# Recent Advances in Cerenkov Luminescence and Tomography Imaging

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*(Invited Paper)*

***Abstract*—Molecular imaging can provide qualitative or quantitative physiological and pathological knowledge at the cellular and molecular levels for biomedical research, which has experienced a remarkable growth in recent years. Among molecular imaging techniques, optical molecular imaging has attracted considerable attention in view of its excellent performance and high cost-effectiveness. However, heretofore the experimental objects of optical imaging technique are mainly small animals like rats and mice, and optical imaging can hardly be used for clinical research not only because of the limited tissue penetration, but also due to the scarcity of appropriate imaging probes. As a newly emerging and very promising imaging modality, Cerenkov luminescence employs light signals emitting from radionuclides used in nuclear imaging based on Cerenkov radiation, which can provide more potential optical imaging probes for clinical application and facilitate the realization of multimodality imaging. In this paper, the fundamentals of Cerenkov luminescence are first introduced, and then Cerenkov luminescence imaging and tomography as well as the corresponding biomedical applications are represented. Finally, limitations of Cerenkov luminescence and the discussion of this contribution are covered.**

***Index Terms*—Cerenkov luminescence imaging (CLI), Cerenkov luminescence tomography (CLT), molecular imaging, multimodality imaging, nuclear imaging, optical imaging, optical tomography, radionuclides.**

Manuscript received May 14, 2011; revised July 3, 2011; accepted July 4, 2011. Date of publication July 14, 2011; date of current version June 1, 2012. This work is supported by the National Basic Research Program of China (973 Program) under Grant 2011CB707700, the Knowledge Innovation Project of the Chinese Academy of Sciences, Beijing, China, under Grant KGCX2-YW-907, the National Natural Science Foundation of China under Grant 81027002, 81071205, 30970778, the Fellowship for Young International Scientists of the Chinese Academy of Sciences under Grant 2010Y2GA03, the Chinese Academy of Sciences Visiting Professorship for Senior International Scientists under Grant 2010T2G36.

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Digital Object Identifier 10.1109/JSTQE.2011.2161757

## I. Introduction

As a newly emerging interdisciplinary of modern molecular biology and advanced *in vivo* imaging technology in the early twenty-first century, molecular imaging can noninvasively visualize the physiological and pathological changes of living organisms at the cellular and molecular levels using suitable imaging probes and appropriate imaging instruments, which can promote the research of biological and medical processes for diseases like cancer as well as drug development, especially for personalized medicine in the future [1]– [3]. As we all know, there are various imaging modalities for different biomedical information including anatomic structure information such as x-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (US), and functional imaging knowledge, such as optical imaging (OI), positron emission tomography (PET), single photon emission computed tomography (SPECT), and functional magnetic resonance imaging (fMRI) [4]. Furthermore, multimodality imaging techniques integrating the strengths of two or more modalities have been widely studied to provide the prospect of improved diagnostics, therapeutic monitoring, and preclinical research with the help of hybrid multimodality imaging probes [5].

Among molecular imaging techniques, optical molecular imaging, such as fluorescence and bioluminescence imaging, has attracted considerable attention in view of its high sensitivity, excellent temporal resolution, simple operation, and high cost-effectiveness [6], [7]. The spatial resolution of conventional 2-D optical imaging is relatively low because of highly scattering property of the biological tissue, and quantitative analysis cannot be performed [8]. Therefore, planar optical imaging has been developed to 3-D optical tomography, and the depth of targeted molecular probes can be resolved simultaneously. However, the penetration depth of emitting photons is generally several millimeters even if the imaging spectrum is located in the near-infrared light window of the biological tissue (650–900 nm), so the clinical application for deep tissues is limited if there is no effective auxiliary method like endoscopic technique [2]. Furthermore, the biggest challenge of optical imaging for clinical application is the probes, especially for transgenic bioluminescence imaging using luciferase [10]. To our best knowledge, only two optical molecular imaging probes, indocyanine green (ICG), and fluorescein sodium, have been approved for clinical application by the US Food and Drug Administration (FDA) [9]. More information of FDA approved contrast agents can be

obtained from molecular imaging and contrast agent database (MICAD) (http://www.ncbi.nlm.nih.gov/books/NBK5330/). Therefore, further progresses in constructing new optical imaging probes and refining data acquisition methods are urgently needed to better exploit the potential of optical imaging.

