Translational Research of Optical Molecular Imaging for Personalized Medicine

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Abstract: In the medical imaging field, molecular imaging is a rapidly developing discipline and forms many imaging modalities, providing us effective tools to visualize, characterize, and measure molecular and cellular mechanisms in complex biological processes of living organisms, which can deepen our understanding of biology and accelerate preclinical research including cancer study and medicine discovery. Among many molecular imaging modalities, although the penetration depth of optical imaging and the approved optical probes used for clinics are limited, it has evolved considerably and has seen spectacular advances in basic biomedical research and new drug development. With the completion of human genome sequencing and the emergence of personalized medicine, the specific drug should be matched to not only the right disease but also to the right person, and optical molecular imaging should serve as a strong adjunct to develop personalized medicine by finding the optimal drug based on an individual's proteome and genome. In this process, the computational methodology and imaging system as well as the biomedical application regarding optical molecular imaging will play a crucial role. This review will focus on recent typical translational studies of optical molecular imaging for personalized medicine followed by a concise introduction. Finally, the current challenges and the future development of optical molecular imaging are given according to the understanding of the authors, and the review is then concluded.

Keywords: Drug development, molecular imaging, multi-modality fusion, optical imaging, personalized medicine, translational research.

INTRODUCTION

As we all know, early diagnosis and treatment of diseases not only can alleviate the suffering of the patient, but can also reduce the cost of treatment, which has promoted the emergence of molecular imaging, non-invasively elucidating biological processes at molecular and cellular levels in intact organisms including the human body, and the general consensus that molecular imaging is regarded as the next generation medical imaging technique in 21st century has been obtained in the medicine field [1-6]. With persistent efforts in the study of molecular imaging, many imaging modalities have been developed or matured, such as nuclear imaging techniques, optical imaging techniques, magnetic resonance imaging (MRI), *etc*. [5, 7]. In the above imaging modalities, although optical imaging is still far from being fully exploited, it has attracted remarkable attention in the biomedical discipline especially in preclinical research, and many two-dimensional and three-dimensional optical imaging modalities have been proposed and developed for different applications [8-13].

In the early research of optical imaging techniques, considering imaging efficiency and simplicity, two-dimensional transmitted and scattered light distribution on the object surface has been acquired to qualitatively represent the change of the internal target, and the corresponding quantitative and localization information cannot be resolved [14]. Conventional fluorescence imaging (FI) and bioluminescence imaging (BLI) belong to the above planar imaging techniques. In order to compensate for the disadvantages of planar imaging modalities, three-dimensional tomographic optical imaging technologies have been developed to realize the quantitative analysis of the labeled targets, such as the evolution of X-ray computed tomography (CT) based on radiography [15-17]. In biomedical research, the following tomographic optical imaging techniques, fluorescence molecular tomography (FMT), bioluminescence tomography (BLT), diffuse optical tomography (DOT), and Cerenkov luminescence tomography (CLT), are being gradually used for tumor mechanism study and drug efficacy evaluation [17-26].

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Table 1. Comparison of Different Molecular Imaging Modalities

Modality	Spectrum	Spatial Solution	Temporal Solution	Depth	Dimension	Cost	Clinical Application
MRI	Radiowaves	10-100 μm	Minutes-hours	No limit	Three	High	Yes
CT	X-ray	50μm	Minutes	No limit	Three	Medium	Yes
PET	γ-ray	1-2 mm	Minutes-hours	No limit	Three	High	Yes
SPECT	γ-ray	1-2 mm	Minutes-hours	No limit	Three	Medium	Yes
FMI	Visible light or near-infrared	Several mm	Minutes	<10 cm	Two	Low	Yes
FMT	Visible light or near-infrared	<1 mm	Minutes-hours	<10 cm	Three	Low	Yes
BLI	Visible light	Several mm	Minutes	Several cm	Two	Low	No
BLT	Visible light	<1 mm	Minutes-hours	Several cm	Three	Low	No
CLI	Cerenkov radiation	Several mm	Minutes	Several cm	Two	Low	Yes
CLT	Cerenkov radiation	<1 mm	Minutes-hours	Several cm	Three	Low	Yes

Comparison of different molecular imaging modalities is summarized in Table **1**.

The main differences between planar optical imaging and tomographic optical imaging are the light transfer model and the inverse reconstruction algorithm which are not required in two-dimensional optical imaging because only simple overlay needs to be performed between photographic and luminescent images, including hardware structure differences regarding the imaging system. It is well known that the light transfer model and the reconstruction algorithm of optical tomography should be further studied because of its severe ill-posedness caused by the complex light propagation in biological tissues and limited measurements compared with so many variables that need to be solved [10, 27]. On the other hand, although both planar optical imaging and tomographic optical imaging have the same shortcoming in that the penetration depth of diffuse photons is limited at an order of magnitude of several centimeters, the corresponding biomedical applications have been expanded to diagnosis before surgery, navigation in surgery, and evaluation after surgery in view of its high cost-effectiveness and simple operation [28-30].

As described above, optical molecular imaging techniques have been successfully employed for biomedical research, especially for many preclinical applications like tumorigenesis and metastasis study, drug development and evaluation, using an animal model of the human disease [1, 2]. First of all, the novelty of molecular imaging including optical imaging technique lies in the fact that it can *in vivo* visualize the region of interest in a living subject consecutively without interference of its normal life functions. In other words, the experimental living animal does not need to be killed for *in vitro* measurement with traditional physical methods, and the number of animals for the imaging experiment is greatly reduced, thus the animal welfare is better ensured. In comparison with traditional molecular imaging modalities, optical imaging technique has its own advantages, such as low cost, high sensitivity, and excellent temporal resolution. Furthermore, optical molecular imaging can reflect specific changes to differentiate pathological from normal tissues using physiological and/or metabolic information with the help of the acquired functional images. It is the biggest difference between traditional means of nonspecific imaging techniques and optical molecular imaging.

