penetration compared with CL under a certain activity of $^{68}$Ga.

![Fig. 2. Diagrammatic overview of the penetration phantom (a). CLFI and CLI at various depths (b). Quantitative analysis of the relationship between various depths of tissue stimulated liquid and the optical intensity of the CLFI and CLI (c). The ratio of CLFI optical intensity to CLI optical intensity according to different depths of tissue stimulated liquid (d). CLFI and CLI of various activities of $^{68}$Ga at 4 mm depth (e). Quantitative analysis of the relationship between various activities of $^{68}$Ga and the optical intensity of the CLFI and CLI (f).](image)

3.3. CLFT and CLT of the phantom

CLI and CLFI images are shown in Fig. 3(d). Quantitative analysis of the optical intensity of the CLI and CLFI (Fig. 3(e)) indicates that the intensity of CLFI is substantially higher than that of CLI ($1.35 \pm 0.09 \times 10^6$ vs. $0.38 \pm 0.05 \times 10^6$ p/s/cm$^2$/sr, $p = 0.0009 < 0.001$), while the radioactivity of the two phantoms (Fig. 3(c)) shows no significant difference when verified by PET/CT imaging quantitative analysis ($175.2 \pm 18.4$ vs. $173.6 \pm 9.7$ kBq/mL, $p = 0.8323 > 0.05$). The reconstructed CLT and CLFT images are shown in Fig. 3(f). The similarity degree (the degree of position coincidence between the reconstructed phantom and the real phantom) for the CLT and CLFT was $0.20 \pm 0.04$ and $0.41 \pm 0.03$, respectively, which strongly suggests
that the CLFT was more accurate than the CLT (p = 0.0008 < 0.001). An enlargement of the reconstruction results for the CLT and CLFT is shown in Fig. 3(g).

3.4. CLFT and CLT of the mice

For the athymic nude mice models implanted with $^{68}$Ga or $^{68}$Ga-RENPs, the $^{68}$Ga activities for the experimental and control models were 177.8 ± 8.9 and 178.1 ± 4.5 kBq/mL, (p = 0.9740 > 0.05). Their optical intensities, however, were 1.15 ± 0.2 × 10^6 p/s/cm^2/sr and 3.30 ± 0.32 × 10^6 p/s/cm^2/sr (p = 0.0017 < 0.01). The reconstructed results showed that the degree of similarity for the CLT and CLFT implanted models were, respectively, 0.16 ± 0.04 and 0.43 ± 0.11 (p = 0.0293 < 0.05). Besides, for the mice injected with only $^{68}$Ga-NGR or with $^{68}$Ga-NGR + RENPs”, the degree of similarity for their bladders based on CLT and CLFT, respectively, were 0.25 ± 0.07 and 0.49 ± 0.12 (p = 0.0288 < 0.05). The bladder reconstruction results are shown in Fig. 4(k). The in vivo 3D reconstruction results further
confirmed that CLFT can provide more accurate spatial distribution information for radiopharmaceuticals.

![Image](image1)

**Fig. 4.** PET/CT images of the phantoms implanted with only $^{68}$Ga and with $^{68}$Ga mixed with RENPs (a). Quantitative analysis of the radioactivity of the implanted models (b). CLI of the phantoms implanted with only $^{68}$Ga and CLFI of the phantoms implanted with $^{68}$Ga and RENPs (c). Quantitative analysis of the optical intensity of the implanted phantoms (d). Reconstruction results for the phantoms implanted with only $^{68}$Ga or mixed with RENPs (e). Quantitative analysis of the degree of similarity for CLT and CLFT based on the implantation models (f). PET/CT images of the mice injected with $^{68}$Ga-NGR only or with $^{68}$Ga-NGR with RENPs (g). Quantitative analysis of the radioactivity of the mice (h). CLI of the bladders of the mice injected with $^{68}$Ga-NGR only and CLFI of the bladders of the mice co-injected with $^{68}$Ga-NGR and RENPs (i). Quantitative analysis of the optical intensity of the bladder phantoms (j). Reconstruction results of the bladders of the mice injected with $^{68}$Ga-NGR or with $^{68}$Ga-NGR with RENPs (k). Quantitative analysis of the degree of similarity for CLT and CLFT based on the injected mice (l).