Nuclear imaging is an important molecular imaging modality commonly used in clinical diagnosis and research, in which PET and SPECT are two most typical and successful imaging techniques. High-energy charged particles such as $\beta^+$, $\beta^-$, and $\gamma$ are released during the decay of various radionuclides, and after about a millimeter propagation, the positron will annihilate with a nearby electron and emit a pair of 511 keV $\gamma$ photons [4], [10]. Nuclear imaging has excellent tissue penetration and high sensitivity, but the temporal resolution and cost-effectiveness are lower in comparison with optical imaging, which limits its application for basic research. Moreover, the spatial solution of nuclear imaging is about 1 mm [4]. It is worth noting that the biggest advantage of nuclear imaging compared with traditional optical imaging is a variety of approved radionuclides with different decay modes for clinical application. Therefore, it is very significant to develop a novel imaging modality for acquiring the similar functional or metabolic information obtained in nuclear imaging.

In recent years, as a novel and promising *in vivo* molecular imaging modality, Cerenkov luminescence has become a research focus in the biomedical imaging field, which combines optical and nuclear imaging together using the same probe based on Cerenkov radiation [10]–[12]. As a well-known physical phenomenon, although Cerenkov radiation was found several decades ago, Cerenkov luminescence imaging (CLI) was first reported and introduced into small animal imaging field in 2009 by Robertson *et al. In vitro* and *in vivo* experiments were performed to validate that the detected light was derived from Cerenkov radiation, and the imaging results were in good agreement with the measurements by PET [10]. This interesting imaging technique can also offer a simple and quantitative measurement method of beta particles in microfluidic applications [13]. The depth of positron emitting radiotracers was determined using spectrally resolved data and diffusion equation or the known spectral distribution of the Cerenkov light source [14], [15]. Furthermore, Cerenkov luminescence tomography (CLT) has been studied to reconstruct 3-D distribution of the internal radiotracers using the permissible source region method and multispectral information, which has been validated by the homogeneous or heterogeneous phantom and the mouse model [16]–[18]. When the assumption that the velocity of charged particles traveling in tissues exceeds the speed of light in the same medium is satisfied, Cerenkov radiation in the visible spectrum will be generated [10]. However, most of emitting photons are produced in the blue light spectrum, and the light intensity is inversely proportional to the square of the wavelength [10], [19]. Therefore, third-order simplified spherical harmonics approximation of the radiative transfer equation (RTE) has been employed to model Cerenkov photon propagation in the biological tissue more accurately [19], [20]. On the other hand, quantum nanoparticles [21], [22] or fluorescence reporters [23] could be coupled to produce longer wavelength light. In addition, a variety of radionuclides that can generate charged particles such as $\beta^+$ and $\beta^-$ were utilized to verify the ability to emit the low-energy light by commercially available optical imaging instrument [11]. More detailed contents will be described in the following sections. In summary, Cerenkov luminescence not only can offer more approved probes for optical imaging, even for clinical application, but also can provide an economical and practical substitutive modality of nuclear imaging for basic biomedical research.

This paper is organized as follows: the fundamentals of Cerenkov luminescence are first introduced in the next section. In the third section, the research progresses of planar Cerenkov luminescence imaging are depicted. Followed by 3-D Cerenkov luminescence tomography, some applications of Cerenkov luminescence imaging and tomography are presented. Finally, limitations of Cerenkov luminescence and the discussion of this contribution are covered.

## II. Principle of Cerenkov Luminescence

Cerenkov luminescence will be produced by a charged particle uniformly traveling in a medium with the velocity exceeding that of light. The more and more comprehensive quantitative theory has afforded an exhaustive interpretation of all the peculiarities of the physical phenomenon.

The prediction of Cerenkov radiation came long ago. Heaviside investigated the possibility of a charged object moving in a medium faster than electromagnetic waves in the same medium becomes a source of directed electromagnetic radiation in 1887 [24]. Kelvin gave a famous talk entitled "Nineteenth century clouds over the dynamical theory of heat and light," in which he also addressed the emission of particles was possible at a speed greater than that of light in 1900 [25]. Somewhat later, from 1904 to 1905, Sommerfeld proposed the similar hypothetical radiation with a sharp angular distribution [26]. Unfortunately, these investigations were forgotten for many years with the coming of the theory of relativity. In accordance with the prevailing view, particles are unable to move at the speed of light, still less to exceed it.

