Bioluminescence tomography with structural information estimated via statistical mouse atlas registration

BİN ZHANG,¹ WANZHOU YIN,¹ HAO LIU,¹ XU CAO,²,³ AND HONGKAI WANG¹,4

¹School of Biomedical Engineering, Dalian University of Technology, Dalian, Liaoning 116024, China
²Engineering Research Center of Molecular and Neuro Imaging of the Ministry of Education & School of Life Science and Technology, Xidian University, Xi’an, Shaanxi 710071, China
³caoxu@life.xidian.edu.cn
⁴wang.hongkai@dlut.edu.cn

Abstract: Due to an ill-posed and underestimated characteristic of bioluminescence tomography (BLT) reconstruction, a priori anatomical information obtained from computed tomography (CT) or magnetic resonance imaging (MRI), is usually incorporated to improve the reconstruction accuracy. The organs need to be segmented, which is time-consuming and challenging, especially for the low-contrast CT images. In this paper, we present a BLT reconstruction method based on a statistical mouse atlas to improve the efficiency of heterogeneous model generation and the accuracy of target localization. The low-contrast CT image of the mouse was first registered to the statistical mouse atlas model with the constraints of mouse surface and high-contrast organs (bone and lung). Then the other organs, such as the liver and kidney, were determined automatically through the statistical mouse atlas model. The estimated organs were then discretized into tetrahedral meshes for BLT reconstruction. The linearized Bregman method was used to solve the sparse inverse problem of BLT by minimizing the regularization function (L1 norm plus L2 norm with smooth factor). Both numerical simulations and in vivo experiments were conducted, and the results demonstrate that even though the localization of the estimated organs may not be exactly accurate, the proposed method is feasible to reconstruct the bioluminescent source effectively and accurately with the estimated organs. This method would greatly benefit the bioluminescent light source localization for hybrid BLT/CT systems.

© 2018 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

OCIS codes: (170.3010) Image reconstruction techniques; (100.6950) Tomographic image processing; (170.6280) Spectroscopy, fluorescence and luminescence.

