New Optical Molecular Imaging Systems

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Abstract: Molecular imaging has become a research focus in recent years, which provides an effective information acquisition, analysis and processing methodology at cellular and molecular levels for biomedical study. As an important molecular imaging technique, optical molecular imaging, especially fluorescence and bioluminescence imaging, has attracted remarkable attention in tumor study and drug development for its excellent performance, non-radiativity and high cost-effectiveness in comparison with conventional imaging modalities. Generally speaking, optical molecular imaging is regarded as the combination of traditional medical imaging technology and modern molecular biology, in which the advanced optics, biology, information, medicine, and other techniques are being married to non-invasively obtain *in vivo* physiological and pathological information sensitively, quantitatively, and specifically. Furhtermore, with the research of imaging theories, algorithms and molecular probes, optical imaging systems have been rapidly developed for biomedical study in molecular imaging discipline, including planar imaging systems, tomographic imaging systems, multimodality fusion systems and so on. This review focuses on some typical optical molecular imaging systems, especially for *in vivo* small animal use. It also provides a brief discussion on the future development and application of the optical molecular imaging systems.

Keywords: Optical molecular imaging, planar imaging; tomographic imaging, bioluminescence tomography, fluorescence molecular tomography, multimodality fusion.

INTRODUCTION

Molecular imaging (MI) is a newly emerging and rapidly developing biomedical imaging discipline, which can transform the internal complex biological interactions, physiological and pathological change information at cellular and molecular levels to macroscopic *in vivo* visualization [1-4]. Furthermore, as an important preclinical technique, small animal molecular imaging can provide an effective information acquisition and assay methodology for observing function of specific genes, origin and development of living subjects and diseases, treatment effect and dynamic change of drugs [5-10].

Optical molecular imaging uses optical probes to track and report specific biologic events. In comparison with conventional imaging techniques like X-ray computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT) etc., small animal optical molecular imaging has attracted increasing attention due to its excellent performance, non-radiativity, and high cost-effectiveness [3, 4, 11, 12]. Optical imaging technique can study gene expression and protein function dynamically by detection of photon emissions from the biological tissues [4, 10]. These photons detected during imaging are generated either from fluorescent sources or from bioluminescent sources. Although the photons generation are various in different optical imaging modalities, the emission spectrum in the optical engineering field is generally in the so-called near-infrared (NIR) light window of the biological tissue ([650-900]nm), in which the photons can travel several centimeters in tissue in view of the low absorption in the above spectral window [3]. Nonetheless, the quantity of photons arrived at the surface of the small animal is still very limited due to the low intensity of the internal light source and the absorption and scattering of the biological tissues, so the capture of the surface light signal should be performed by a high sensitivity detector, such as cooled charge-coupled device (CCD) camera and photomultiplier tube (PMT). The external interference of ambient light should be avoided using a light-tight imaging chamber. In addition, in order to ensure the normal physiological functioning of the living small animal, most commercial optical imaging systems include a gas anesthesia device and a thermostat device. At present, the commonly used macroscopic information based on bio-photonics is the 2D planar image using photographic method, which is the projection of the light distribution on the small animal surface to the image plane of the photon detector [3]. This planar imaging method is simple and direct, and the corresponding imaging instrumentation is relatively cheap. However, the depth of the internal light source can not be resolved because photon attenuation in the biological tissue is a highly nonlinear function of the depth parameter and the whole small animal contains different organs [13, 14]. Furthermore, photons propagating in the biological tissue are strongly scattered before either being totally absorbed by the tissue or escaping from the surface where they are captured, and the scattered photons have no preferential direction or orientation, so the spatial resolution of planar

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optical imaging is very poor [3]. Therefore, although photographic methods are simple and practical, the potential of optical imaging technique have not been fully reflected.

In order to overcome the limitations of planar optical imaging, tomographic optical imaging approaches have been studied to obtain 3D spatial information of the internal light source [15-19]. The generally employed tomographic imaging technologies are multi-view and multi-spectral data acquisition, incorporated with the appropriate mathematical model that depicts light transport in the biological tissue [13, 20]. Furthermore, the geometry of the small animal should be fused for 3D source reconstruction, which can be determined by computer vision technology with multi-view photographic images. In addition, the surface topography or anatomical structure information of the small animal can also be obtained through a laser scanner or a micro-CT imaging system [21].