Cerenkov experiments performed at the suggestion of Prof. S. Vavilov opened a door to the true physical nature of Cerenkov luminescence in 1930s. There is indeed a large number of luminescence phenomena, even if only for radioactive materials. Cerenkov distinguished them by the excitement method, the fluorescent lifetime, the character of the spectrum, properties of the luminescent substances, and other peculiarities [27]. The newly discovered light is called as Cerenkov luminescence. The phenomena was theoretically interpreted by Frank and Tamm in the framework of classical electrodynamics [28]. Identical relations between the frequency of the radiated light and the direction of its emission was obtained by both the classical and the quantum methods [29]:

$$\frac{n(\omega)\omega}{c}\cos\theta = \frac{\omega \pm \omega_0}{v} \tag{1}$$

where $\omega_0$ is the natural frequency, and $\theta$ is the angle between the path of the moving electron at the speed of $v$ and Cerenkov radiation in a certain frequency $\omega$. $n(\omega)$ is the refractive index

of a particular medium. The standard radio $\beta$ is equal to $v/c$, and $\cos\theta = 1/(n(\omega)\beta)$. When $n(\omega) > 1$ for visible light, the propagation speed of the light waves in the medium, $c/n(\omega)$, is smaller than the velocity of light in the vacuum $c$. As a result, the Cerenkov radiation spectrum is determined by the velocity of the moving system $v$, its natural frequency $\omega_0$ and the phase velocity of light $c/n(\omega)$ in a medium. And the Vavilov–Cerenkov effect is possible, if

$$vn(\omega)/c > 1. \tag{2}$$

When the speed of moving system nears the speed of light, the threshold energy for the production of Cerenkov radiation by use of (2) is

$$E = mc^2(1/\sqrt{1-n^2(\omega)} - 1) \tag{3}$$

where $m$ is the particle mass. As a matter of fact, $n(\omega)$ is a function of the radiated frequency. When Cerenkov radiation occurs, the transfer of energy is related without the phase but precisely with the group velocity [29]:

$$u = c/\left(n(\omega) + \omega\frac{dn(\omega)}{d\omega}\right). \tag{4}$$

And the Tamm–Frank radiation condition is satisfied for any velocity owing to a frequency interval, after Fermi considered a charge moving uniformly in a medium with dielectric constant [30]. The first quantum consideration of the Cerenkov radiation was obtained by Ginzburg in 1940 [31], which coincides with the classical expression given by Tamm and Frank. The number of photons produced per unit path length $ds$ of a particle with charge $ze$ and per unit energy interval of the photons is expressed as

$$\frac{d^2N}{dsd\lambda} = \frac{4\pi^2e^2z^2}{hc\lambda^2}\sin^2\theta \tag{5}$$

where $e$ is the elementary charge; $\lambda$ is wavelength of photons; and $h$ is the Planck constant. Practical Cerenkov radiator materials are dispersive. Photons propagate at the group velocity $u$. In a nondispersive medium, this is simplified to $u = c/n(\omega)$. Using charge coupled device (CCD) with an ideally 100% efficiency in the 400–700 nm spectrum, the number of photons can be computed by the following formula:

$$\frac{dN}{ds} = \frac{4\pi^2e^2z^2}{hc}\left(\frac{1}{\lambda_1} - \frac{1}{\lambda_2}\right)\sin^2\theta. \tag{6}$$

In order to mathematically address that these Cerenkov photons are the blue glow, it is necessary to consider that the light energy, $W$, is produced per unit path length [29]

$$\frac{dW}{ds} = \frac{z^2e^2}{c^2}\int_{\beta n(\omega)>1}\omega\sin^2\theta d\omega. \tag{7}$$

Since the spectrum is continuous, $n(\omega)$ can be reduced to 1.0 considering $n(\omega)$ is a constant. And then, the differentiating with respect to the angular frequency, $\omega$ is formulated as

$$\frac{dW}{d\omega} = \frac{z^2e^2s\omega}{c^2} \tag{8}$$

which can be equivalently expressed in wavelength as follows:

$$\frac{dW}{d\lambda} \propto \frac{1}{\lambda^3}. \tag{9}$$

In the real experiment, the photons can be lost by geometrical acceptance, for instance of transparency of the quartz window, reflectivity of the mirror and the Winstone cones [32], and the quantum efficiency of the CCD camera.

Further studies of Cerenkov luminescence have been rapidly developed. Jelley presented a review of experimental and theoretical investigations of the Cerenkov radiation in 1955 [33]. The source-theoretic description of this effect was given for zero temperature in an isotropic homogeneous medium in 1976 [34]. The exact power spectrum of the Cerenkov radiation with the radiative correction in electromagnetism and gravity was derived at finite temperature [35]–[37]. Charged particles traveling through nonlinear medium can emit Cerenkov radiation, associated with two velocities, one above and the other below a certain speed threshold [38]. The radiation structure inside the Cerenkov cone was further illustrated, and the visible Cerenkov light region is found to be surrounded by the low-intensity infrared region and by the high-intensity one corresponding to high-energy photons [39]. Supposing the microscopical structure of medium and interaction of moving charges with it, the two-photon production by motion of a charged particle can be described in terms of quantum electrodynamics [40], which breaks through the limitations of Frank formulation.