References and links

1. M. Keyaerts, V. Caveliers, and T. Lahoutte, “Bioluminescence imaging: looking beyond the light,” Trends Mol. Med. 18(3), 164–172 (2012).
2. C. H. Qin, J. C. Feng, S. P. Zhu, X. B. Ma, J. H. Zhong, P. Wu, Z. Y. Jin, and J. Tian, “Recent advances in bioluminescence tomography: methodology and system as well as application,” Laser Photonics Rev. 8(1), 94–114 (2014).
3. C. Darne, Y. Lu, and E. M. Sevick-Muraca, “Small animal fluorescence and bioluminescence tomography: a review of approaches, algorithms and technology update,” Phys. Med. Biol. 59(1), R1–R64 (2014).
4. B. Zhang, K. K. Wang, J. Yu, S. Eslami, I. Iordachita, J. Reyes, R. Malek, P. T. Tran, M. S. Patterson, and J. W. Wong, “Bioluminescence tomography-guided radiation therapy for preclinical research,” Int. J. Radiat. Oncol. Biol. Phys. 94(5), 1144–1153 (2016).
5. J. Shi, T. S. Udayakumar, K. Xu, N. Dogan, A. Pollack, and Y. Yang, “Bioluminescence tomography guided small-animal radiation therapy and tumor response assessment,” Int. J. Radiat. Oncol. Biol. Phys. S0360-3018(18)30182-2, epub ahead of print (2018).
6. W. Cong, G. Wang, D. Kumar, Y. Liu, M. Jiang, L. Wang, E. Hoffman, G. McLennan, P. McCray, J. Zabner, and A. Cong, “Practical reconstruction method for bioluminescence tomography,” Opt. Express 13(18), 6756–6771 (2005).
7. 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. Express 14(17), 7801–7809 (2006).
8. G. Wang, Y. Li, and M. Jiang, “Uniqueness theorems in bioluminescence tomography,” Med. Phys. 31(8), 2289–2299 (2004).
9. 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. Hao, and J. Tian, “In vivo quantitative bioluminescence tomography using heterogeneous and homogeneous mouse models,” Opt. Express 18(12), 13102–13113 (2010).
10. S. Yahiyannejad, P. V. Granton, N. G. Liewews, L. Gilmour, L. Dubois, J. Theys, A. J. Chalmers, F. Verhaegen, and M. Vooijs, “Complementary use of bioluminescence imaging and contrast-enhanced micro-computed tomography in an orthotopic brain tumor model,” Mol. Imaging 13(1), 1–8 (2014).
11. M. A. Naser, M. S. Patterson, and J. W. Wong, “Algorithm for localized adaptive diffuse optical tomography and its application in bioluminescence tomography,” Phys. Med. Biol. 59(8), 2089–2109 (2014).
12. P. Wu, K. Liu, Q. Zhang, Z. Xie, Y. Li, N. Ning, X. Yang, X. Li, and J. Tian, “Detection of mouse liver cancer via a parallel iterative shrinkage method in hybrid optical/microcomputed tomography imaging,” J. Biomed. Opt. 17(12), 126012 (2012).
13. H. Yan, Y. Lin, W. C. Barber, M. B. Unlu, and G. Guislen, “A gantry-based tri-modality system for bioluminescence tomography,” Rev. Sci. Instrum. 83(4), 043708 (2012).
14. A. D. Klose, B. J. Beattie, H. Dehghani, L. Vider, C. Le, V. Ponomarev, and R. Blasberg, “In vivo bioluminescence tomography with a blocking-off finite-difference SP3 method and MRI/CT coregistration,” Med. Phys. 37(1), 329–338 (2010).
15. A. Ale, V. Ermolayev, E. Herzog, C. Cohrs, M. H. de Angelis, and V. Ntziachristos, “FMT-XCT: in vivo animal studies with hybrid fluorescence molecular tomography-X-ray computed tomography,” Nat. Methods 9(6), 615–620 (2012).
16. B. Li, F. Maafi, R. Berti, P. Pouliot, E. Rheaume, J. C. Tardif, and F. Lesage, “Hybrid FMT-MRI applied to in vivo atherosclerosis imaging,” Biomed. Opt. Express 5(5), 1664–1676 (2014).
17. J. Zhang, D. Chen, J. Liang, H. Xue, J. Li, Q. Wang, D. Chen, M. Meng, Z. Jin, and J. Tian, “Incorporating MRI structural information into bioluminescence tomography: system, heterogeneous reconstruction and in vivo quantification,” Biomed. Opt. Express 5(6), 1861–1876 (2014).
18. H. Park, P. H. Bland, and C. R. Meyer, “Construction of a probabilistic atlas and its application in segmentation,” IEEE Trans. Med. Imaging 22(4), 483–492 (2003).
19. C. Platero and M. C. Tobar, “A multi-atlas segmentation using graph cuts with applications to liver segmentation in CT scans,” Comput. Math. Methods Med. 2014, 182909 (2014).
20. M. Baiker, J. Milles, J. Dijkstra, T. D. Henning, A. W. Weber, I. Que, E. L. Kaijzel, C. W. Löwik, J. H. Reiber, and B. P. Lelieveldt, “Atlas-based whole-body segmentation of mice from low-contrast Micro-CT data,” Med. Image Anal. 14(6), 723–737 (2010).