With the above advantages, optical molecular imaging provides us with unprecedented opportunities to promote the evolvement of basic biomedical research and clinical practice, especially for drug development. Based on the results of optical molecular imaging, we can elucidate mechanisms of drug activity and effects during preclinical and clinical drug development more directly and more clearly in comparison with traditional experimental methods, which evaluate drugs by measuring the structural changes in the diseases without consideration of detailed mechanisms of action, and the preclinical research process will also be accelerated using high throughput imaging of animal models. In the assessment of target expression for drug development, optical molecular imaging can provide localization and quantitative information of specific targets after the determination of their presence on the basis of the confirmation of the targets according to prior biomedical research. Although optical molecular imaging has been used in preclinical studies for drug development, such as efficacy evaluation of the compound and pharmacokinetics research, yet it should be noted that optical molecular imaging plays an important role in compound screening due to its imaging abilities of high sensitivity, high throughput, and high cost-effectiveness [2, 31].

Furthermore, with the development of medical research and sequencing of the human genome, the eve of personalized medicine has approaching to match the specific drug to the appropriate person under the assumption that diseases from different people are heterogeneous and unique, including their causes and responses to drugs, therefore different patients should be diagnosed and treated individually [32-34]. Overall, the current expectation and future goal in the molecular medicine discipline of the postgenomic era, is that

specific drugs best suited for an individual should be determined based on pharmacogenetic and pharmacogenomic information obtained using optical molecular imaging techniques or other methods.

This review focuses on the translational research of optical molecular imaging for personalized medicine, especially for the development of personalized drug. The rest of this paper is organized as follows. The next section presents optical molecular imaging including computational methods, prototype systems, and basic applications, followed by a simple principle depiction of two mainstream optical imaging techniques. Recent advances in biomedical applications of optical molecular imaging regarding personalized medicine are then reviewed. Subsequently, other typical translational researches of optical molecular imaging are briefly introduced. Finally, this paper is concluded and future prospects are predicted.

OPTICAL MOLECULAR IMAGING

As a newly emerging and very important field, the development of personalized medicine should be merged with modern bio-molecular technologies and advanced imaging instruments to better study biological and medical processes, as well as diagnosing and treating diseases. Among these techniques and instruments, although optical molecular imaging is being further studied and tending to mature, it has been commonly used for biomedical study, especially for drug development and efficacy evaluation based on the research progress of computational methods, prototype instruments, and basic biomedical experiments in optical molecular imaging discipline. As mentioned above, optical molecular imaging can be classified as planar and tomographic imaging modalities according to the spatial dimension of the final results, and recent advances in optical molecular imaging will be concisely introduced in the following paragraphs of this section.

The breakthrough in computational methods regarding optical molecular imaging laid the foundation for its application in the biomedical discipline, especially for three-dimensional tomographic imaging because traditional planar optical imaging only requires accomplishing a simple overlay without consideration of complex inversion reconstruction. Before the study of computational methods, the appropriate light transfer model should be selected because it is the basis of follow-up programs [35-37]. It is well known that the radiative transfer equation (RTE) can accurately model light propagation in biological tissues, but it is computationally too expensive to be used in practical applications due to its integro-differential nature, especially for the complex heterogeneous organisms with irregular internal structure [35]. Therefore, simpler approximation models to the RTE have been proposed and applied, such as the diffusion equation (DE) suitable for the highly scattering domain, the discrete ordinates (S_N) and the spherical harmonics (P_N) approximation appropriate for the low scattering region [38-47]. Although the diffusion equation is the most prevalent model to represent light propagation in the biological tissue, some assumptions must be satisfied for the validity of the diffusion equation, especially for the prerequisite that light scattering dominates over absorption. In other words, the validity of the diffusion equation will be limited if the absorption coefficient is comparable to or greater than the reduced scattering coefficient. Furthermore, the accuracy of the diffusion equation will be greatly reduced in some particular domains like near the source and near the surface. Therefore, higher order approximations to RTE have been proposed to solve the above issues and used for tissue optics, such as S_N method, P_N and SP_N approximations. However, different light transfer models have their own characteristics and advantages, so the hybrid transfer model for optical tomography based on tissue specificity of different organs has been presented, and the corresponding contribution is under review.

Based on the appropriate photon transport model in biological tissues, inverse reconstruction algorithms are then explored to quantitatively determine spatial and temporal distribution of the labeled targets inside the disease animal model. The two most important performance indexes of the reconstruction algorithm are efficiency and accuracy. On the other hand, tomographic reconstruction of the internal light source is severely too ill-posed to ensure the uniqueness and stability of the solution, so sufficient *a priori* knowledge should be fused to improve both the robust performance and computational efficiency of the source reconstruction as well as the localization accuracy [27]. The commonly used *a priori* knowledge consists of functional information like optical properties of different tissues, anatomical structure derived from CT reconstruction, permissible source region used for reducing the volume of the imaging domain, and spectrally resolved data employed to increase detectable measurements [48-50]. After years of research, a system of calculation methodology including many analytical, statistical, and numerical methods has been preliminarily established [35]. Among these solution techniques, the finite element method (FEM) based numerical approaches have become the natural choice and the most typical algorithm for tomographic optical imaging due to its ability to model complicated inhomogeneous regions [35]. However, it is difficult and time-consuming to generate the finite element mesh of irregular objects with arbitrarily shaped internal structure, so meshless method based numerical approaches have been introduced into the bio-photonics field to avoid the computationally expensive three-dimensional meshing [35, 51]. Although a wide variety of photon propagation models and inversion reconstruction algorithms have been proposed and developed currently, they should be further studied for either improvement of imaging precision or advancement of computation efficiency.

At present, the available optical imaging systems are mainly composed of laboratory prototypes and commercial instruments, and these imaging systems can also be divided into two categories, planar and

tomographic imaging systems, according to whether the imaging results contain the depth information of the labeled targets in the living animal or not. Most of the commercial instruments in the optical molecular imaging field only obtain planar luminescent light distribution on the surface of the animal model, and then the luminescent information is overlaid with the photographic image reflecting two-dimensional shape of the animal model. Therefore, the above planar imaging systems can only achieve qualitative analysis of the internal labeled targets, and the corresponding localization and quantitative information in which the biomedical researchers are interested cannot be resolved, so the tomographic optical imaging systems have been constructed and developed like the emergence of CT based on radiograph. However, the inverse reconstruction of the labeled targets is strongly ill-posed because of its own characteristics, so the system structure should be designed for conveniently collecting more measured data. For example, multi-view luminescent light signals on the animal model surface can be acquired with the help of a rotation stage or several mirrors, and the collection of multi-spectral measurements usually requires a set of band-pass or cutoff filters except the application of multiple optical probes [20, 52, 53].

