In Vivo Quantitative Reconstruction Studies of Bioluminescence Tomography: Effects of Peak-Wavelength Shift and Model Deviation

Junting Liu, *Member, IEEE*, Duofang Chen, Xiangsi Li, Xiaopeng Ma, Haichao Chen, Weiwei Fan, Fu Wang, Xiaochao Qu, Jimin Liang, *Member, IEEE*, Feng Cao, and Jie Tian*, *Fellow, IEEE*

***Abstract*—Bioluminescence tomography is a novel optical molecular imaging technology. The corresponding system, theory, and algorithmic frames have been set up. In the present study, we concentrated on the analysis of quantitative reconstruction deviation from peak-wavelength shift of luminescent source and the deviation of heterogeneous mouse model. The findings suggest that the reconstruction results are significantly affected by the peak-wavelength shift and deviation of anatomical structure animal models. Furthermore, the model deviations exhibit much more influence than the wavelength shift on the reconstruction results.**

***Index Terms*—Bioluminescence tomography (BLT), model deviations, peak-wavelength shift, quantitative reconstruction.**

I. Introduction

BIOLUMINESCENCE tomography (BLT) is an emerging optical molecular imaging modality, which has cost-effectiveness, good molecular specificity, and high sensitivity for noninvasive 3-D *in vivo* imaging. It can be used to monitor physiological and pathological processes by using bioluminescent light-emitting probe in small living animal [1]. This tomographic technique can perform absolutely quantification through reconstructing 3-D maps of bioluminescence in small animal on the basis of sophisticated algorithms [2]. Wang *et al.* also described that the significant advantages of BLT technology are its high sensitivity and specificity [1]. Furthermore, the specificity is determined by the characteristics of firefly luciferase reporter gene. In addition, the sensitivity can be determined by using a highly sensitive charge-coupled device (CCD) camera. More than this, the background noise is very low in BLT because of no external light source for excitation [3]. In the past several years, BLT technology has made great strides in its theory and algorithmic frames independently [4]. How to evaluate the performances of BLT system and algorithm? What are the main factors to affect the reconstruction results? However, so far, few investigations have been done to determine where the reconstruction errors come from and which the crucial factors are.

In this study, we concentrated on evaluating the effects of peak-wavelength shift and deviation of anatomical structure models on BLT reconstruction results based on our dual-modality BLT/MicroCT imaging system and *hp* finite-element method (*hp*-FEM) reconstruction algorithmic frame [5], [6]. The peak wavelength will shift as temperature changes, and optical properties of biological tissues are usually different at different peak wavelengths. Although bioluminescent reporter-gene assays have distinct advantages over fluorescent assays for high sensitivity and specificity, the emission wavelength of luciferases and fusion luciferases are sensitive to the environment temperature. The spectral emission peak of firefly luciferase is red-shifted from 578 to 612 nm when temperature is increased from 25 °C to 37 °C, and the peak-wavelength shift arrives at about 15 nm when the temperature changes from 25 °C to 29 °C [7]. Wang, *et al.* also reported the spectral red shift at different temperature by ultrasound heating [8]. In addition, the peak-wavelength measurement of the Glowproducts luminescent source implanted in a mouse body was performed at different temperature to demonstrate the spectral red shift, and the data was listed in Table I. It is worth emphasizing that the peak-wavelength shift often occurs in small animal experiments at different body temperature under different anesthetic conditions. Besides, influences of heterogeneous and homogeneous animal models on *in vivo* BLT reconstruction have been reported by our group earlier, submillimeter-level deviation of localization in reconstruction has been confirmed with appropriate optical parameters in accurate living-mouse model [1], [6]. Overall, we attempted to find the key determinant of BLT reconstruction deviation, including the changes of optical properties due to the wavelength shift and the spatial distribution of heterogeneous model relative to source position.