As we all know, different types of imaging techniques can provide images of the structure or function with differing resolutions on the spatial and temporal scales, and by a different sensitivity for measuring properties related to morphology or function [22, 23]. No one modality can image all of the anatomical structure and functional processes of livings. The inherent defects of one modality can be made up by other imaging methods. Therefore, fusion of imaging modalities which integrate the strengths of two or more modalities and at the same time eliminate one or more weaknesses of an individual modality has attracted considerable interest in the past 20 years, such as PET-CT, and SPECT-CT. As will be described later, the same story also takes place in the optical molecular imaging field.

The review is organized as follows. The next section introduces planar optical imaging systems, and then tomographic imaging systems are described. Followed by multimodality fusion systems, some other typical optical imaging systems are presented. Finally, conclusions and future prospects are provided.

PLANAR IMAGING SYSTEMS

Planar optical imaging has been commonly applied in biomedical research in view of its simplicity and immediacy, which uses the photographic principle to obtain 2D transmitted and scattered light distribution on the surface of the small animal by optical detection instrumentation. Although planar optical imaging can not solve the depth parameter of the internal light source, it can provide relative quantification information to reflect the development trend and the probable organ of the imaging target. The term of relative quantification means that the signals are dependent of depth and sample-type, but can be validated in experiments [2]. Depending on the probes types, planar optical imaging is roughly divided into two categories: fluorescence imaging and bioluminescence imaging.

In fluorescence imaging, the fluorophore probes inside the biological tissue absorb the excitation photons with a specific wavelength produced by an external light source, and then re-emit some photons at a longer wavelength. The external excitation light source is indispensable in fluorescence imaging. Based on the type of the excitation source, fluorescence imaging can be commonly assorted into three modes, including time domain (TD) mode, continuous wave (CW) mode and frequency domain (FD) mode [3]. The continuous wave mode, also called steady-state method, employs the excitation light source with constant intensity, and it can obtain excellent detection characteristics, high cost-effectiveness, and optimum signal-to-noise performance. The major drawbacks of steady-state method are the inability to determine fluorescence lifetime and the difficulty of resolving the tissue absorption from scattering. The frequency domain mode, also called modulated light excitation technology, generally uses the modulated intensity light with the frequency range between 70 MHz and 150 MHz [24]. The measurement of the emission light intensity can reveal the distribution of the internal fluorophore probes, and the detection of the phase shift of the photons wave-front away from the excitation source or the fluorophore can determine the fluorescence lifetime information. The signal-to-noise ratio (SNR) of the modulated light excitation technology is reduced because of the application of the high frequency excitation light, but it is less affected by the surround light than the aforementioned continuous wave mode. In bio-photonics field, time domain optical imaging is also widely studied, especially for the independent measurements of tissue absorption, scattering, and fluorochrome lifetime. This technique uses an ultrafast laser pulse to illuminate the small animal, and the arrival time distribution of the transmitted fluorescence photons on the tissue boundary is detected and then employed to separate absorption and scattering coefficients, quantify the fluorescence emitter, and provide fluorescent lifetime. Besides, time domain imaging method is equivalent to the modulated light excitation technology through Fourier Transform and the inverse Fourier Transform.

According to the mode of data acquisition, fluorescence imaging can be classified as reflectance imaging and trans-illumination, as shown in Fig. (**1A** and **1B**) respectively [3]. In the reflectance imaging mode, the excitation light source and the detection instrumentation are placed on the same side of the small animal. However, in the trans-illumination imaging, the emission fluorescent photons excited by the external light source on one side propagate from the fluorochrome and transmit through the opposite side of the small animal. Ntziachristos *et al*. [25] studied the data normalization methods in reflectance and trans-illumination mode, and the experiment results showed that the normalized trans-illumination presented significant advantages over planar reflectance method and over nonnormalized ones.

Comparatively speaking, bioluminescence imaging employs luciferase enzymes, which can catalyze the biochemical reactions of substrate luciferin with oxygen, ATP and Mg^{2+} to generate bioluminescent photons [26]. Bioluminescence imaging can be approximately regarded as the continuous wave technology because the internal bioluminescent source is continuously on during the imaging experiment. The bioluminescent signal on the external surface of the small animal can be directly collected by a CCD camera. Therefore, bioluminescence imaging is a simple diffuse pattern without consideration of the external excitation light source, as show in Fig. (**1C**).