In addition, the multi-modality fusion optical molecular imaging system can not only provide more types of image information, but it can also reduce the ill-posedness of optical tomography effectively and improve the reconstruction accuracy largely, such as the integration of optical and structural imaging systems, the combination between the optical and radionuclide imaging systems. The panorama of BLT based multi-modality fusion imaging system developed by our group is shown in Fig. (**1A**) [50]. It can perform optical and micro-CT imaging, and two-dimensional multi-view photographic and luminescence images, three-dimensional geometric shape and internal anatomical structure can be acquired using this system. It mainly contains a cryogenic cooled back-illuminated CCD camera with a focus lens, a light-tight imaging chamber (not shown in the figure), a micro-focus X-ray source tube, and an X-ray flat panel detector. It should be noted that a three-dimensional programmable stage including two motorized translation stages and a motorized rotation stage is shared by optical and micro-CT imaging. Fig. (**1B**) shows the schematic diagram of multi-modality fusion imaging, and Fig. (**1C**) illustrates the fusion flow based on different image information [54]. Furthermore, the multi-modality fusion system at hardware and software levels has become the mainstream development of optical imaging, and it will be of excellent performance and high cost-effectiveness as well as point-and-shoot operation.

As is well known, the clinical application of optical molecular imaging is limited in view of its small penetration depth and few approved optical probes. According to the document [1], the maximum penetration depth of optical molecular imaging is lower

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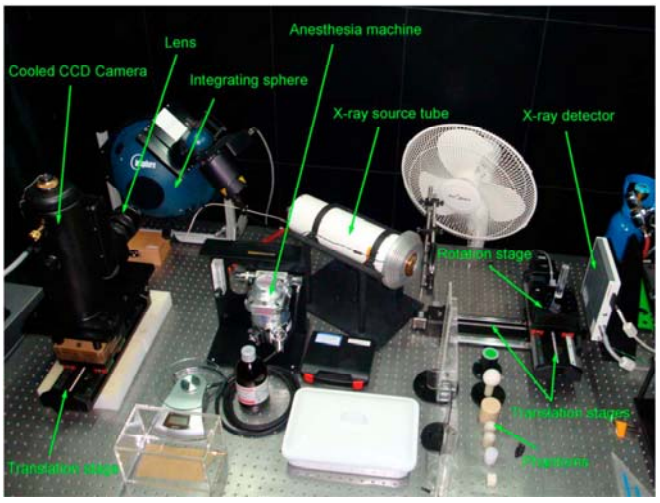

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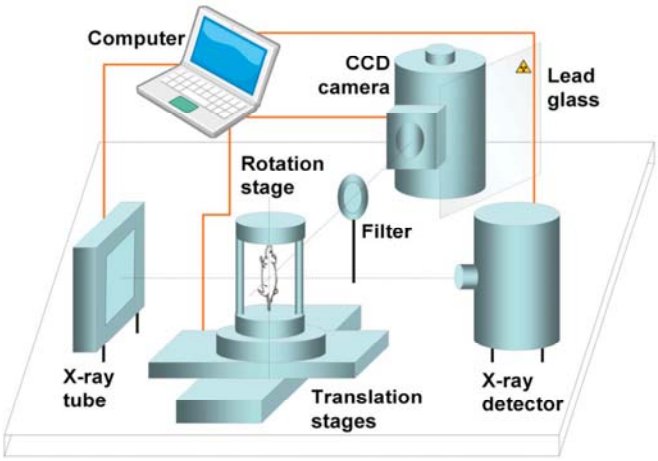

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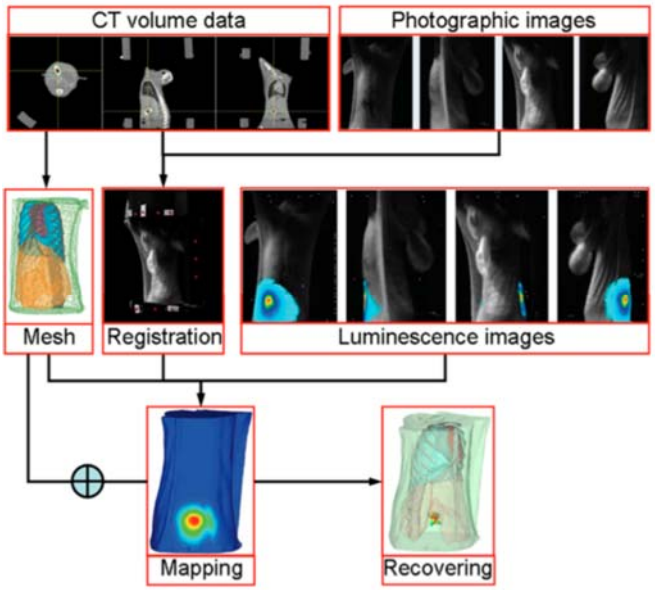

Fig. (1). (**A**) Bioluminescence tomography-based multi-modality imaging system [50]; (**B**) The schematic diagram of multi-modality fusion imaging; (**C**) The fusion flow based on different image information [54].

than 10 centimeters. Searching the Molecular Imaging and Contrast Agent Database, only two optical molecular imaging probes, indocyanine green (ICG) and fluorescein sodium, have been approved for clinical application by the U.S. Food and Drug Administration (FDA). Therefore, current optical imaging techniques and systems are mainly utilized for preclinical drug evaluation and oncology research based on disease small animal model. For example, tumorigenesis, cancer metastasis, and tumor regression can be *in vivo* detected and evaluated using optical molecular imaging [1]. For drug development, assessment of target expression, compound screening, efficacy and toxicity evaluation, especially for personalized medicine, can be promoted largely depending on *in vivo* optical molecular imaging, and it can also increase efficiency and reduce costs of drug discovery [2].