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J. Liu, D. Chen, X. Li, X. Ma, H. Chen, F. Wang, X. Qu, and J. Liang are with the Life Sciences Research Center, School of Life Sciences and Technology, Xidian University, Xi'an 710071, China (e-mail: liujunting@fingerpass.net.cn; dfchen@xidian.edu.cn; lixiangsi1113@gmail.com; maxp88@163.com; haichaoch@gmail.com; fuwang.whu@hotmail.com; xiaochaoqu@gmail.com; jimleung@mail.xidian.edu.cn).

W. Fan and F. Cao are with the Department of Cardiology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China (e-mail: fww2142008458@gmail.com; wind8828@gmail.com).

*J. Tian is with the Life Sciences Research Center, School of Life Sciences and Technology, Xidian University, Xi'an 710071, China, and also with the Institute of Automation, Chinese Academy of Sciences, Beijing 100190, China (e-mail: tian@ieee.org).

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TABLE I
PEAK-WAVELENGTH SHIFT AT DIFFERENT TEMPERATURE

Temperature (℃)	25	29	32	36	39	45
Peak wavelength (nm)	631	636	638	644	646	657

The deviation of anatomical structure models also can increase the reconstruction errors. In this study, we want to obtain quantitative influences of peak-wavelength shift and model deviations on reconstruction results. Furthermore, we hope that the quantitative results will guide us to choose appropriate bioluminescent reporter gene and establish accurate enough anatomical structure animal models.

II. MATERIALS AND METHODS

A. Materials

1) BLT Prototype System: We adopted a dual-modality ZKKS-Direct3D molecular imaging system (jointly developed by Guangzhou Zhongke Kaisheng Medical Technology Company, Ltd., Xidian University, and Institute of Automation, Chinese Academy of Sciences), which combines a MicroCT to get anatomical structure information of small animal. A highly sensitive CCD camera (Princeton Instruments PIXIS 2048B, Roper Scientific, Trenton, NJ) was employed to acquire multiview images around the mouse. Besides, a Nikon Micro–Nikkor 55-mm *f*/2.8 manual focus lens was mounted on the CCD camera. The axis direction of camera lens runs parallel to the high-precision electronic driving translation stage and vertical to the MicroCT X-ray central projection direction. The MicroCT imaging is performed by employing an X-ray tube (OXFORD INSTRUMENTS series 5000 Apogee X-ray tube, X-ray technology. Inc., CA) with a focal spot size of 35 μm, which is accompanied by a high-resolution flat panel X-ray detector (HAMAMATSU C7921CA-02, Hamamatsu City, Japan) incorporating a 1032 $\times$ 1012 pixel photodiode array with a 50-μm pixel pitch. We can get the high-quality 3-D anatomic structure information based on Feldkamp–Davis–Kress cone-beam reconstruction algorithm on GPU acceleration scheme [9]. A Matrx VIP 3000 anesthesia machine (Matrix Medical Inc., MN) is employed to keep mouse sedated and alive during the whole experiment.

2) Optical Parameters: The optical parameters were calculated based on literature [10]. We analyzed the optical parameters in the spectra range from 600 to 700 nm, and we find that tendencies and rates of change are different in different organs at the same wavelength range. All the optical parameters and their tendencies for six organs at different wavelengths were shown in Fig. 1.

3) Anatomical Structure Animal Models: A mini glow-luminescent light stick (Glowproducts, Victoria, BC, Canada, emission wavelength about 630 nm, source flux density 60 nW/mm^3) was selected as the known source. Its emission wavelength is very close to that of the luciferase. A 7 μL luminescent solution was drawn by pipette gun and injected into a plastic catheter of 1.4 mm in diameter and 4.5 mm in length (the total power is 415 nW), and then, the catheter was implanted into the mouse abdomen. (All animal procedures were in accordance with the Fourth Military Medical University approved animal protocol.) In order to get a satisfactory anatomical structure information, the mouse was scanned by MicroCT system on the holder in the upright attitude, and then the segmentation was done to obtain different mouse organs (such as heart, lungs, liver, and kidneys), using commercially available software Amira 4.1.1 (Mercury Computer system, Inc. Chelmsford, MA).