## III. Cerenkov Luminescence Imaging

Cerenkov luminescence imaging is an approach to utilize Cerenkov radiation escaping from the surface of living organisms injected with medical isotopes with optical imaging techniques. When radiotracers labeled with high-energy particle emitters are involved in the metabolism of organisms, a sequence of signals can be detected *in vivo* in the form of waves or particles. These biophysical signals have access to the prediction of the concentration of radioactive nuclides in various biological tissues. The distribution and retention of radionuclides by various organs in the body is the basis and premise of imaging organs functionally and structurally. The biomedical information from radionuclide imaging noninvasively maps biomolecular or biologic processes within single cells or even whole organs. There are at least two types of particles from radioactive nuclides, which could be detected in a noncontact way. One is the gamma ray by the PET scanner, SPECT scanner, and gamma camera; the other is the Cerenkov photon by the CCD camera. Taking the $^{18}$F radioactive decay for example, after the release of a certain positron, its ultra high-speed movement generates Cerenkov radiation, until it annihilates with an electron and produces a pair of gamma photons. The visible Cerenkov light is so faint that the high-performance CCD camera is needed during *in vivo* optical imaging, including sensitivity, quantum efficiency, readout noise and so on.

Appearance of CLI opens a door to nuclear imaging without a gamma ray detector. Robertson first made use of Xenogen devices (IVIS 100 or 200, Caliper Life Sciences, Alameda, CA) and 2′-deoxy- 2′-[$^{18}$F]fluoro-D-glucose ($^{18}$F-FDG),

investigated the spectrum and decay of luminescence for $^{18}$F-FDG solutions with different activities, and demonstrated Cerenkov luminescence that is consistent with the $^{18}$F-FDG distribution throughout the mouse. The fact that the Cerenkov light output from different degrees was consistent with the quantification from the PET scan has been proved in [10, Fig. 4]. The CLI imaging results were verified with those of PET (micro-PET R4, Siemens Medical). Assuming that the refractive index of biological tissues $n$ is a constant [41], we can calculate the threshold speed (0.7 c) and energy (262 keV) for the production of Cerenkov radiation by a $^{18}$F positron according to (2) and (3). The experimental values of the photon yield were consistent with the theoretical value. Liu evaluated the *in vivo* CLI of $^{18}$F, $^{90}$Y, $^{131}$I labeled compounds by the IVIS Spectrum small animal imaging system (Caliper Life Sciences, Hopkinton, MA), and displayed the Cerenkov spectrum using a narrow band emission filter (20 nm bandwidth) [11]. Park normalized the Cerenkov light intensities of various radiotracers and summarized the relative luminescence intensity (IVIS 200, Caliper Life Sciences, Inc., USA) [12]. The aforementioned parameters of radionuclides have an important reference value for the choice of molecular imaging probes with optical techniques. Ruggiero performed the first examples of dual optical CLI and PET of *in vivo* tumor-specific uptake using a metallolabeled antibody. The dynamic distribution of $^{89}$Zr has been comprehensively evaluated in a small animal. Details of temporal images of $^{89}$Zr-DFO-J591 uptake recorded in dual subcutaneous LNCaP (PSMA-positive) tumor bearing severe combined immune deficient mice between 24 and 96 h after administration are given in [42, Fig. 4].

Cerenkov emission spectrum is a continuum. Utilization of Cerenkov spectrum helps to calculate the depth of light source, with the help of the multispectral imaging system. Suppose that the index of refraction is given and Schwinger source theory works, the number of Cerenkov photons in the source can be expressed in analytic form. Assuming that the diffusion approximation occurs in biological tissues, Spinelli and colleagues gave the method to estimate the depth of the source inside the animal in this framework [14], [15]. Of course, these data came from a single perspective, based on a list of planar images captured with several emission filters.

As a matter of fact, the transmission of the Cerenkov luminescence is weak for small animal imaging. In order to modulate the luminescence spectrum for better tissue penetration [43], biochemistry experts have opened a cross talk between Cerenkov luminescence and fluorescent agents. When quantum dots (QDs) is illuminated by radiation luminescence as an internal light source, the excited QDs can produce fluorescence for *in vivo* imaging [21]. Dothager discovered highly red-shifted photonic emissions in Cerenkov radiation, coupled with PET isotopes $^{18}$F or $^{64}$Cu and high Stokes-shift quantum nanoparticles (Qtracker 705) [22]. Lewis converted Cerenkov luminescence to longer wavelengths with the simultaneous utilization of fluorescence reporters [23]. In addition, we can achieve multimodality imaging through modifying the reporter gene/nuclear reporter probe system. For example, [herpes simplex virus type-1 thymidine kinase (HSV1-tk) and 9-(4-$^{18}$F-fluoro-3-[hydroxymethyl] butyl) guanine ([$^{18}$F]FHBG)] [44] could be successively imaged by CLI and PET [45].