Fortunately, a newly emerging optical molecular imaging modality, Cerenkov luminescence imaging technique based on Cerenkov radiation, has attracted considerable attention because of its significant impact on both preclinical and clinical imaging [24-26, 55-58]. Cerenkov radiation is well-known physical phenomenon and introduced into bio-photonics field in 2009 [55]. Cerenkov radiation can be generated when a charged particle travels through a medium with a velocity greater than the corresponding speed of light, and the visible photon is emitting from the near ultraviolet through the visible spectrum [55]. The distributed light intensity has been proved to be inversely proportional to the square of the wavelength [55]. Utilizing the existing optical molecular imaging instrument, Cerenkov luminescence imaging can be accomplished easily [56]. Cerenkov luminescence imaging employs the approved radioactive probes used in nuclear imaging field to emit luminescent photons, so this technique can alleviate the challenges of optical molecular imaging that evolves from preclinical to clinical application. Although Cerenkov luminescence imaging is closer to clinical application than traditional optical molecular imaging modalities, most of emitting photons are in the blue band, so the appropriate light transfer model and computational methods should be considered, especially for CLT. Furthermore, in comparison with nuclear imaging, Cerenkov luminescence imaging has better temporal resolution and cost-effectiveness. As a simple and economical technique, Cerenkov luminescence imaging can be used for the design and synthesis of radionuclide probes, and assessment of compound pharmacokinetics and efficacy can also be achieved. Except the aforementioned novel imaging modality, the existing optical imaging techniques have been further explored for clinical application, especially for fluorescence molecular imaging. More gratifying, in recent study, conventional optical molecular imaging technique and system have been successfully used for the surgical navigation of ovarian cancer [30]. Therefore, we believe that optical molecular imaging will become one of the most important tools for the biomedical research, especially for personalized medicine.

APPLICATIONS OF OPTICAL MOLECULAR IMAGING IN PERSONALIZED MEDICINE

The drug development process is a lengthy, high-risk and costly endeavor including assessment of target expression, lead compound optimization, preclinical drug evaluation, clinical phase I-III and final FDA approval [2]. Optical molecular imaging can be used at various stages in the drug development process which may help reduce the time and material costs and allow the selection of the most promising drug candidates into the final FDA approval process.

Target Expression

Drugs often kill tumor cells by a target that was expressed on the tumor cell surface. Many factors are involved in tumor progression, such as epidermal growth factor receptor (EGFR), transforming growth factor β, vascular endothelial growth factor family and so on. During the rapid development of personalized medicine, optical molecular imaging has already played an increasingly important role in evaluation of target expression.

One approach to evaluate target expression is to use an imaging probe to directly interact with the target(s) under consideration. For example, for a drug being developed against an EGFR, which is a transmembrane glycoprotein composed of a single polypeptide chain, an imaging probe that directly binds to EGFR (for example, Cy5.5-Erbitux in fluorescence imaging) can be used for assessing target expression. Chen's group used Erbitux-Cy5.5 to demonstrate the EGFR expression level in breast cancer xenografts [59]. His group used Cy5.5-conjugated GX1 peptide in a subcutaneous U87MG glioblastoma xenograft model to investigate tumor-targeting efficacy and the results suggested that Cy5.5-GX1 was a promising molecular probe for optical imaging of tumor vasculature [60], and the imaging results are shown in Fig. (**2**). Otherwise, fluorescent dye-labeled E $[PEG_4\text{-}c(RGDfK)]_2$ had been developed to image integrin $\alpha_v\beta_3$ expression by Liu and his coworkers [61]. In addition, other research groups, such as Gershwin's and Zuniga's group, had monitored the transforming growth factor-β using fluorescent dye [62, 63]. As there are already a large number of optical imaging probes that are directed against a large number of known tumor targets, optical molecular imaging allows confirmation of many targets for personalized drug developments. However, for relatively new targets, the sensitivity and specificity of detection and interaction with the target need to be improved which depends on the improvement of the imaging system and the probe.

Reporter gene imaging is another approach used to evaluate target expression, which involves simultaneous co-expression of the therapeutic target gene and a reporter gene which are often driven by the