### 3.5. Quantitative study of radiopharmaceuticals based on CLT and CLFT

In the quantitative analysis experiment, we took the tubes containing $^{68}$Ga (14.8 MBq) and RENPs-$^{68}$Ga (10 mg/mL, 14.8 MBq) as standard references. Then we calculated the activities of $^{68}$Ga (7.4 MBq) and RENPs-$^{68}$Ga (10 mg/mL, 7.4 MBq) using CLT and CLFT. On the basis of standardized analysis that described in the materials and methods section, we found that the quantitative activity error for $^{68}$Ga-RENPs was $-2.5 \pm 0.11\%$. For $^{68}$Ga, however, it was $-21.3 \pm 3.7\%$ ($p = 0.0124 < 0.05$). Further quantitative analysis experiments indicated that CLFT could also provide more accurate activity information for $^{68}$Ga radiopharmaceuticals than CLT (Fig. 5).
4. Discussion

Cerenkov luminescence imaging features prominently in the anticipated development of molecular imaging and radiopharmaceuticals. However, the most intensive CL signal distributes in the ultraviolet and blue range, which is easily scattered and absorbed by biological tissues [1, 23]. The possible applications of CLI are therefore limited by poor penetration and CL’s low intensity. As natural properties of light, red and NIR photons are more effective at penetrating biological tissue. As a result, research has focused on how to translate CL into red or NIR photons, thus improving CLI penetration. Various materials have been explored in this regard, including quantum dots and nanocrystals [24, 25]. The Y$_2$O$_3$:Eu$^{3+}$ nanoparticle is a rare-earth oxide that has a main excitation peak at 300 nm and a main emission peak at 660 nm. This makes it a promising candidate for excitation by CL because, when excited in this way, it emits red light. In this study, we successfully enhanced CL penetration and intensity by using Y$_2$O$_3$:Eu$^{3+}$ RENPs. We found that Y$_2$O$_3$:Eu$^{3+}$ RENPs could, indeed, be excited by CL, resulting in a significantly enhanced fluorescence intensity that was over 3 times better within the detection range of CCD cameras. Besides, CLF (Cerenkov luminescence excited fluorescence) intensity has a positive linear correlation with the radioactivity of $^{68}$Ga and a concentration of Y$_2$O$_3$:Eu$^{3+}$ RENPs. This helps with quantification analysis of CLFT. We found that CLFI could detect deeper optical signals than CLI, which will lead to better quality CLFI and CLFT for deep tissues.

RENPs have been considered as biocompatible agents for biological applications [17, 26] for many years. In this study, we saw that Y$_2$O$_3$:Eu$^{3+}$ RENPs are globular nanoparticles with a diameter of less than 60 nm and an excitation spectrum that distributes in the 300 nm range, which coincides with an intense region of the Cerenkov spectrum [17]. Y$_2$O$_3$:Eu$^{3+}$ RENPs in this study are excited by Cerenkov luminescence which could be demonstrated by the open/ blocked experiment (Fig. 1(f)). However, some other nanophosphors also can be excited directly by ionizing radiation [27]. Thus, the excitation mechanism of different nanophosphors mostly depends on the nanophosphor itself. When examining the excitation mechanism for Y$_2$O$_3$:Eu$^{3+}$ RENPs we found that RENPs are equipped with a down-conversion characteristic and can be excited by pure $\beta$ decay radionuclides. Compared to other conversion methods, Y$_2$O$_3$:Eu$^{3+}$ RENP conversion offers a number of advantages: (1) It has a high output, which means that one high-energy photon can convert into several low-energy photons with a longer wavelength; (2) Long wavelength photons are equipped with better penetration and a good signal-to-noise ratio when compared to shorter wavelength photons; (3) Y$_2$O$_3$:Eu$^{3+}$ RENPs can be used for imaging therapeutic nuclides such as Y$^{90}$.