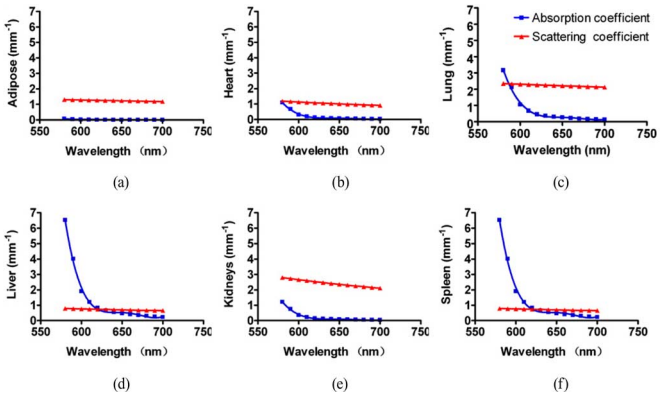

Fig. 1. Optical parameters (absorption coefficient and reduced scattering coefficient) of different organs have different tendencies and rates of change. (a) Adipose tissue. (b) Heart tissue. (c) Lung tissue. (d) Liver tissue. (e) Kidney tissue. (f) Spleen tissue.

B. Methods

1) Quantitative Reconstruction Algorithm Frame: Our BLT prototype system works in continuous-wave mode and photons propagation in the scattering media can be well described by the steady-state diffusion equation. The diffusion approximation with a modified diffusion coefficient has been verified through steady-state measurement in highly scattering media [11]. BLT source reconstruction based on adaptive *hp*-FEM method was adopted in this study. Using adaptive mesh refinement strategy and intelligent permissible source region, we can obtain more accurate information about the location and density of sources. With this method, the robustness, stability, and efficiency were improved [5]. More robust total power unit was employed, which was obtained by integrating the area of reconstructed source density in present paper. In order to get the quantitative reconstruction result, we also calibrated our CCD camera by an integrating sphere of 12 in diameter. Our calibrated camera is used for data acquisition on the mouse surface; the image pixel gray level can release the mouse-surface power or photons flux information; in the processing of calibration, we consider the field of view and the distance from CCD camera to the mouse surface in our calibration study. The relationship involves the image pixel gray value, the exposure time, and the position parameters, etc. The final calibration formula for the CCD camera is given by

$$E = 10^4 \times \left[\frac{0.0001(v+20)}{t_e} + 0.0009\right] \times \frac{(R-d)\times 59.72}{5.7R} \quad (1)$$

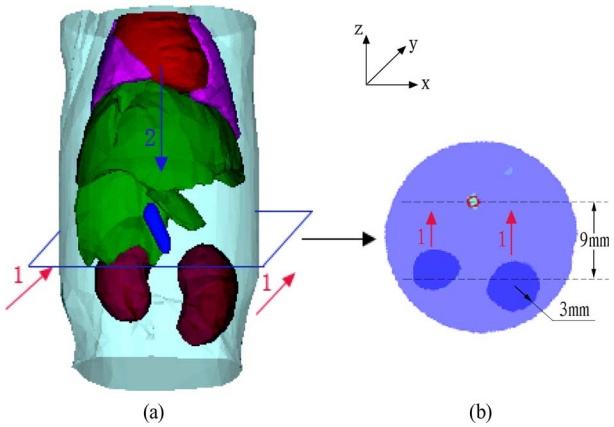

Fig. 2. Anatomical structure model distortions and movements. (a) Anatomical structure model: red region is heart, pink region is lung, green region is liver, two brown regions are kidneys, and blue region is the implanted luminescent source. (b) One slice in the z-axis direction of anatomical structure model. The red circle represents the actual source.

where E (nW/mm^2) is the irradiance intensity on the mouse surface, v is the pixel gray value of the luminescent image by CCD camera, t_e (s) is the exposure time for the luminescent images acquisition, R (mm) is the distance from the mouse surface to the edge of the cylindrical lens, d (mm) is the distance from the mouse surface to the center of the lens front face.