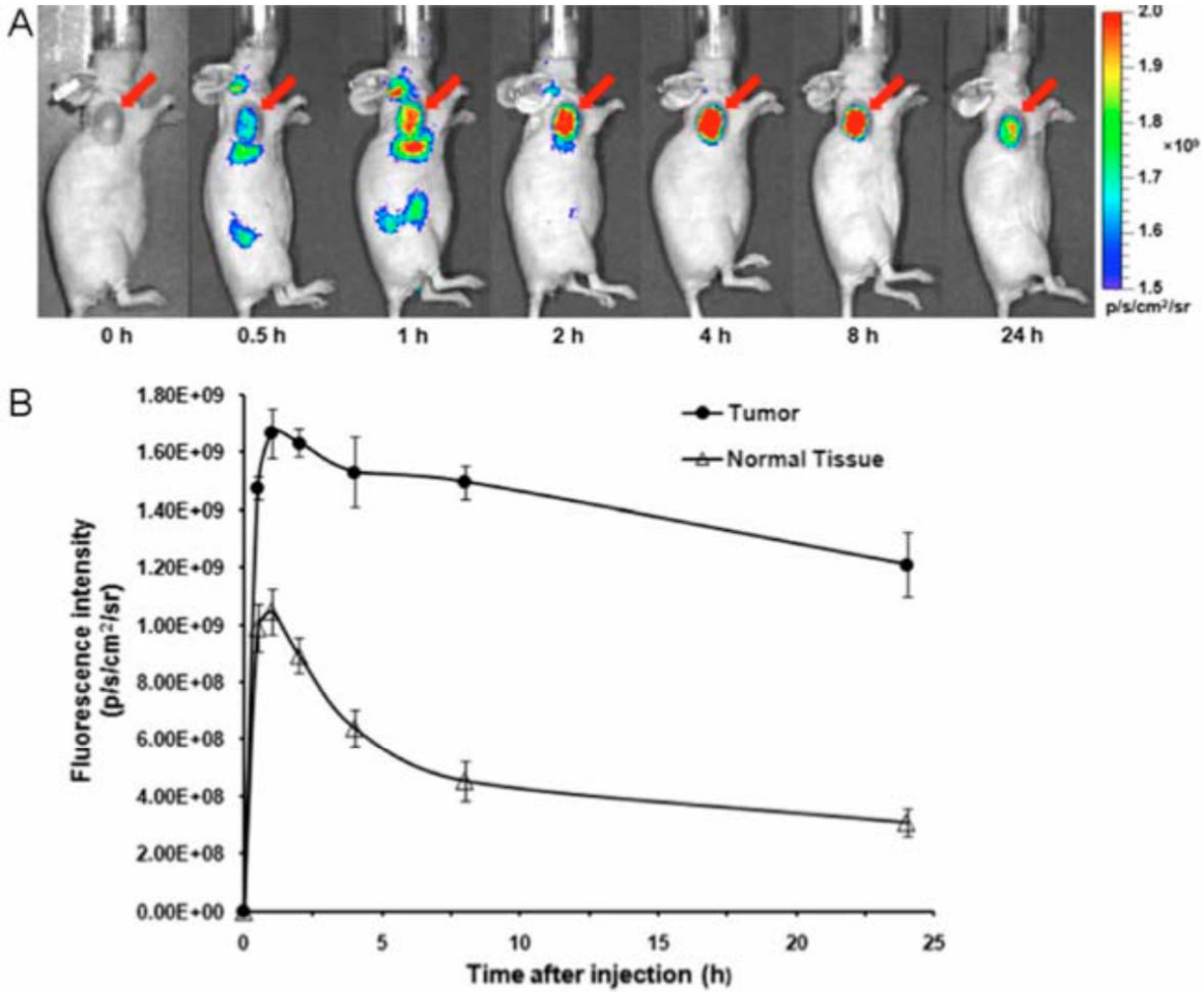

Fig (2). *In vivo* fluorescence imaging of subcutaneous U87MG tumor-bearing nude mice after intravenous injection of 1.0 nmol of Cy5.5-GX1. The tumor can be clearly visualized as indicated by arrows from 0.5 to 24 h p. i. The fluorescence intensity was recorded as per second per centimeter squared per steradian (p/s/cm2/sr). b Quantification and kinetics of *in vivo* targeting character of Cy5.5-GX1. Tumor fluorescence washout was slower than that in normal tissue [60].

same promoters. The reporter genes that encode for enzymes, such as firefly luciferase (Fluc) and rinilla luciferase (Rluc), can be introduced to certain gene fragments for research which can produce bioluminescence in the presence of its specific substrate. Tse's group used dynamic BLI to semiquantitatively monitor changes in vascular permeability through Fluc repoter gene technology which facilitated the study of tumor angiogenesis in animal models of disease [64]. Our group had reconstructed the three dimensional location of the liver cancer *in situ* using tumor cells marked with Fluc reporter gene [65]. In addition, Gambhir's group had also developed the bioluminescence image technology using Renilla luciferase enzyme/protein by injecting the substrate coelenterazine in living mice [66]. In their following research, they had used Rluc reporter gene technology to monitor the protein-protein interactions. Afterwards, many groups used the reporter gene technology to do much work on the study of tumor mechanism and drug evaluation [67-69].

Compound Screening

After a target was chosen and identified, the next stage is typically high throughput screening of large-scale compounds for their ability to search the target. In traditional research, high throughput screening is restricted to experimental verification of cell cultures. Optical molecular imaging with its high sensitivity, high throughput capabilities and low costs allows compound screening in cell experiments and animal models which make the results more convincing. Livingston's group had done some research on homeostasis under hypoxic conditions which were maintained through a coordinated transcriptional response mediated by the hypoxia-inducible factor (HIF) pathway and required coactivation by CBP and p300 transcriptional coactivators [70]. Through a target-based high throughput screen using bioluminescent imaging, they identified chemotin as a disrupter of HIF binding to p300 by disrupting the structure of the CH1 domain of p300 and precluding its interaction with HIF and thereby attenuating hypoxia-inducible transcription. The

corresponding experimental data is shown in Fig. (**3**). Tran *et al.* accomplished high throughput antiangiogenic compound screening using transgenic zebrafish with fluorescent blood vessels [71]. Zhang *et al.* performed high throughput compound screening to identify clinically useful ABCG2 inhibitors based on bioluminescence imaging technique [72]. Improgo *et al.* designed a cell viability assay for high throughput screening of anti-small cell lung carcinoma (SCLC) agents using bioluminescence imaging [73]. Ei-Deiry's group detected the p53-mediated transcription *in vitro* and *in vivo* based on an HCT116 human colon carcinoma xenograft model using bioluminescence molecular imaging [74]. Then, his group used Calcein AM, D-luciferin and Mitotracker Red FM as a counterstain to visualize dye-effluxing cells and the results suggested that it was possible to image and quantitatively analyze putative CSC populations within the tumor microenvironment and that the loss of pro-apoptotic and tumor suppressing genes such as Bax or p53 enriched such tumor-prone populations [75]. In addition, Goodman's group used bioluminescence and immunohistochemisty to confirm the chemotaxis of reporter cells and their differentiation into mature osteoblasts in the presence of infused particles [76]. Their research proved that injection of a CCR1 antagonist decreased reporter cell recruitment to the ultra high molecular weight polyethylene (UHMWPE) particle infusion site and increased osteolysis [76].

In recent years, many groups used a split-protein strategy to assess protein-protein interactions *in vivo* using optical molecular imaging [77-80]. Generally, the protein/enzyme was cleaved into amino-terminal and carboxy-terminal segments and each segment was linked to the two interacting proteins. When the two proteins interacted physically in a cell culture or in mouse models, two parts of the enzyme could produce signals which could be captured by optical molecular imaging. Gambhir's group quantified and imaged homodimeric protein-protein interactions in mammalian cells and in living mice using a split synthetic renilla luciferase (hRLUC) complementation-based