## IV. Cerenkov Luminescence Tomography

Cerenkov luminescence escaping from the surface of living organisms is made the use of tomography imaging with optical techniques, which is termed as Cerenkov luminescence tomography. Process tomography includes several modalities, such as electrical (impedance, capacitance, inductance), radiation (optical, x-ray, positron electromagnetic, magnetic resonance), and acoustic (ultrasonic, photoacoustic) [46]. Optical techniques are nonintrusive in nature and safe as the transducer does not require direct physical contact with the measured [47]. Optical tomography is to reflect the absorption, scattering, and emission of radiation in media by use of low-energy visible or near-infrared light, which relies on the fact that various disease processes and most physiological changes affect the optical properties of the biological tissue [48]–[51]. The differences in optical properties between healthy and pathological tissues provide the contrast for this imaging technology. In the imaging experiment, medical isotopes are injected in the body, and the Cerenkov luminescence is transformed into digital signals by a CCD camera, which prepares for Cerenkov tomographic processing. The reconstructed distribution of the light source inside the media can help the diagnosis of pathological dysfunction in clinical and preclinical imaging.

CLT is a typical reverse problem. The mathematical framework of a complete algorithm process for CLT can be broken down into two parts: a forward model for light propagation in biological tissues, and an inverse model for reconstructing the medical isotopes inside the media from predicted and measured partial boundary measurements.

The forward model describes the phenomena of energy transfer by optical radiation in media, which has been of interest for a long time. The phenomenological foundations were developed and radiative transfer theory was advanced for optical tomography [52]–[57]. The fundamental integro-differential equation for the radiance $\phi$ is the RTE approximated from Maxwell's equations:

$$\left\{\hat{s}\cdot\nabla+\mu_a+\mu_s+\frac{\partial}{\partial t}\right\}\phi(r,\hat{s},t)=q(r,\hat{s},t)+\mu_s\int(p(\hat{s}',\hat{s})\phi(r,\hat{s}',t))d\Omega'. \tag{10}$$

Radiance $\phi$ is defined as the spectral radiance integrated over a narrow frequency range with units of $\mathrm{W}\cdot\mathrm{m}^{-2}\cdot\mathrm{sr}^{-1}$; $r$ denotes position; $\hat{s}$ indicates unit direction vector; $t$ denotes time; $\mu_a$ is the absorption coefficient; $\mu_s$ is the scattering coefficient; $d\Omega$ denotes a differential solid-angle element around direction $\hat{s}$; $d\Omega'$ denotes a differential solid angle element around direction $\hat{s}'$; $p(\hat{s}',\hat{s})$ is the phase function, and the product $p(\hat{s}',\hat{s})d\Omega$ represents the probability of light with propagation direction $\hat{s}'$ being scattered into $d\Omega$ around direction $\hat{s}$. $q(r,\hat{s},t)dvd\Omega$ denotes the energy produced by a source in the volume element within the solid element per unit time. The use of the RTE in relation to real media is based on some physical simplifications,

where the direct use of the Maxwell electro-magnetic theory is difficult [58]. Similarly, the mathematical difficulties that arise in solving the complete RTE have resulted in the appearance of a series of approximation methods for solutions to the RTE. For example, the Monte Carlo (MC) computation model [59] has been developed as a numerical model to study the propagation of light in tissues [60]–[64]. The MC method is prohibitively costly in computational time, such as photon paths with several hundred interactions in length, and sufficient statistics with many millions of photons to be followed. MC that provides a solution of the inverse problem in some simple cases does not allow to form the fast algorithms for inverse problems [66]. Taking into account the feature of electromagnetic energy scattering on particles that allows for separating the angular and spatial variables in the Maxwell theory [67], the spherical harmonics method [68] helps us to obtain the approximate solution of the RTE by using the initial assumption on a special form of the unknown solution [69]. The first-order solution, the simplest formula, is $P_1$ approximation, or the diffusion equation (DE), in the framework of the spherical harmonics method:

$$\left\{\nabla \cdot D(r)\nabla - \mu_a c - \frac{\partial}{\partial t}\right\}\Phi(r,t) = -q(r,t). \tag{11}$$