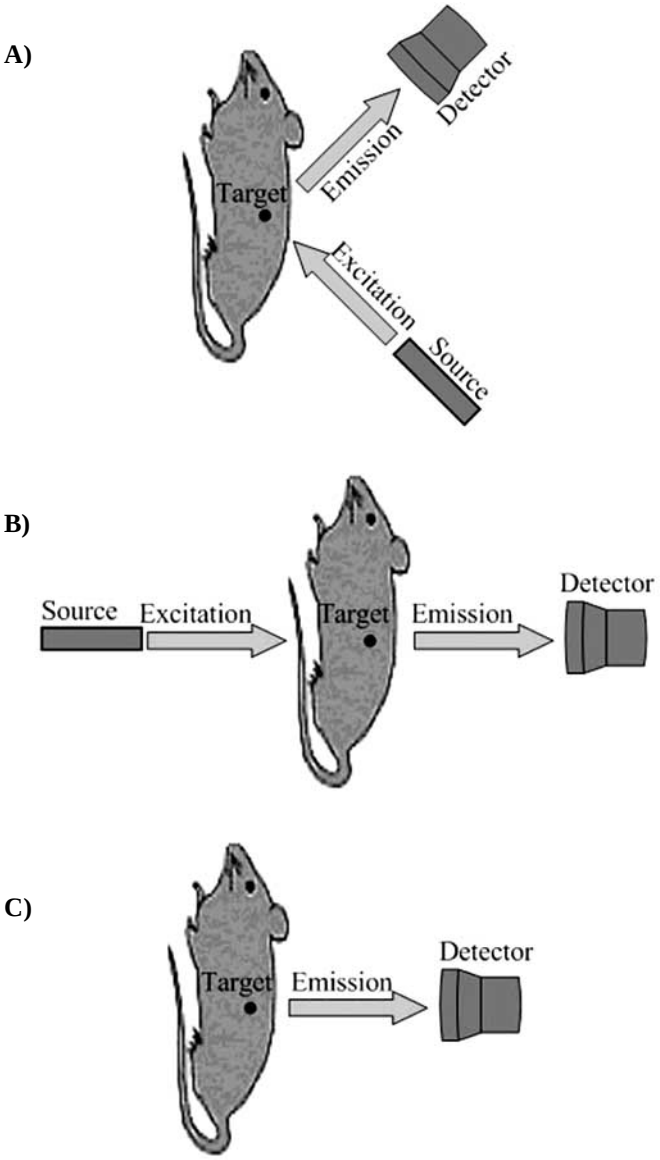

Fig. (1). Three planar optical imaging modes. **(A)** Fluorescence reflectance imaging; **(B)** Fluorescence trans-illumination; **(C)** Bioluminescence imaging.

Fluorescence and bioluminescence imaging have been widely used as effective *in vivo* small animal imaging tools and many commercial products have been found in market [27-29]. In ref. [30], the authors compared fluorescence and bioluminescence imaging performance quantitatively on an IVIS Imaging System 100 Series (Xenogen Corporation-Caliper, Alameda, CA, USA). In this instrument, a back-illuminated cooled CCD camera is used for bioluminescence and CW fluorescence imaging in the wavelength range of 400-950 nm. The results indicated that autoluminescence and autofluorescence were the important limitation to detection sensitivity of reporters. Compared with fluorescence imaging, bioluminescent signals were low, but it provided superior signal to background ratios because of its very low autoluminescence levels. Maestro imaging system, developed by Cambridge Research & Instrumentation Incorporation, uses liquid crystal tunable filter (LCTF) approach with 10 nm wavelength intervals for multi-spectral imaging. Using multi-spectral information and spectrum separation technique, Maestro can obtain high sensitivity through removing auto-fluorescence from the fluorophores of interest. This increased contrast enables imaging of smaller tumors with weak fluorescent light earlier in their growth cycle. Besides, Maestro can image up to nine kinds of fluorophores, which can realize multi-target imaging on a single small animal. Maestro is specialized for fluorescence imaging, and the signals are brighter than bioluminescence imaging, so its CCD camera is just cooled to 0^{o}C. While, for bioluminescence imaging, the CCD camera need to be cooled into a deeper temperature, -90^{o}C for example.