21. A. J. Chaudhari, A. A. Joshi, F. Darvas, and R. M. Leahy, “A method for atlas-based volumetric registration with surface constraints for optical bioluminescence tomography in small animal imaging,” Proc. SPIE 6510, 651024 (2007).
22. M. Baiker, M. Staring, C. W. Löwik, J. H. Reiber, and B. P. Lelieveldt, “Automated registration of whole-body follow-up MicroCT data of mice,” Med Image Comput Comput Assist Interv 14(Pt 2), 516–523 (2011).
23. D. Konrad, D. Dufour, F. Bourgoin, P. Berghofener, O. A. Tamayo, H. Green, M. C. Gregoire, and O. Salvado, “Mouse whole-body organ mapping by non-rigid registration approach,” Proc. SPIE 7965, 79650E (2011).
24. W. P. Segars, B. M. Tsui, E. C. Frey, G. A. Johnson, and S. S. Berr, “Development of a 4-D digital mouse phantom for molecular imaging research,” Mol. Imaging Biol. 6(3), 149–159 (2004).
25. B. Dogdas, D. Stout, A. F. Chatziioannou, and R. M. Leahy, “Digimouse: a 3D whole body mouse atlas from CT and cryosection data,” Phys. Med. Biol. 52(3), 577–587 (2007).
26. W. Wan, Y. Wang, Y. Qi, C. Liu, W. Ma, J. Li, L. Zhang, Z. Zhou, H. Zhao, and F. Gao, “Region-based diffuse optical tomography with registered atlas: in vivo acquisition of mouse optical properties,” Biomed. Opt. Express 7(12), 5666–5680 (2016).
27. S. Ren, H. Hu, G. Li, X. Cao, S. Zhu, X. Chen, and J. Liang, “Multi-atlas registration and adaptive hexahedral voxel discretization for fast bioluminescence tomography,” Biomed. Opt. Express 7(4), 1549–1560 (2016).
28. H. Wang, D. B. Stout, and A. F. Chatziioannou, “Estimation of mouse organ locations through registration of a statistical mouse atlas with micro-CT images,” IEEE Trans. Med. Imaging 31(1), 88–102 (2012).
29. H. Wang, D. B. Stout, and A. F. Chatziioannou, “A deformable atlas of the laboratory mouse,” Mol. Imaging Biol. 17(1), 18–28 (2015).
30. G. Wang, W. X. Cong, Q. S. Yang, Q. Tian, S. P. Zhu, J. M. Liang, M. Barroso, and X. Intes, “Innovation and fusion of X-ray and optical tomography for mouse studies of breast cancer,” Proc. SPIE 9967, 99671R (2016).
31. A. D. Klose and E. W. Larsen, “Light transport in biological tissue based on the simplified spherical harmonics equations,” J. Comput. Phys. 220(1), 441–470 (2006).
32. K. Liu, Y. Lu, J. Tian, C. Qin, X. Yang, S. Zhu, X. Yang, Q. Gao, and D. Han, “Evaluation of the simplified spherical harmonics approximation in bioluminescence tomography through heterogeneous mouse models,” Opt. Express 18(20), 20988–21002 (2010).
33. C. Kuo, O. Coquoz, T. L. Troy, H. Xu, and B. W. Rice, “Three-dimensional reconstruction of in vivo bioluminescent sources based on multispectral imaging,” J. Biomed. Opt. 12(2), 024007 (2007).
34. H. Dehghani, S. C. Davis, S. Jiang, B. W. Pogue, K. D. Paulsen, and M. S. Patterson, “Spectrally resolved bioluminescence optical tomography,” Opt. Lett. 31(3), 365–367 (2006).
To overcome the challenges in segmentation of low-contrast organs in CT images, several studies have presented to facilitate the estimation of internal organs using atlas-based methods [18–23]. In those studies, the CT images were registered to an atlas constrained by high-contrast features (such as the body surface, bone and lung) at first, followed by estimating the low-contrast organs from the registered atlas. Baiker et al. [20] presented an automated whole-body segmentation method for low-contrast micro-CT mouse images based on a modification of the MOBY mouse atlas [24]. Similarly, Xiao et al. [23] registered the skin, bone and lung from the Digimouse mouse atlas [25] to the mouse CT image by a non-rigid
registration method, and then estimated the other organs through thin-plate spline transform. Recently, Wan et al. [26] proposed to recover the mouse anatomical structures by registering the Digimouse mouse atlas to the CT image with non-rigid registration, and then employed the estimated organs to guide DOT reconstruction for revealing the optical properties of the inner organs. These organ estimation methods mainly use a single atlas for registration, which is not sufficient for compensating the variations of the organ structure caused by the differences of weight, sex and strain among mice.

