New *in Vivo* Optical Molecular Imaging Modalities

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Abstract

Recent advances in optical molecular imaging technology have led to great improvements in image resolution, and are increasingly being applied to non-invasively delineate *in vivo* physiological and pathological processes at cellular and molecular levels. It provides the potential for the understanding of integrative biology, earlier detection and characterization of disease and the evaluation of treatment. This paper focuses on the typical *in vivo* optical molecular imaging modalities as well as their potential clinical applications and future development.

1. Introduction

Optical molecular imaging (OMI) is a rapidly developing multidiscipline that involving molecular biology, mathematics, chemistry, physics, information science, medicine and pharmacology. OMI can realize real-time noninvasive dynamic *in vivo* imaging for biology organic, providing an effective method of information acquiring and analysis processing for disease progress, drug efficacy and dynamic change, as well as gene function. With the research of OMI, significant breakthroughs have been made in imaging theories, algorithms, instruments, key technologies and biomedical applications, especially two review articles in Nature series journals [1, 2] that described the applications to cancer research and drug development.

OMI offers *in vivo* dynamical visualization of the functions and can explore specific molecules as the source of image contrast. The *in vitro* approach has been less successful in deciphering physiological whole-body contributions of proteins, in which redundancies and differences in regulation can alter the outcome. Historically, conventional imaging techniques such as radiography, computed tomography, ultrasonography and magnetic resonance imaging were developed to visualize nonspecific macroscopic physical, physiological or metabolic changes in tissues. They could not identify the specific molecular events responsible for disease [3].

This paper introduces several new typical *in vivo* OMI modalities, including bioluminescence tomography (BLT), fluorescence molecular tomography (FMT), photoacoustic tomography (PAT), optical coherence tomography (OCT), Corenkov luminescence tomography (CLT), optical projection tomography (OPT), confocal laser scanning microscopy (CLSM), selective plane illumination microscopy (SPIM), and stochastic optical reconstruction microscopy (STORM). Finally, conclusions and future prospects are presented.

2. Typical *in Vivo* Optical Molecular Imaging Modalities

Emerging as an important alternative to molecular imaging, OMI has attracted much attention because of its excellent performance and low cost. The new *in vivo* OMI modalities include BLT, FMT, CLT, PAT, OCT, OPT, CLSM, SPIM, STORM, etc.

2.1 Bioluminescence Tomography

The development and widespread implementation of bioluminescence imaging has resulted in biological research undergoing a significant advance in the ability of investigators to determine cell growth, metastasis detection, drug discovery and development. BLT is capable of acquiring three-dimensional information of the light sources. Compared with other imaging modalities, the advantages of BLT are sensitivity, speed, non-invasiveness, low cost and background noise.

The bioluminescent signal is emitted when luciferin is combined with luciferase in the presence of oxygen and ATP, and luciferase enzymes are generally from the firefly, click beetle and Renilla reniformins [4]. This signal has

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different emission spectra, roughly from 400nm to 750nm, which can be detected by sensitive low light imaging systems. Bioluminescent photon propagation in biological tissue is governed by the radiative transfer equation (RTE) which has been utilized as the forward model of BLT. However, RTE is computationally expensive. In most reported papers, the forward solution is predominantly based on either the finite element method [5] or finite volume method and the boundary element method [6]. Due to the heavy computational and memory requirements of meshes, adaptive finite element methods have been used [7].

There has been a great effort lately devoted to developing the BLT model and reconstruction algorithms, but the current technique has not fully explored the potential of this approach. Multiple scattering of photons that propagate through heterogeneous biological tissues makes the BLT reconstruction problem highly ill-posed. Wang et al. first theoretically proved that bioluminescent sources can be uniquely and accurately recovered by incorporating sufficient a priori information to alleviate ill-posedness [8]. Currently, multiple types of a priori information have been verified and extensively applied in BLT reconstruction algorithms, such as anatomical information, permissible source region, spectral information and so on [4, 5, 9-11].

Although there are many challenging problems, it is believed BLT will be enhanced to meet the needs of biological research by novel probes, sensitive data acquisition, multi-modality multi-mode fusion, an accurate forward model, advanced reconstruction methods and prior knowledge based on regularization, etc.

2.2 Fluorescence Molecular Tomography

Opening new pathways for the characterization of biological processes in living animals at cellular and molecular levels, FMT is currently applied to phantom experiments and *in vivo* small animal experiments. In comparison with other molecular imaging approaches, fluorescence molecular imaging can obtain high sensitivity detection with low instrumentation expense.

The basic substance that the fluorescence probe absorbs and which emits photons is the fluorochrome. Fluorochromes mostly belong to a group of aromatic compounds, resulting from the fluorescence reaction caused by indole rings on the tryptophan residues. Fluorochrome atoms in the ground state absorb the incident photons at the appropriate wavelength and generate the transition of the energy level. However, the atoms that are on the high energy level are unstable and will decay to the ground state after some time, and then emit the photons at a longer wavelength than the excitation light [12].