Here, $D(r) = c/(3(\mu_a + (1-g)\mu_s))$; $g = \int_{4\pi}(\hat{s}' \cdot \hat{s})p(\hat{s}', \hat{s})\, d\Omega$ is the scattering anisotropy; fluence rate or intensity $\Phi$ denotes the energy flow per unit area per unit time, which is the radiance $\phi$ integrated over the entire $4\pi$ solid angle. The validity of DE is demonstrated by experimental work, which are approximate for types of applications considering $\mu_a \ll (1-g)\mu_s$ [70]–[73]. There are some limitations of DE in real media: the medium must be scattering dominated; light propagation cannot be modeled accurately in the proximity of the collimated light sources and boundaries [74], [75]. Due to these limitations, the DE fails to produce accurate estimates for light propagation close to the sources and boundaries, and in cases in which the turbid medium contains low-scattering or nonscattering void-like regions [76], [77]. The discrete-ordinates ($S_N$) method [78], spherical harmonics ($P_N$) methods [79], [80], coupled RTE and diffusion approximation model [81], and the simplified spherical harmonics ($SP_N$) approximation to the RTE [82]–[85] have been implemented for use in turbid tissue optics. In addition, the Gaussian quadratures method has also access to the approximate solution of the RTE by the Gaussian quadratures and subsequent transformation of the initial integro-differential equation into the system of ordinary differential equations [57], [86]. In chief, many of approximate formulas could be derived from a specific problem for forming an inverse algorithms. In summary, assuming that $N$ possible source locations inside the animal and $M$ detector locations on the surface, the measured data can be finally modeled within the framework of the forward model as

$$A(\lambda)S(\lambda) = B(\lambda) \tag{12}$$

where $A(\lambda) \in R^{M \times N}$ is the monochromatic forward model at wavelength $\lambda$; $S \in R^N$ is the source distribution; $B$ is the surface measurement with the $M$ source locations.

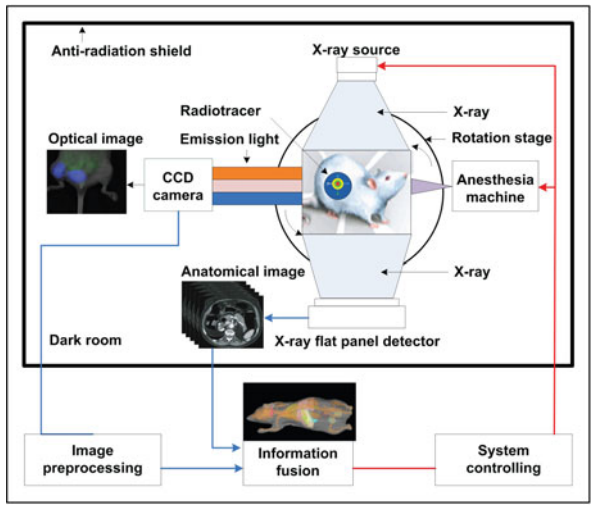


Fig. 1. Schematic representation of the *in vivo* imaging system [17].

The inverse model [87], [88] is to transform the measured data into accurate approximation of the spatial distribution of Cerenkov light source inside the tissue. Due to the lack of analytical inversion formulas [89], [90], this transformation could be done through numerical optimization

$$f(\eta, S) = \frac{a}{2} \parallel AS - B \parallel^2 + \frac{b}{2}R(\eta) \tag{13}$$

where $f$ denotes the objective function used to quantify the difference between the measurements and model predictions; $a$ and $b$ are constant; $R(\eta)$ denotes a regularization term so as to impose additional constraints on the parameter $\eta$. Many of regularization theories have been proposed on the development of methods of approximation of pseudo solutions and on evaluating the accuracy of approximation algorithms [91], [92], such as Tikhonov regularization [93], Landweber iteration [94], total variation regularization [95], and via the Lasso [96]. A survey of new approaches to the ill-posed problems have been used in the finite element (FEM) framework [97], [98], the permissible source region strategy [99], the generalized graph cuts method [100], and the meshless method [101]. The advantage of the FEM approach is its versatility that makes it applicable to complex geometries and highly heterogeneous parameter distributions [102], [103].

With CLT, quantitative and location analysis of the radiopharmaceutical distribution becomes feasible. Li firstly reported CLT with the DE to model the *in vivo* Cerenkov photon propagation in the homogeneous mouse model [16]. Since 2009, Zhong performed *in vivo* tomographic imaging of $^{18}$F-FDG in the heterogeneous mice. The functional and anatomic information of the radioactive probe in mice was detected by a small animal imaging system with a CCD camera and a micro-CT (see Fig. 1), and fused in a FEM model on basis of the DE and the Tikhonov regularization (see Fig. 2) [17]. Hu proposed a multimodality validation strategy [18] to verify the results of CLT with $Na^{131}I$, which regards SPECT as the criterion (see Fig. 3). Considering the weighted Cerenkov radiation spectrum toward blue bands of the electromagnetic spectrum [33],