2) Spectra Selection: Bioluminescence emission spectra focus on the range of 580–700 nm wavelength *in vivo* [12]. The *in vivo* spectra of several luciferases revealed a significant attenuation of light at wavelengths below 600 nm, especially in deep tissue. Most available luciferases have wavelengths of maximum emission above 600 nm. Therefore, we only analyze the spectral range from 600 to 700 nm, and select several typical wavelengths, such as 600, 620, 630, 640, 650, 660, 680, and 700 nm.

3) Anatomical Structure Model-Distortion Strategy: We suppose that the emission peak wavelength and corresponding optical parameters are accurate, and the main error is from construction of the small animal model. The anatomical structure mouse model was contorted to simulate the model deviations. First, we presume that main organs move along z-axis direction. In our experiments, the kidneys were artificially moved 1, 2, 3, 4, and 6 mm toward the source in y-axis direction, respectively. Then, we can obtain the reconstruction deviations. Second, most of the organs above the source were shifted downward 2, 4, 6, 8, and 10 mm. Thus, we can find whether the reconstruction deviations are determined by the spatial distribution of main organs relative to the actual source position. The present anatomical structure mouse model is shown in Fig. 2. The red and blue arrows indicate the moving direction of the kidneys and the moving direction of the main organs, respectively.

III. Experiments and Results

A. Reconstruction Deviation With Peak-Wavelength Shift

In this experiment, we supposed that the mouse model was accurate. A mouse model of implanted luminescent source was established for source reconstruction with shifted emission peak wavelength. Multiview photographs and luminescent images were acquired by CCD camera from four directions in intervals of 90°. The implanted source was reconstructed based on *hp*-FEM reconstruction algorithm with the optical parameters at wavelength from 600 to 700 nm and 10 or 20 nm apart for the heterogeneous mouse model. The actual position coordinate of the luminescent source is (29.3 , 22.3, and 26.6 mm), which was obtained by our MicroCT system. The reconstruction results based on heterogeneous mouse model had a localization deviation of about 0.3 mm and a quantitative deviation less than 6.3% at the wavelength of 630 nm. Especially, we focused on the changing of quantitative deviation with the emission peak-wavelength shift. Figs. 3 and 4 showed that quantification is more sensitive than localization with the wavelength shift. When the peak wavelength changes 10 nm from source emission peak wavelength of 630 nm, we can obtain the deviation within 15% in quantification. At the same time, the localization deviation is acceptable. A large number of experiments show that the body temperature changes several degrees because of anesthesia, although we have adopted the constant temperature heating unit in the mouse holder.

The reconstruction results suggest that the wavelength shift of light-emitting probe may be optimized for BLT imaging. With the development of luciferases commercialization, we can select luciferase reporter genes of little wavelength shift whose range

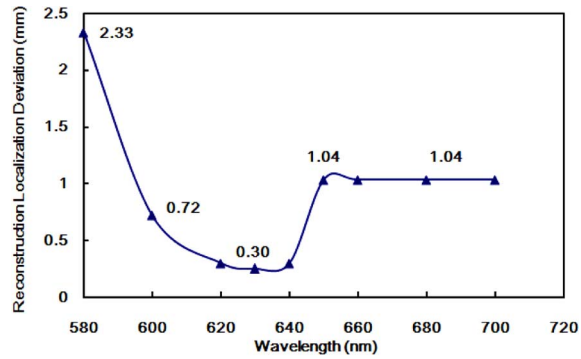

Fig. 3. Reconstructed localization deviation based on accurate heterogeneous mouse model. The blue line represents the reconstructed localization deviation of the heterogeneous mouse model.