TOMOGRAPHIC IMAGING SYSTEMS

Planar optical imaging, both fluorescence imaging and bioluminescence imaging, only provide relatively quantitative results, and there is no depth information of the internal source. Extending planar optical imaging to 3D is attracted and promising. The aim of tomographic optical imaging is not only to reconstruct the 3D position of the light source in tissue, but also to determine the quantitative distribution. Tomographic optical imaging relative to planar optical imaging is compared to the X-ray CT relative to X-ray radiography [31, 32]. But there are two significant differences between X-ray CT and optical tomography. The first aspect is that the optical photons are highly scattered in tissues while X-ray can be regarded as rectilinear propagation. The second one is the boundary effect caused by the refraction of visual light at the tissue surface which may change the propagation direction of photons notably. For these reasons, the mathematical model of optical photons propagation in tissue is more complicated than the X-ray, so is the inverse problem. Here the inverse problem refers to reconstruct the light source from some planar images acquired from the outside of the tissue. The radiative transfer equation (RTE) is usually adopted to model the photons transport in the biological tissue [33]. Because RTE is difficult to solve, its first-order approximation, diffusion equation, is often used to depict the photons propagation in tissue [34], and the boundary condition also should be considered carefully.

Fluorescence molecular tomography (FMT) [14] and bioluminescent tomography (BLT) [13] play active roles in optical tomographic imaging. The study on FMT is a little earlier than BLT. In original FMT systems [14], fibers are used to deliver and detect photons and the matching fluids are employed to improve fiber coupling and simplify the boundary conditions. Data processing for such setups is relatively simply and it is suitable for basic feasibility study. However, the number of the fibers limits the data acquisition, and the matching fluids add additional photons diffusion. Therefore, the use of fibers and matching fluids not only make the systems complicated, but also affect the reconstruction results.

The theory of free-space propagation of diffuse light plays an important role in the development of the FMT systems [35-37]. By the free-space propagation theory, researchers developed noncontact FMT systems [38-40], in which lens coupled CCD camera was adopted instead of

fiber-based detection system. Such systems obtain a high dense sampling over the tissue surface, which is benefit to optical tomography. But fixed geometry is also employed with or without matching fluids, which makes it inconvenient to acquire multiple views around the tissue. In order to solve such problem, free-space 360° FMT systems appeared [41-43]. In these setups, fix geometry limitation is removed and complete-angle (360°) projections with high spatial sampling of photon signals are acquired. Such systems are flexible to operation and they can improve imaging quality compared with the former systems.

It should be noted that all the above systems work in CW mode, which has the advantages in high cost effectiveness, good SNR and simple operation, but can only provide fluorescence strength [32]. The systems in time domain or frequency domain mode not only offer fluorescence output but also the lifetime [44]. The eXplore Optix (Advanced Research Technologies-GE Healthcare, St. Laurent, QC, Canada) is the first commercial TD small animal fluorescence imaging system, which can provide the fluorescence intensity and lifetime in the small animal using time-correlated single photon counting (TCSPC) technique. The wavelength of the excited light generated by the pulsed laser diode can be customized to the desired fluorescence excitation spectrum, and the detector used in the imaging system is a PMT which is sensitive to the spectral band of 450-900 nm. When the small animal is positioned on the heated plate, the surface shape of the small animal can be obtained using the digital camera on the top and the profilometer camera on the side. Furthermore, eXplore Optix can derive the depth and concentration information of fluorescent objects inside the small animal using temporal point spread function. However, the diameter of the illumination spot is about 1 mm, so the scanning speed is reduced largely, and the region of interest is generally selected. In ref. [45], the performance comparison of eXplore Optix and IVIS 200 (Xenogen Corporation-Caliper, Alameda, CA, USA), which works in CW mode, has been done through phantom studies. The results showed that the eXplore Optix was log order more sensitive at detecting minute concentrations of Cy 5.5, and it had better spatial resolution than IVIS 200. Compared with the IVIS 200, the main two disadvantages of the eXplore Optix are that it needs a longer acquisition time and has a lower throughput. A similar results have been obtained by Zerda *et al.* [46], who compared the performance of one TD mode imaging system (eXplore Optix) and two CW mode systems, Maestro *in-vivo* imaging system (CRI, Woburn, MA, USA) and IVIS-Spectrum (Xenogen Corporation-Caliper, Alameda, CA, USA).