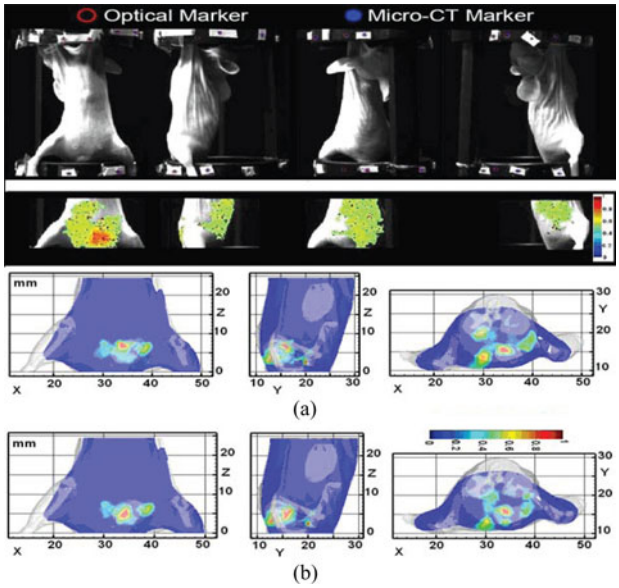


Fig. 2. Slices of the *in vivo* flux density distribution through the reconstructed source center successively. (a) Three slices from 3D views in homogeneous model. (b) Three slices in homogeneous model [17].

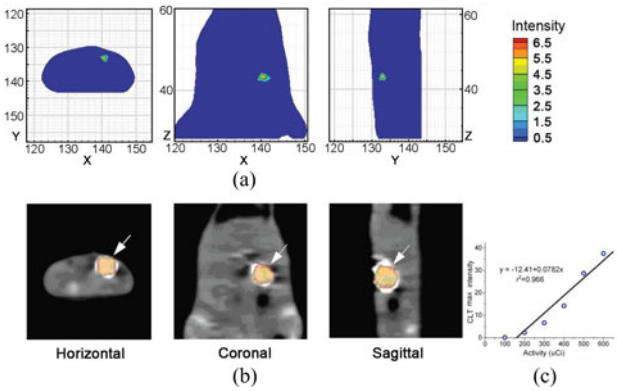


Fig. 3. Reconstruction results of 300 $\mu$Ci $Na^{131}I$ radioactive source. (a) and (b) are the reconstruction results in horizontal, coronal, and sagittal views of CLT and SPECT imaging, respectively; (c) correlation analysis between CLT reconstructed maximum intensity and the radiotracer activity. There was a robust correlation between activity versus the maximum intensity ($r^2 = 0.966$) [18].

the large absorption coefficients at these wavelengths make the DE as a light propagation model less accurate [43]. The third-order simplified spherical harmonics ($SP_3$) approximation to the RTE in the FEM framework is employed for modeling Cerenkov photon propagation in a small animal (see Fig. 4). With the increasing use of small animals for research, whole-body imaging has become a trend [104], [105], so elastic-net penalties was employed for whole-body CLT reconstruction based on the $SP_3$ forward model [19].

## V. Applications of Cerenkov Luminescence Imaging and Tomography

At present, although Cerenkov radiation is mainly utilized for *in vivo* small animal imaging, it provides a potential imaging technique for clinical research and application. Images of living

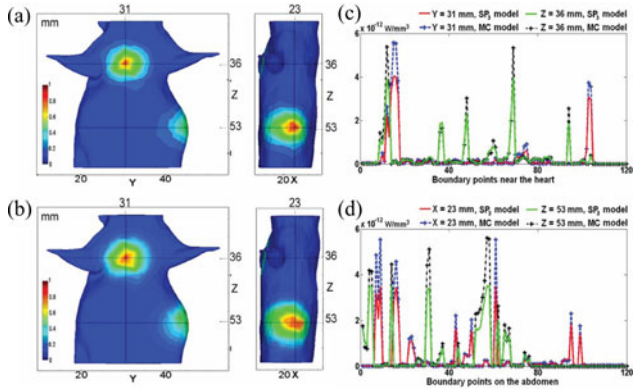


Fig. 4. Energy densities from both MC simulations and $SP_3$ approximation. (a) and (b) are optical energy density distribution on the surface derived from the forward simulation by MC simulations and the $SP_3$ model, respectively; (c) and (d) are the energy densities at all boundary points along those four slices from both MC simulations and $SP_3$ approximation [19].