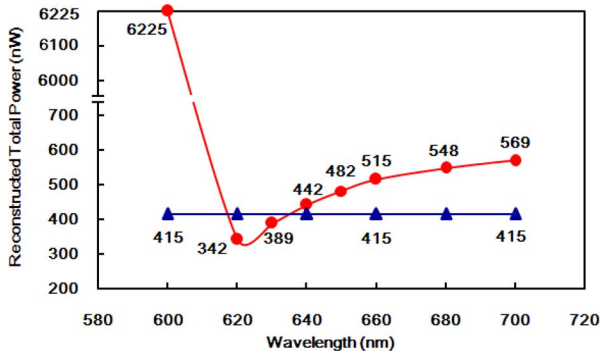

Fig. 4. Reconstruction results of quantification in heterogeneous mouse model with the wavelength range from 600 to 700 nm. The red line represents the reconstructed total power (nW) of heterogeneous mouse models and the blue line represents the actual total power of luminescent source.

TABLE II
RECONSTRUCTION DEVIATION BASED ON ANATOMICAL MODEL DISTORTION

Distortion Strategy	Localization Deviation (mm)	Total Power (nW)	Quantitative Deviation (%)
D_{ktz} = 0mm	0.3	441.9	7
D_{ktz} = 1mm	1.9	348.9	21
D_{ktz} = 2mm	3.4	68.8	84
D_{ktz} = 3mm	7.6	13.4	97
D_{ktz} = 4mm	6.8	95.0	79
D_{ktz} = 6mm	5.9	78.2	82
D_{mod}= 2mm	3.6	1101	149
D_{mod}= 4mm	3.9	1245	182
D_{mod}= 6mm	2.6	2478	461
D_{mod}= 8mm	2.9	23170	5142
D_{mod}= 10mm	2.5	16045	3530

Note: D_{ktz} = the distance of kidneys were moved only in y-direction, D_{mod} = the distance of main organs were moved only in $-z$-direction. Localization deviation represents the distance between coordinate of reconstructed source and that of the actual source. Quantitative deviation = (measured total power – actual total power)/actual total power.

of emission peak-wavelength shift should be controlled in ± 10 nm at experimental body temperature.

B. Reconstruction Deviation With Anatomical Structure Model Distortion

In order to find the reconstruction deviation of spatial distribution of heterogeneous organs relative to the actual position of source and corresponding optical properties of organs, we reconstructed the source with the anatomical structure mouse model distortion strategy as described earlier. First, the kidneys were moved in y-axis direction as shown in Fig. 2. It is clear from Table II that the reconstruction deviations are much larger than the moving distance in location, which can guide us to construct the accuracy anatomical structure animal model. Meanwhile, the effect is not very serious in quantification; the relative deviation can be controlled within 100%. Second, the main organs were moved downward to the source. According to the reconstruction results, both in localization and quantification in Table II, it has great influences on the localization and quantification. Moreover, the quantitative deviation is much more sensitive than the localization deviation when the main organs were moved downward to the implanted source.

IV. DISCUSSION AND CONCLUSION

In the present study, we try to find the influences of the emission peak-wavelength shift of luminescent light and deviation of anatomical structure model on reconstruction results. On one hand, variations in temperature will cause peak-wavelength shift and the shift will lead to errors in localization and quantification for BLT reconstruction. Therefore, peak wavelengths with shift less than ± 10 nm are expected to be used in BLT technology. On the other hand, the deviation of anatomical structure model has marked effects on the localization and quantification. We also find the reconstruction deviation is determined by the spatial distribution of main organs relative to the actual position of source. Although some researchers have done many studies on nonrigid registering in optical imaging to obtain more accurate anatomical structure without MicroCT or MRI [13], we believe that submillimeter accuracy in position for the heterogeneous mouse model is required; thus, we can obtain ideal reconstruction results.

In conclusion, we have gained a basic understanding of the influence of light probe peak-wavelength shift and the deviation of anatomical structure mouse models on reconstruction results. The experiments suggested that the deviation of the model has much more influence on the reconstruction precision than the emission peak-wavelength shift does. Further studies of finite-element mesh error and system robustness are still needed. Overall, BLT seems to be a powerful and cost-effective tool, and has a promising future for development.