Recently, Ren et al. [27] presented a multi-atlas registration method to estimate the internal organs from the mouse CT images, and then the organs were discretized into hexahedral voxels for BLT reconstruction. The multi-atlas was constructed from 50 manually segmented CT images. For the mouse to be analyzed, the similarity between the mouse surface and the 50 multiple atlases were calculated, and the internal organs were estimated from the best 10 atlases. However, the influence of the estimated organs on BLT reconstruction was not investigated.

Different from aforementioned method, we have constructed a statistical mouse atlas based on 103 healthy mice of different sexes, strains, weights and postures, which demonstrates better capability for compensating anatomical variations [28, 29]. The statistical shape model and conditional Gaussian model were used to learn and capture the inter-subject shape variations and inter-organ shape correlations. For registration, the mouse surface and high-contrast organs (bone and lung) from CT images were fitted using the statistical shape model at first, the low-contrast organs were subsequently estimated using the conditional Gaussian model. The registration accuracy with the statistical mouse atlas is promising. The obtained Dice coefficients > 0.7 for the lungs, heart, liver and kidney. Although the estimated organs may be not completely accurate, the high Dice coefficient illustrates its potential for organ estimation in low-contrast CT images and will benefit the BLT reconstruction by providing the organ information. But the feasibility of using statistical mouse atlas for BLT reconstruction has not been investigated before.

In this paper, we studied the feasibility of using the statistical mouse atlas for BLT reconstruction, aiming to effectively promote mouse CT image segmentation and facilitate BLT reconstruction for the multimodality BLT/CT systems. First, the organs in the torso region were automatically segmented through registration of the statistical mouse atlas to the CT images. Then the segmented organs were discretized into 3D meshes and each organ was assigned with corresponding optical properties. The third-order simplified spherical harmonic equation (SP3) which was more accurate than the diffuse equations (DE) in modeling the bioluminescent light propagation was used in the forward model. Multispectral bioluminescence images and a linearized Bregman method with combined L1 and L2 regularization were used as the optimization scheme to reconstruct the bioluminescent source distribution. Numerical simulations and in vivo experiments were conducted to evaluate the BLT localization accuracy for bioluminescent sources in different organs. The results demonstrate that the statistical mouse atlas is feasible for BLT reconstruction and can provide acceptable accuracy for localizing bioluminescent sources.

2. Materials and method

A hybrid BLT and micro-CT system is used for small animal imaging, as described previously [27, 30]. Briefly, the X-ray tube, X-ray detector panel and CCD camera are mounted on a gantry which rotates around the animal stage. The mouse CT images are obtained by rotating the gantry 360 degrees around the mouse. Multispectral BLIs are acquired by a CCD camera with different bandpass filters at single projection. After data acquisition, the BLIs are mapped on the surface of the mesh generated from the CT image through a transformation matrix which is measured using a calibration phantom [4], then the BLT reconstruction is performed following the procedure shown in Fig. 1. The details of the procedure are listed as follow.
2.1 Mouse organ localization through the registration of a statistical mouse atlas

In order to overcome the problem of soft tissue organ segmentation in mouse CT images, we propose an efficient method to recover the organ localization, i.e., the anatomical structure of a mouse is determined by registering a statistical mouse atlas to the targeted CT image with non-rigid registration. Then the estimated organs are employed to guide BLT reconstruction. The statistical mouse atlas is established using the statistical shape model based on 103 manually segmented mouse micro-CT images, as shown in our previous work [28, 29]. The procedure is concisely depicted in Fig. 1(a). In this study, the BLT reconstruction based on the statistical mouse atlas is described as follows. After the 3D CT image is obtained, the mouse surface and high-contrast organs (bone and lung) are easily segmented through intensity thresholding and registered by fitting the statistical mouse atlas. The low-contrast organs are subsequently estimated from the registered high-contrast organs using the conditional Gaussian model, as shown in Fig. 1(b). Then the segmented organs are discretized into tetrahedral meshes and the multispectral 2D BLIs are mapped onto the surface of the mouse, as shown in Figs. 1(c-d). With the discretized meshes and the light propagation equation in biological tissue, the 3D spatial distribution of the bioluminescent source in the mouse can be linearly related to the light signal on the mouse surface. Finally, the source distribution is reconstructed by optimizing the objective function.