Reconstruction is a crucial part of FMT. It is also a challenging area because the predomination of scattering in the photon transportation leads to the ill-posedness of the problem. Generally, the inverse reconstruction problem of FMT is to find the distribution of the fluorophore within the object to be imaged-based on the pre-calculated weighing matrix and the boundary measurement. The conjugate gradient (CG) method has been adopted successfully with less storage and computation [13]. Normally, the linear CG method and the nonlinear CG method [14] are two types of CG methods that are usually being applied. In order to take advantage of both of them, a penalized linear and nonlinear combined conjugate gradient method for the reconstruction of FMT is presented [15].

It is known that the measurement performance can be further improved by better optimizing the arrangement of the illumination source and field of view. More and more reconstruction algorithms with a high accuracy and computational efficiency are under research to adapt to different experimental situations. Besides, researchers are trying to find new fluorescence dyes in order to acquire a signal with a high SNR [16]. A light source that can launch excitation signals at a larger spectrum region to satisfy different fluorescent probes is being developed as well [17].

2.3 Photoacoustic Tomography

The photoacoustic effect was first discovered by Alexander Graham Bell and was reported in 1880. Research in PAT became active only after the invention of the computer, ultrasound transducer and pulse laser. PAT is a hybrid imaging technique, based on the photoacoustic effect. It combines the merits of both optical imaging and ultrasound imaging. PAT has several distinctive advantages, which makes it a promising imaging modality. In recent years, many

papers have been published to exploit the potential usage of PAT. Wang et al. [18] reported the functional *in vivo* imaging ability of PAT in 2003. In 2009, Razansky et al. [19] reported their research in imaging fluorescent proteins using multi-spectral PAT.

2.4 Optical Coherence Tomography

OCT is an optical signal acquisition and processing method allowing extremely high-quality, micrometer-resolution, and three-dimensional images from within the optical scattering media to be obtained, which has been widely used in the biological and medical fields for about twenty years. The technique of OCT is based on light, rather than sound or radio frequency, so it delivers a high resolution. Using the OCT technique, scattered light can be filtered out and the glare can be completely removed. Since OCT can be used to image living tissue *in situ*, non-invasive and real-time detection of micro-structures can be applied in the medical field, such as the *in vivo* images of the human retina [20], intra-coronary imaging, and gastrointestinal examination [21].

2.5 Other Optical Molecular Imaging Modalities

Besides what have been mentioned above, here are also many other optical imaging systems, such as CLT, OPT, CLSM, SPIM, STORM, etc.

CLT provides a new OMI strategy in a non-invasive manner within living subjects using radioactive probes [22]. Resting on the Vavilov-Cerenkov effect, *in vivo* optical images can be obtained for a variety of radionuclides. OPT is a relatively new technique for volumetric visualization of transparent or slightly opaque objects on the cellular level up to small organisms. The principle of OPT is similar to computed tomography, but in contrast one uses visible light instead of X-rays. OPT is limited to transparent specimens as it is necessary to minimize light scattering through the sample. CLSM is a type of high-resolution fluorescence microscopy that overcomes the limitations of conventional wide-field microscopy and facilitates the generation of high-resolution 3D-images from relatively thick sections of tissue. CLSM relies on the confocal effect to enhance both lateral and in-depth resolution in contrast to conventional microscopy [23]. Images generated by CLSM have been used in the research of the articular cartilage, bone, muscle, tendon, ligament and menisci. SPIM is a newly developed method that allows one to measure large-size specimens with an improved semiconfocal illumination. SPIM permits the rapid capture of three-dimensional images so that transient biological phenomena can be detected. Its ability to detect dynamic processes in a large specimen with a resolution of ~1 μm is of major importance [24]. STORM as a kind of super-resolution optical imaging, uses photo-switchable fluorescent probes to temporally separate the otherwise spatially overlapping images of individual molecules, allowing the construction of high-resolution images of ~20 nm lateral and ~50 nm axial resolutions [25].

3. Conclusions and Future Prospects

As the application of molecular imaging expands, the information acquired by the current single-modality imaging is relatively limited. Therefore, OMI technology will be developed from the single-modality to the multimodality. The fundamental theory will be improved from organism heterogeneous property to tissue specificity; the reconstruction algorithm will be enhanced from feasible region to dynamic global region; the imaging system will be developed from two- and three-dimensional to high-dimensional and multimodality; the mathematical model will be facilitated from diffusion approximation to high-order approximation; the molecular probe will be boosted from single function to multiple functions and multiple targets; the application object will be changed from small animal to large one, even human body, such as the early breast cancer accurate diagnosis and antitumor drug efficacy evaluation, ect.

4. Acknowledgments

This paper is supported by the Project for the National Basic Research Program of China (973) under Grant No. 2011CB707700, the National Natural Science Foundation of China under Grant No.81027002, 81071205, 30970778,

the Hundred Talents Program.

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