Compared with fluorescence molecular tomography, bioluminescence tomography is more ill-posed due to the absence of the external excitation source. It can be regarded that the BLT works just in steady-state mode. The uniqueness research on BLT shows that *a priori* knowledge has a quite important effect in source reconstruction [15]. Currently, anatomical information [47], photons distribution of on the surface [47, 48], multi spectrum information [16, 49, 50] and the sparse characteristics of the source [51] are utilized as *a priori* knowledge. The reconstruction algorithms are very vital to tomographic optical imaging. The first BLT system employs multiple planar views to reconstruction [13, 47, 48]. The mouse or phantom is fixed on a rotation stage and a high sensitivity CCD camera is used to acquire bioluminescence imaging. Four views can be acquired by rotating the stage at a 90° interval. This technology is simple in principle and easy to understand, but it takes a long time to acquire multi views. For this reason, some researchers proposed improved methods to realize multiview detection in a shorter time, such as via the mirrors' reflection [49].

Spectrally-resolved measurement is other important technology for BLT [16, 49, 50], which utilizes a *priori* information that photons with different wavelengths have different absorption and scattering characteristics. A principle illumination of the spectrally-resolved method is shown in Fig. (**2**). Assume that two monochromatic sources with the same intensity but different frequencies, red light and green light for example, are embedded in tissues at the same depth. It is well known that green light is more absorbed by tissues than the red light. If the detected intensity ratio of red light and green light outside the tissue is 1:1, it indicates that the sources are close to the tissues surface. If the ratio is 2:1, it indicates that the sources are relative deep in the tissues. If the ratio is 4:1, it confirms that the sources must be deeper than the other two cases, because the attenuation of green light is far greater than the red light. In order to acquire spectrum data, filters with different wavelength center are consecutively used before the CCD to remove the photons outside the corresponding wavelength range. It is obvious that the spectrally-resolved measurement and multiview detection can be integrated together in a BLT system. Wang *et al.* [52] proposed a system which could acquire multiview and multispectral data simultaneously.

MULTIMODALITY FUSION SYSTEMS

There is no doubt that multimodality is an obvious trend in molecular imaging [22, 53]. Each imaging modality can only provide one or a few aspect information of the interesting organism. Combining two or more modalities together may supply mutual needs or offset mutual lacks.

As for the optical imaging, it is a very sensitive and powerful tool for molecular imaging in small animals, and it is non-radiativity and relatively inexpensive compared with nuclide imaging and MRI. However, optical-based imaging suffers from limited structural information. Fusing structural imaging, such as X-ray image, CT or MRI, with optical imaging is a feasible approach to overcome this drawback. And many groups have focused on this area.

Some manufacturers add an X-ray source to original planar optical instruments and then optical imaging and X-ray radiography can be acquired in the same setups [29, 54]. As CCD camera can not directly detect X-ray photons, a scintillator screen is utilized to convert X-ray photons to visible photons, then the CCD camera which is used for optical imaging can also be used for X-ray imaging indirectly. The inside structure of an optical/X-ray imaging instruments, IVIS Lumina XR (Xenogen Corporation-Caliper, Alameda, CA, USA), is show in Fig. (**3**) [55]. The X-ray source energy range is optimized for small animal imaging and an aluminum filter cuts off low energy X-ray to reduce exposure in animals. During optical imaging, data acquisition is identical with pure planar optical imaging. In X-ray imaging mode,

the scintillator screen is utilized to convert X-ray to visible photons which can be imaged by the CCD camera. Merging optical image with X-ray image brings optical signal into anatomical context. The X-ray image gives a high resolution 2D structure of bones and contrast agent, which is benefit for localizing and determining the disease state of an animal.