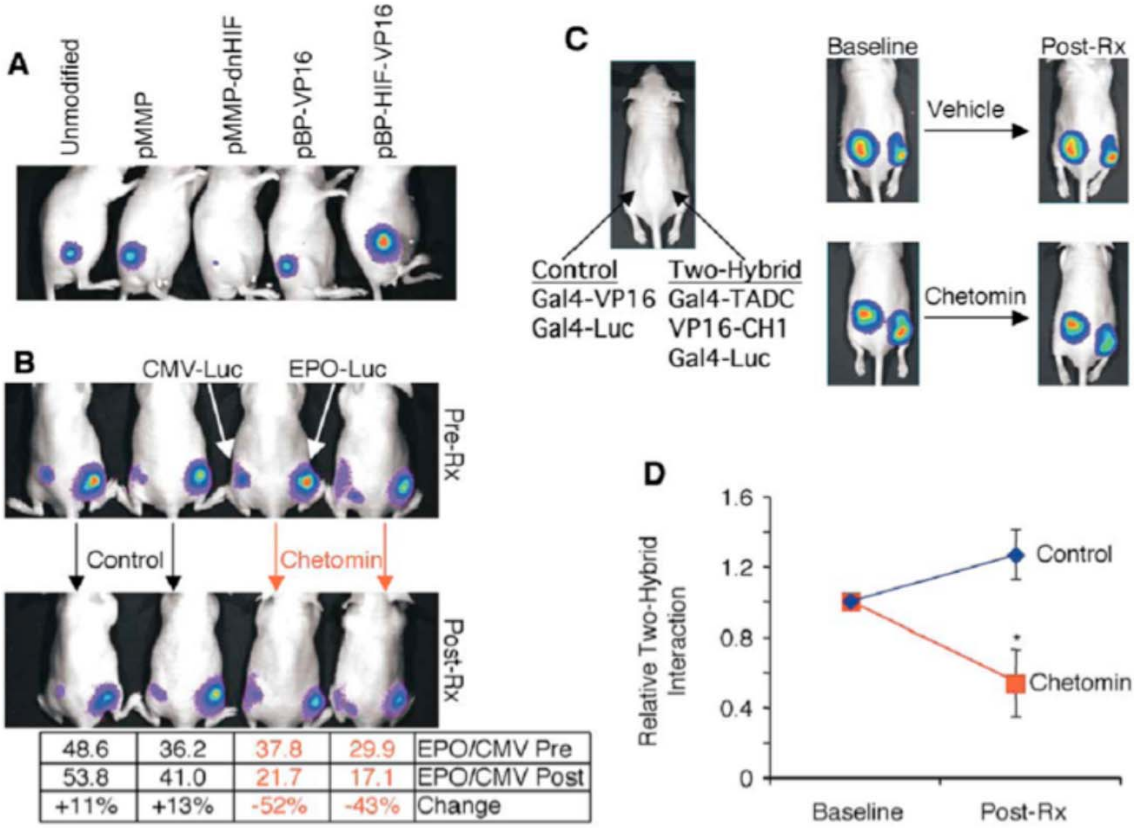

Fig. (3). Chetomin disrupts HIF pathway activity *in vivo* [62]. (**A**) *In vivo* imaging of HIF-1 activity. HepG2 cells with stably integrated EPO-Luc reporter were implanted without further modification or after infection with retroviruses encoding a dominant-negative HIF polypeptide (pMMP-dnHIF), dominant-positive HIF-1αallele (pBP-HIF-VP16), or controls for retroviral infection (pMMP, pBP). Mice were imaged 48 hr after implantation of cells. (**B**) *In vivo* effects of chetomin. Hypoxia reporter cells (EPO-Luc) were implanted on the right flank, and constitutive control cells (CMV-Luc) were implanted on the left flanks of mice. Animals were imaged prior to (Pre-Rx) and after (Post-Rx) i. v. administration of two doses of chetomin (2mg/kg) or vehicle control. HIF-1-specific activity was determined by calculating the ratio of EPO-Luc/CMV-Luc. (**C**) *In vivo* imaging of TADC-CH1 protein-protein interaction. Mice were inoculated with two-hybrid reporter cells in the right flank and constitutive control cells in the left flank. After baseline imaging, mice were treated with two doses of chetomin (2mg/kg) or vehicle control, followed by reimaging 24 hr after initial imaging. (**D**) For each animal, TADC-CH1 two-hybrid interaction (right flank) was normalized to the constitutive control (left flank) and expressed relative to the baseline ratio for each animal; mean±SEM; n=4; *p<0.05.

bioluminescence assay [77]. Then, his group proposed a novel fusion protein approach for efficient high throughput screening of small molecule-mediating protein-protein interactions in cells and living animals using bioluminescence imaging. The results demonstrated that in this fusion system, rapamycin induced heterodimerization of the FRB and FKBP12 moieties occurred rapidly even at very low concentrations (0.00001 nmol/L) of rapamycin [79].

Exploratory Preclinical Research

Once a compound was chosen at the stage of compound screening, the next stage is to establish drug efficacy at a dose lower than that associated with toxicities. Extensive work in the past decade has shown that optical molecular imaging is valuable in this respect. A range of optical molecular imaging techniques including Cerenkov luminescence imaging developed in recent years has been applied to monitor drug action. Most of these studies focused on the efficacy evaluation of the antitumor drug. The tumor cells are usually transfected into a luciferase gene in order to be detected by the optical molecular imaging system (such as the bioluminescence imaging system and fluorescence imaging system) after drug administration. Tian's group used bioluminescence imaging to evaluate the antitumor efficacy of cyclophosphamide (CTX) quantitatively based on two groups of HCC-LM3-fLuc human hepatocelluar mice models [81]. The results showed that CTX could induce a 25.25±13.13% and 35.91±25.85% tumor growth inhibition rate on days 9 and 12 post-treatment respectively. The results were tested and verified by the results using small-animal positron emission tomography (PET) (the results are shown in Fig. **4**). Wang's group evaluated the antitumor efficacy of 111In-VNB-liposome based on SCID mice models bearing the HT-29/luc xenografts using the bioluminescence imaging system [82]. Lin's group evaluated the response to therapy based on the multiple myeloma mice models using bioluminescence imaging [83].

In addition, the Cerenkov luminescence imaging as a less expensive, easier-to-use, and higher-throughput alternative to other nuclear imaging modalities (such as PET) has been used to monitor drug action. Cheng's group used Cerenkov luminescence imaging and PET to evaluate the efficacy of bevacizumab in 6 mice implanted with H460 xenografts bilaterally in the shoulder region with ^{18}F-FLT injection [58]. The results showed that on the ^{18}F-FLT scans, both Cerenkov luminescence imaging and PET revealed significantly decreased signals from H460 xenografts in treated mice from pretreatment to day 3 which were moderately increased to unchanged signals in untreated mice. Similarly, they had done the same research using the PC3 xenograft mouse models. All this research proved that Cerenkov luminescence imaging and PET exhibited excellent correlations and demonstrated the use of Cerenkov luminescence imaging for monitoring cancer treatment in the future.