subjects with a wide diversity of radioactive probes have been used to diagnose and treat a variety of diseases, including cancer, heart disease, gallbladder, and brain disorders [1], [106]– [109]. These examples establish a stone foundation for applications of CLI/CLT in the clinic [110]. The biodistribution of $^{18}F$, $^{89}Zr$, $^{90}Y$, and $^{131}I$-labeled probes and the quantitative imaging performance of CLI were evaluated [11], [42]. CLI/CLT could play a more important part in theranostic nanomedicine, promoted by the development of nanoparticle-based agents [111], [112]. It will lead multimodality imaging, diagnostic and therapeutic compounds into nanoformualtions [113]. CLI/CLT may be a method to visualize tumors during surgery using medical isotopes. It is difficult to completely remove the tumor from normal tissue dependent on the ability of the surgeon [114]. A way to objectively assess tumor margins during surgery in patients would be of great value, such as MRI-guided clinical staging, presurgical planning, and intraoperative fluorescence-guided surgery [115]. The CLI/CLT technique has the advantage in types of clinical imaging probes, comparing with the fluorescence imaging. In addition, CLI/CLT may provide an imaging and testing method for the synthesis of novel radiolabeled probes [116], [117].

## VI. Limitations of Cerenkov Luminescence Imaging and Tomography

Imaging sensitivity of CLI/CLT need to be considered for *in vivo* applications, due to blurring effect and low levels of the transmitted light caused by the strongly absorption and scattering of the emitted Cerenkov light within the biological tissue. Although the luminescence spectrum can been modulated for better tissue penetration, Cerenkov radiation could stimulate local autofluorescence that is then subsequently detected [10], [40]. Physical effects of this synergy phenomenon will increase the depth of light penetration and complicate the localization of objected signal. The modification of the Cerenkov spectrum with quantum nanoparticles or coupling with fluorescence agents needs to add metal nanoparticles or genetically modified operation, which makes it more difficult for the

application of molecular probes to the preclinical study and the clinics. The permeability and bio-security of the probe would become new academic problems. As a new imaging approach, the choice of molecular probes, radioactivity, and other parameters for specific applications need further preclinical and clinic trials.

## VII. Summary and Discussion

Molecular imaging has attracted remarkable attention and become an indispensable research tool for the biomedical study and clinical application in the past few years, with which the biological processes can be shown specifically, noninvasively, and sensitively using the corresponding probe and instrument. Due to the inexpensive and simple properties, optical imaging has been commonly applied to basic research of life science discipline, especially the tumorigenesis study and drug development, but the design and synthesis of probes have become one of the biggest challenges for further progress of optical imaging. As a maturely developed imaging technique, nuclear imaging has widely used in clinical diagnosis and disease therapy for many years. However, the cost of purchasing and maintaining nuclear imaging instrument is very high, so the scope of application is limited. Based on Cerenkov effect of radionuclides, a new optical molecular imaging modality, Cerenkov luminescence, not only establishes a bridge between optical and nuclear imaging, but also fuses the complementary advantages of the aforementioned two modalities. Furthermore, Cerenkov luminescence provides an effective multimodality imaging strategy based on the same radiotracer. In Cerenkov luminescence, the light intensity is correlated with the radionuclide activity and the energy of emitting charged particles. Therefore, the imaging sensitivity can be improved using the higher energy charged particles emitting radionuclides. In addition, another hypothesis that the low energy light in radionuclide radiation generate the detectable optical signals was also proposed and validated using several radiotracers [11].

Based on Cerenkov effect, planar Cerenkov luminescence imaging and 3-D Cerenkov luminescence tomography have been proposed and studied. Through the introduction of CLT, quantitative analysis can be realized, and the spatial resolution is largely improved. In contrast, CLI mainly performs the qualitative analysis. Cerenkov luminescence is a potential clinical optical molecular imaging technique, but the inherent properties of optical imaging still hinder its accessibility of clinical application, such as low-tissue penetration. Moreover, CLT is ill-posed severely because of complex photon propagation in the biological tissue and limited boundary measured data with noise, so the accurate inverse reconstruction methods are becoming more important [102], [118]. Overall, although there are many difficulties to be solved, Cerenkov luminescence is a very promising optical imaging modality for clinical diagnosis and treatment with the help of approved radiotracers [119], especially for superficial diseases such as breast and lymph tumor. Furthermore, Cerenkov luminescence can also be used as a simple and economical tool for the design and synthesis of radionuclide probes.

In conclusion, Cerenkov luminescence has the following advantages: high sensitivity, excellent spatial and temporal resolution, high cost-effectiveness, and potential clinical application. In this paper, the recent main research progresses of Cerenkov luminescence and tomography imaging are reviewed in detail. Heretofore, CLI and CLT have been applied in basic research and preclinical imaging, and the attempts to clinical application are under way. All of these predict an attractive prospect of Cerenkov luminescence.

## Acknowledgment

Authors C. Qin and J. Zhong contributed equally to this paper.

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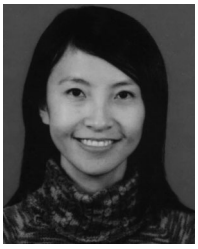

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