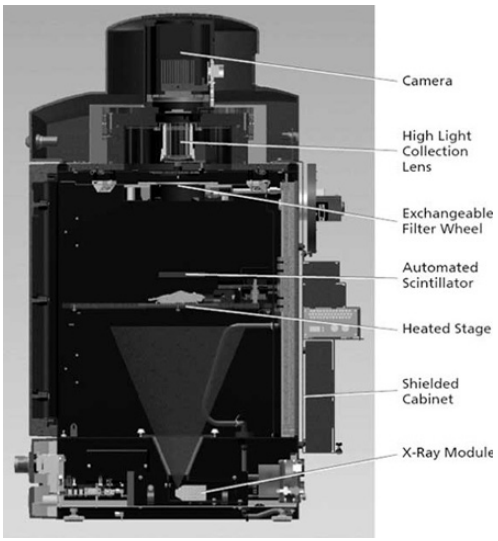

Fig. (2). Illumination of the spectrum solved method principle.

For tomographic optical imaging, structure imaging not only provides anatomic information, but also can be used to reduce ill-posedness in 3D reconstruction. As is mentioned in the last section, BLT is more ill-posed than fluorescence tomography. Alexandrakis *el al.* [56] pointed that reconstruction of BLT inverse problem would become less accurate when the light source became deeper if the small animal was supposed as homogenous. Through micro-CT or MRI, we can acquire the animal's anatomic data and segment them into several organs, such as bone, heart, lung, liver, and muscle. Though CT or MRI data provide little optical properties information of tissue or organs, we can refer to related literatures for optical properties of these tissues to reduce the ill-posedness of BLT inverse problem.

Fig. (**4**) shows a schematic diagram of combined micro-CT and BLT system. The figure shows that the micro-CT subsystem and the BLT subsystem share the same mechanism that is composed by motorized moving stage and the motorized rotation stage. During experiment, the small animal is put on the rotation stage. As there is no need to move the small animal during each imaging process, it is convenient for post processing of the imaging data.

Integrating fluorescence tomography with other multi-modalities attracts much attention. Usually, in noncontact optical tomography, other modalities information is not only useful but also necessary. Schulz *et al.* [36, 39] employed a 3D photogrammetric camera to capture the 3D tissues surface in FMT systems. In ref. [57], a dual-modality system for fluorescence tomography and X-ray computed tomography of small animals has been developed. A free-space FMT equipment was integrated onto the rotating gantry of a commercial micro-CT (eXplore Locus, General Electric HealthCare, London, ON, Canada). To our best known, it is "the first system developed to offer a fully integrated FMT and XCT components delivering intrinsically coregistered datasets" [57]. The CT data are semiautomatic segmented and used in the fluorescence tomography reconstruction. Experimental results show multimodality approach is superior to standalone implementation.

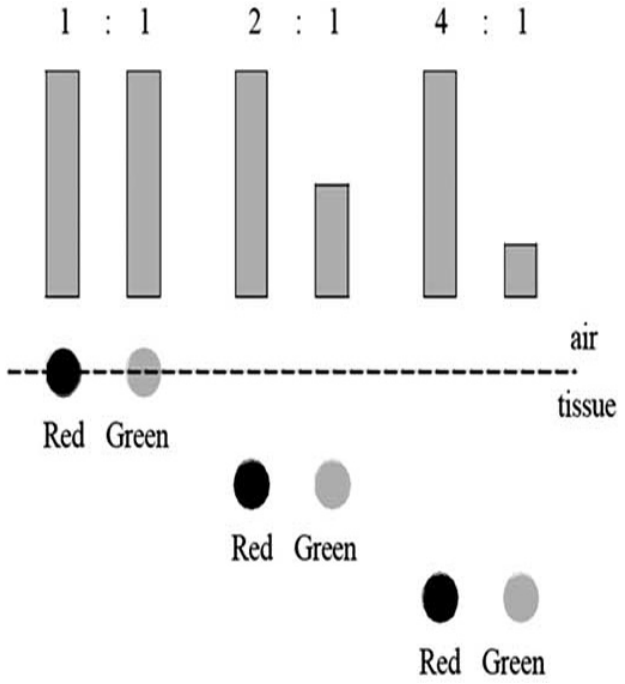

Fig. (3). Inside structure of the IVIS Lumina XR (Ref. [55]).