OTHER TRANSLATIONAL RESEARCH OF OPTICAL MOLECULAR IMAGING

Optical molecular imaging could not only be used in personalized medicine, but it could also be used in detection of malignant tumors, such as cancer detection using ICG fluorescence. Frangioni's group described the successful clinical translation of a new NIR fluorescence imaging system for image-guided

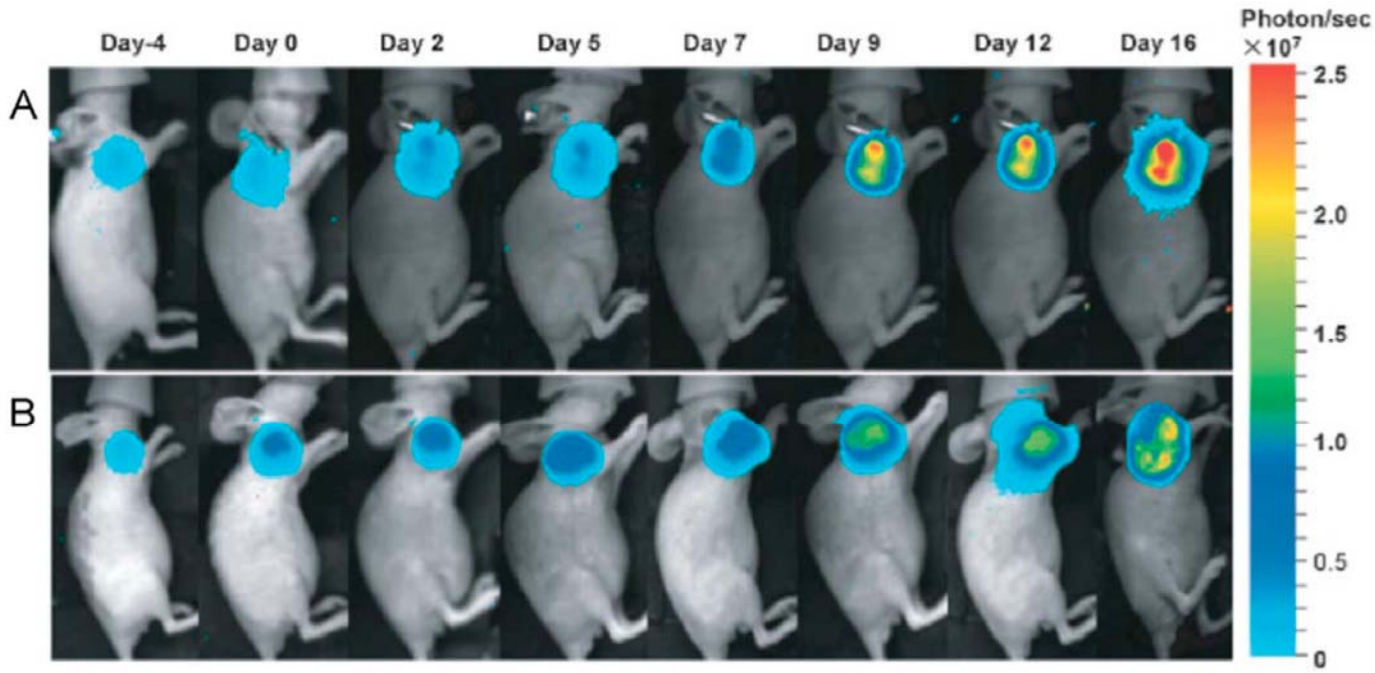

Fig. (4). Serial bioluminescence images of the HCC-LM3-fLuc tumor-bearing nude mice that underwent saline (**A**) or cyclophosphamide (**B**) treatment [70].

oncologic surgery [84]. Fukamizu's group detected a sentinel lymph node (SLN) in skin cancer patients using ICG fluorescence [85]. The results showed that SLN biopsy using ICG fluorescence could achieve a high identification rate and allowed effective observation for several hours, and this method could become a useful option for the detection of other tumors including skin cancer, breast cancer [86, 87], vulvar cancer [88], cervical cancer [89], colorectal cancer [29] and gastric cancer. Hunerbein's group used this method in the detection of breast cancer and the results showed that this method allowed transcutaneous visualization of lymphatic vessels and intraoperative lymph node detection without a radioisotope [86]. In addition, optical molecular imaging was also used in functional brain imaging. Liu's group had done some basic research work in this area to overcome the poor optical contact of the optodes with the scalp due to obstruction by hair in functional near infrared spectroscopy and imaging [90].

Besides all the preclinical exploratory research on medicine and tumor, Ntziachristos's group had firstly applied the fluorescence imaging in the clinical surgical navigation [30]. The overexpression of folate receptor-α (FR-α) in 90-95% epithelial ovarian cancers make the investigation of intraoperative tumor-specific fluorescence imaging in ovarian cancer surgery possible using and FR-α-targeted fluorescent agent. In their studies, they conjugated folate with fluorescein isothiocyanate (FITC) through an ethylenediamine spacer to form folate-FITC probe in order to targeting the ovarian cancer. The corresponding imaging system and experiments are shown in Fig. (**5**). The results showed in patients with ovarian cancer, intraoperative tumor-specific fluorescence imaging with an FR-α-targeted fluorescent agent had the potential applications in patients with ovarian cancer for improved intraoperative staging and more radical cytoreductive surgery. As we known, most of the above researches had not been used in the clinical surgery. However, with the development of the probe and imaging technologies, we believed the optical molecular imaging have a wide application range in many clinical research aspects, such as clinical surgery navigation, clinical drug evaluation and so on.