At present, living tomography based on Cerenkov optical signals is faced with the difficulty that CL is not effective for deep tissue because of its low penetration and intensity. As a result, although CLT applications do exist, these are far away from being in vivo. The degree of similarity values for CLT in different phantoms are typically too poor to afford

Fig. 5. CLI and CLFI of the phantom with various activities (a). Relative quantification results based on CLFT and CLT of the phantom with various activities (b). Quantitative activity error for $^{68}$Ga (7.4 MBq) and $^{68}$Ga-RENPs (10 mg/ml, 7.4 MBq) based on CLT or CLFT (c).
accurate location information. However, after being enhanced by Y$_2$O$_3$:Eu$^{3+}$ RENPs, CL can be converted into red CLF, which has better penetration and less absorption and scatter-loss than CL over shorter wavelengths. This significantly improves the quality of CLFI and CLFT. In view of the long acquisition time that results from the low CLFI signal, our CLFT approach relied upon just a single optical projection measurement for 3D reconstruction. The sparse characteristics of RENPs distributions, however, made it possible for CLFT to use regularized L1-normalized methods to overcome the weaknesses of this single view optical inverse problem. This contributed to a better signal-to-noise ratio, which was very important for the quality of the CLFT results. In artificial cylinder phantoms, implanted mice models and mice bladder models, the degree of similarity values based on CLFT were significantly higher than those based on CLT. This indicates that CLFT has a better reconstruction accuracy than CLT, thereby offering important opportunities for reflecting the distribution of radiopharmaceutical probes in future applications. Another interesting approach reported by Pogue et al. [28,29] is using CL of high energy X-ray generated from a clinical linear accelerator (LINAC) to excite phosphors. It uses the long lifetime property of phosphors to separate phosphorescence form the pulse CL and obtains high sensitivity optical imaging of the molecular signatures of tumors. While their results and ours both indicate that the reconstructed signal values are closely related to the concentration of fluorophore as well as the intensity of the Cerenkov radiation.

Results from our relative quantitative analysis indicated that CLFT is able to reflect more accurate uptake information for a radiopharmaceutical. As tumor could uptake RENPs because of the enhanced permeability and retention (EPR) effect which has been well investigated [30]. Its use would therefore make sense in staged diseases and tumor diagnosis/treatment evaluation in future clinical studies and applications. We also found that the quantitative activity error values were negative for both CLFT and CLT. This may imply that there are still optical signal losses in both CLT and CLFT. There are some innovations in our study as following: Firstly, Y$_2$O$_3$:Eu$^{3+}$ has been demonstrated with the character of transforming CL into long wavelength light and further enhancing CL. Secondly, we innovatively tried to do a semi-quantitative research based on optical tomography and the results showed the superiority of CLFT on quantitation compared with CLT. Thirdly, we demonstrated that CLFT could provide more accurate spatial information than CLT depending on different phantoms. There are also some further other notable limitations to this study. For instance, although the luminescence intensity and reconstruction accuracy of CLT has been significantly enhanced, there is still a lot of room for improvement. Besides, in this project we paid close attention on the enhancement effect and tomography reconstruction with phantom studies and the toxicity study was not performed systematically. Even though we observed that the mice injected with RENPs survived as long as the mice without RENPs injection until they were euthanized (4 weeks after injection), biological toxicity study of RENPs was still needed. Our studies in these regards are still ongoing.

5. Conclusion

Y$_2$O$_3$:Eu$^{3+}$ RENPs can significantly enhance the Cerenkov luminescence signal of radionuclides and CLFI and CLFT can provide more reliable spatial and quantitative information of radiopharmaceuticals on the basis of this enhancement. This is likely to improve the possible applications of CLI in the future.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

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