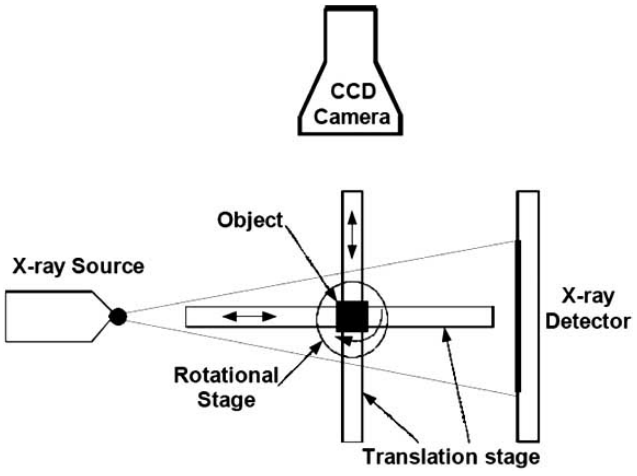

Fig. (4). Fusion of micro-CT and BLT system.

FMT and BLT can also integrate with other optical modalities. Zhang *et al.* [58] combined diffuse optical tomography (DOT) and BLT system, and experimentally demonstrated that BLT reconstruction could be enhanced quantitatively by incorporating prior spatial distribution of optical properties of heterogeneous media obtained from DOT. In ref. [59], Razansky and Ntziachristos fused the photoacoustic tomography (PAT) with FMT. Quantitative optical absorption map was reconstructed by PAT, and then was employed

to compute a diffusion-equation-based forward model for FMT using a finite element solution. It demonstrated an improvement in the quality of FMT reconstructions at the presence of a large absorber. Tan and Jiang [60] proposed DOT guided fluorescence molecular tomography method which combined FMT with DOT to study the impact of heterogeneous optical property distribution. Compared with PAT, DOT offers lower spatial resolution, but it could obtain both absorption and scattering coefficients when *a priori* information coupled with effective normalization schemes is used in constant wave DOT [60].

In ref. [61], Wang *et al*. show a three modalities system sketch map for BLT, including bioluminescence imaging, optical tomography and CT. CT is responsible for providing the 3D structure information of the mouse. Optical tomography like DOT will be used to obtain the *in vivo* optical parameters for each organ/tissue. The bioluminescence imaging equipment acquires the bioluminescence signal emitted from the mouse surface. All the information will be fused together in bioluminescence tomography and a reliable reconstructed bioluminescence source distribution will be given in the end. Compared with current systems, such a sketch combines more modalities information and it is also suitable for FMT. We believe that it will appear in the near future.

Another advance of optical multimodality integration is the combination of optical imaging with nuclide imaging, such as PET and SPECT, to acquire both functional and molecular imaging. Compared with nuclide imaging, optical imaging is very convenient to operation, and it has much better temporal resolution. For example, it often takes several tens of minutes to finish a PET scanning, while a few seconds or minutes are enough for optical imaging. Therefore, nuclide/optical imaging can offset the drawback of nuclide imaging in temporal resolution and it is benefit to dynamically monitor the interesting tracers or markers. Another benefit is that the radionuclide based imaging can be utilized to valid the 3D optical tomographic reconstruction results. For example, Lu *et al*. [62] introduced a PET radionuclide imaging-based strategy to validate the BLT results. In the CT imaging-based strategy, it is difficult to identify the optical source *in vivo* based on anatomical information because of the poor soft tissue contrast in preclinical CT imaging. Combining X-ray computed tomography, positron emission tomography, and spectrally-resolved bioluminescence imaging, Lu *et al*. [62] showed the effectiveness of radionuclide imaging in bioluminescence tomography.

In order to combine separate optical and PET imaging into one instrument, a system, called optical PET (OPET), was designed at the UCLA Crump Institute for Molecular Imaging [56]. It is capable of detecting both high-energy gamma-ray and visible photons by a single detector, which is a scintillation array with an open end and photomultiplier tuber for detection. During PET imaging by the OPET, high energy photons are absorbed by the crystal and fluorescence photons are released, which is the same as general PET imaging. In optical imaging mode, the same crystal acts as a light guide for visible photons. The optical detection sensitivity of the OPET device was tested. It indicated that "this prototype OPET detector module should have comparable sensitivity to IVIS system" [56].