CONCLUSIONS AND FUTURE PROSPECTS

Since the emergence of molecular imaging, it has evolved considerably and become an important tool for biomedical research in living subjects, which has promoted considerable advances in the disciplines of biology, medicine, chemistry, and life sciences. It is a multidisciplinary field in which basic sciences and modern techniques are increasingly merged to non-invasively visualize, characterize, and quantify normal and pathological processes within the living organism. In addition, not only the development of imaging instruments and quantification techniques, but also molecular probes and assays in this field help elucidate molecular mechanisms in biology and medicine. As a cost-effective and sensitive imaging modality, optical molecular imaging provides detailed pictures of what is happening inside the animal model and uses light to obtain functional information at cellular and molecular

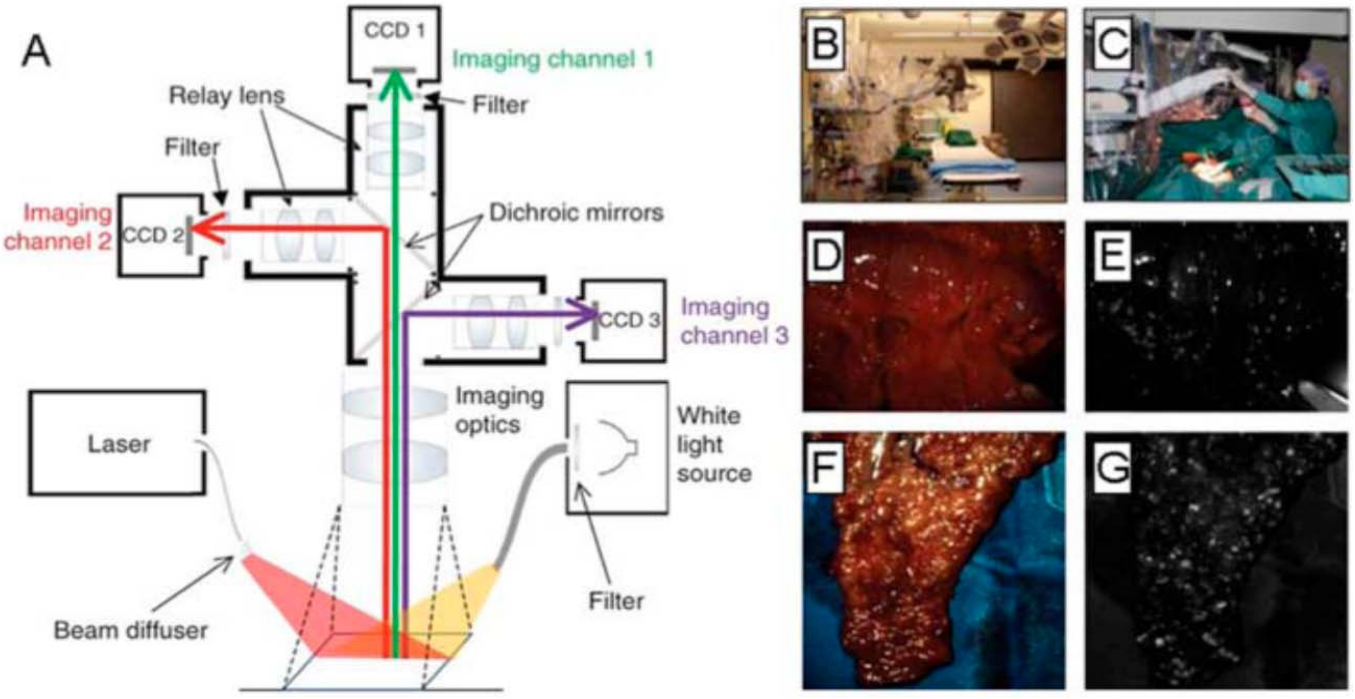

Fig. (5). Intra-operative multispectral imaging system. (**A**) Multispectral fluorescence camera system. (**B**) Positioning before draping. (**C**) Intraoperative application including draping. (**D-G**) Intraoperative screenshots of simultaneously detected and depicted images in color (**D**, **F**) and corresponding fluorescence during surgery (**E**, **G**) in a patient with high-grade serous ovarian carcinoma and extensive peritoneal carcinomatosis (stage III, FR-α positive) [30].

levels derived from tissue composition and bio-molecular processes in the living organism. Exogenous agents injected into the organism body, like ICG and green fluorescent protein (GFP), or endogenous molecules with optical signatures, such as Fluc and cytochrome, are generally employed to emit the detectable photons. In view of excellent advantages and high cost-effectiveness, optical molecular imaging has been widely used for basic biomedical research and new drug discovery, such as tumorigenesis and regression, tumor invasion and metastasis, drug compound screening, drug efficacy evaluation, *etc*. This paper tries to review recent typical translational studies of optical molecular imaging for personalized medicine, which not only embody the scientific interest and significant work of the contributors and their groups from different disciplines including cell biology, biomedical engineering, chemistry, information science, mathematics, medicine, pharmacology and genetics, but also lay a solid foundation for follow-up research and breakthroughs in scientific challenges existing in the development and applications regarding optical molecular imaging.

As mentioned above, the research of optical molecular imaging has made significant progress and wide application, but in our opinion, it is still far from being fully exploited, and greater efforts should be mounted to realize faster development. Firstly, fast reconstruction algorithms and precise mathematical models should be further studied to improve imaging accuracy and efficiency, especially models and methods for endoscopic optical imaging (EOI) need to be emphasized to overcome the limit of penetration depth regarding optical imaging. Secondly, Cerenkov luminescence imaging and CLT employ the approved nuclear imaging probes to accomplish the function of optical molecular imaging, which is very promising for clinical diagnosis and treatment, especially for superficial diseases such as breast and lymph tumors. Finally, an optical based multi-modality fusion imaging system should be designed with consideration to registration and fusion between different modalities in post-processing. In conclusion, although many challenges are still ahead, we believe that optical molecular imaging will become an indispensable tool for research in personalized medicine based on existing studies and future breakthroughs.

ABBREVIATIONS

MRI = Magnetic resonance imaging

FI = Fluorescence imaging

BLI = Bioluminescence imaging

CT = Computed tomography

FMT = Fluorescence molecular tomography

BLT = Bioluminescence tomography

DOT = Diffuse optical tomography

CLT = Cerenkov luminescence tomography

RTE = Radiative transfer equation

DE = Diffusion equation

S_N = Discrete ordinates

P_N = Spherical harmonics

FEM = Finite element method

PET = Positron emission tomography

ICG = Indocyanine green

FDA = Food and Drug Administration

EGFR = Epidermal growth factor receptor

HIF = Hypoxia-inducible factor

hRLUC = Renilla luciferase

CONFLICT OF INTEREST

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

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