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REFERENCES

[1] G. Wang, W. Cong, K. Durairaj, X. Qian, H. Shen, P. Sinn, E. Hoffman, G. McLennan, and M. Henry, "In vivo mouse studies with bioluminescence tomography," *Opt. Exp.*, vol. 14, no. 17, pp. 7801–7809, 2006.

[2] R. Weissleder and M. J. Pittet, "Imaging in the era of molecular oncology," *Nature*, vol. 452, no. 7187, pp. 580–589, Apr. 3 2008.

[3] W. Cong, G. Wang, D. Kumar, Y. Liu, M. Jiang, L. Wang, E. A. Hoffman, G. Mclennan, P. B. McCray, J. Zabner, and A. Cong, "Practical reconstruction method for bioluminescence tomography," *Opt. Exp.*, vol. 13, no. 17, pp. 6756–6771, 2005.

[4] G. Wang, W. Cong, Y. Li, W. Han, K. Durairaj, X. Qian, H. Shen, M. Jiang, T. Zhou, J. Cheng, J. Tian, Y. Lv, H. Li, and J. Luo, "Recent development in bioluminescence tomography," *Curr. Med. Imaging Rev.*, vol. 2, pp. 453–457, 2006.

[5] R. Han, J. Liang, X. Qu, Y. Hou, N. Ren, J. Mao, and J. Tian, "A source reconstruction algorithm based on adaptive hp-FEM for bioluminescence tomography," *Opt. Exp.*, vol. 17, no. 17, pp. 14481–14494, Aug. 17 2009.

[6] J. Liu, Y. Wang, X. Qu, X. Li, X. Ma, R. Han, Z. Hu, X. Chen, D. Sun, R. Zhang, D. Chen, D. Chen, X. Chen, J. Liang, F. Cao, and J. Tian, "In vivo quantitative bioluminescence tomography using heterogeneous and homogeneous mouse models," *Opt. Exp.*, vol. 18, no. 12, pp. 13102–13113, 2010.

[7] H. Zhao, T. C. Doyle, O. Coquoz, F. Kalish, B. W. Rice, and C. H. Contag, "Emission spectra of bioluminescent reporters and interaction with mammalian tissue determine the sensitivity of detection in vivo," *J. Biomed. Opt.*, vol. 10, no. 4, pp. 041210-1–041210-9, Jul./Aug. 2005.

[8] G. Wang, H. Shen, and W. Cong, S. Zhao, and G. Wei, "Temperature-modulated bioluminescence tomography," *Opt. Exp.*, vol. 14, no. 17, pp. 7852–7871, Aug. 21 2006.

[9] G. Yan, J. Tian, S. Zhu, Y. Dai, and C. Qin, "Fast cone-beam CT image reconstruction using GPU hardware," *J. X-Ray Sci. Technol.*, vol. 16, no. 4, pp. 225–234, 2008.

[10] G. Alexandrakis, F. Rannou, and A. Chatziioannou, "Tomographic bioluminescence imaging by use of a combined optical-PET (OPET) system: A computer simulation feasibility study," *Phys. Med. Biol.*, vol. 50, no. 17, pp. 4225–4242, 2005.

[11] J. Ripoll, D. Yessayan, G. Zacharakis, and V. Ntziachristos, "Experimental determination of photon propagation in highly absorbing and scattering media," *J. Opt. Soc. Amer. A Opt. Image Sci. Vis.*, vol. 22, no. 3, pp. 546–551, Mar. 2005.

[12] B. W. Rice, M. D. Cable, and M. B. Nelson, "In vivo imaging of light-emitting probes," *J. Biomed. Opt.*, vol. 6, pp. 432–440, 2001.

[13] K. Marias, J. Ripoll, H. Meyer, V. Ntziachristos, and S. Orphanoudakis, "Image analysis for assessing molecular activity changes in time-dependent geometries," *IEEE Trans. Med. Imaging*, vol. 24, no. 7, pp. 894–900, Jul. 2005.