In OPET system, there are no collimating lenses for the coupling of optical photon and the imaged object should be contacted with or close to the crystal during scanning. Peter *et al*. [63] utilized a microlens array (MLA) for field-of-view definition. They presented a concept for tomographic dual-modality positron/optical small animal imaging. They coupled a MLA to complementary metal oxide semiconductor (CMOS) detector, and a septum mask was used to avoid crosstalk between the output of individual microlens. Though this design may be convenient to integrate with a PET scanner, the sensitivity for optical detection is lower than CCD camera used in the pure optical imaging system. In addition, unlike the OPET instrument, this dual-modality system consists of individual positron detector and optical photons detector.

OTHER OPTICAL IMAGING SYSTEMS

In the above, we mainly focus on the bioluminescence and fluorescence imaging, including their planar imaging, tomographic imaging, and the integration with other imaging methods. There are also many other optical imaging systems, such as optical coherence tomography (OCT), ultrasound modulated optical tomography (UOT) and PAT. PAT attracts more and more attention in biomedicine application, for both clinical and preclinical. Photoacoustic effect, which is also refer to as optoacoustic or thermoacoustic (TA) effect, is the basis of PAT [33]. When an object is irradiated by modulated electromagnetic (EM) radiation, some of the energy is absorbed and converted into heat and further converted to acoustic pressure via thermal expansion. The acoustic pressure is propagated as an ultrasound wave, which can be detected by ultrasonic sensor and then is used for imaging. Both EM pulses and intensity-modulated continuous wave EM waves can be adopted as photoacoustic EM source and the short EM pulses is widely used for its merit in SNR and time resolved characteristic.

Photoacoustics overcomes the drawback of pure optical and ultrasound imaging. Due to high scattering of optical photons, the spatial resolution of optical imaging is low, but it is high sensitive in absorption by molecules, such as oxygenated and deoxygenated hemoglobin. On the other hand, the ultrasound imaging is limited in contrast but provides high spatial resolution. Thus, the photoacoustics combines the high absorption contrast of optical imaging with the high spatial resolution of ultrasound imaging.

In PA imaging, PAT acts as an important role. The spatial resolution of PAT is scalable, depending on the center frequencies and bandwidth of the transducers [64]. Not only structure information, but also functional and molecular imaging could be obtained through PAT [65]. The fusing of PAT and other modalities is also promising [59, 66]. Comprehensive surveys on PA effecting and PAT were given in ref. [64] and [65].

CONCLUSIONS AND FUTURE PROSPECTS

Last ten years witness the rapid development of molecular imaging, which greatly depends on the advances of the biomedical probes, imaging systems and corresponding algorithms. Optical molecular imaging is one of the most important components of molecular imaging. This paper reviews

the states of art of the optical molecular imaging systems. We do not expect to and are not able to include all the progresses of this field in such a review, and we just want to draw an outline of it and give a brief account of the history and the present. Some trends of the optical molecular imaging systems could be learnt from this review.

As the technology progresses, imaging systems are expected to make good advances on spatial resolution, temporal resolution, tissue penetration, sensitivity, throughput and cost. Multimodality will play a key role in future development of imaging systems. Up to now, there are so many different imaging instruments but less standardization in optical molecular imaging field, so establishing a standardized evaluation should be valuable for the whole field development.

Nowadays, molecular imaging techniques have been widely applied in basic research, preclinical imaging, drug development [67], tumor progression monitoring [2], and treatment evaluation, etc. The attempts to clinical application are under way, and some encouraging progresses have been made. All of these predict an attractive prospect of molecular imaging.

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ABBREVIATIONS

MI = Molecular imaging

BLT = Bioluminescence tomography

CCD = Charge-coupled device

CMOS = Complementary metal oxide semiconductor

CT = Computed tomography

CW = Continuous wave

DOT = Diffuse optical tomography

EM = Electromagnetic

FMT = Fluorescence molecular tomography

LCTF = Liquid crystal tunable filter

MLA = Microlens array

MRI = Magnetic resonance imaging

NIR = Near-infrared

OCT = Optical coherence tomography

OPET = Optical PET

PAT = Photoacoustic tomography

PET = Positron emission tomography

PMT = Photomultiplier tube

SNR = Signal-to-noise ratio

SPECT = Single photon emission computed tomography

TCSPC = Time-correlated single photon counting

TD = Time domain

UOT = Ultrasound modulated optical tomography.

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