2.2 BLT reconstruction

The diffusion equation (DE) is widely adopted in modeling light propagation in biological tissues, but it is only valid in the diffusion limit at wavelengths where light scattering dominates absorption (\( \frac{\mu_s}{\mu_s + \mu_a} \approx 1 \)). However, the solution is less accurate at wavelength shorter than 620nm where absorption becomes significant (\( \frac{\mu_s}{\mu_s + \mu_a} < 1 \)) [14]. Here, the 3rd order simplified spherical harmonics function (SP3) to the radiative transfer equation (RTE) is used to model bioluminescence light propagation in biological tissues. The SP3 equation is a high-order approximation to the RTE, which is more accurate than DE [14, 31, 32] and is more suitable for BLT in terms of accuracy and computational effort. The steady state SP3 equations are expressed as follow:
\[
\begin{aligned}
- \nabla \cdot \left( \frac{1}{3\mu_a} \nabla \varphi_1(r) + \mu_a \varphi_1(r) \right) - \frac{2}{3} \mu_s \varphi_2(r) &= \delta(r-r_s) \\
- \nabla \cdot \left( \frac{1}{3\mu_s} \nabla \varphi_2(r) + \left( \frac{4}{9} \mu_a + \frac{5}{9} \mu_s \right) \varphi_2(r) \right) - \frac{2}{3} \mu_a \varphi_1(r) &= -\frac{2}{3} \delta(r-r_s)
\end{aligned}
\]  

(1)

where \( \mu_a \) and \( \mu_s \) are the absorption coefficient and scattering coefficient, respectively, \( g \) is the isotropic parameter, and \( \mu_{\text{iso}} = \mu_a + \mu_s \). \( \delta(r-r_s) \) represents a point light source. \( \varphi_1(r) \) and \( \varphi_2(r) \) are the composite moments, and the photon flux \( \phi_0(r) = \int_{4\pi} \varphi(r, \Omega) d\Omega \) inside tissue is defined as

\[
\phi_0(r) = G(r_s, r) = \varphi_1(r) - \frac{2}{3} \varphi_2(r).
\]

(2)

With the retrospective characteristic that \( G(r_s, r) = G(r_s, r) \), the measurement at position \( r_j \) on the surface can be calculated as follow:

\[
J(r_j) = \Theta \int G(r, r_j) x(r) dr = \Theta \int G(r_j, r) x(r) dr,
\]

(3)

where \( \Theta \) is the gain factor relating to the system, and \( x(r) \) is the unknown bioluminescent source distribution.

Assume the imaged object is discretized into \( N \) mesh nodes, the relationship between measurements at the object surface and the bioluminescence source distribution can be expressed as

\[
\begin{bmatrix}
J_1 \\
\vdots \\
J_M
\end{bmatrix} =
\begin{bmatrix}
G_{1,1} & \cdots & G_{1,N} \\
\vdots & \ddots & \vdots \\
G_{M,1} & \cdots & G_{M,N}
\end{bmatrix}
\begin{bmatrix}
x_1 \\
\vdots \\
x_N
\end{bmatrix},
\]

(4)

where \( [J_1, \ldots, J_M]^T \) is a vector representing the fluence rate measured at the surface \( (M<<N) \), \( [x_1, \ldots, x_N]^T \) is a vector of the unknown bioluminescence distribution. Multispectral images were acquired to improve BLT reconstruction [33, 34], and then Eq. (4) can be rewritten as

\[
\begin{bmatrix}
J(\lambda_1) \\
\vdots \\
J(\lambda_s)
\end{bmatrix} =
\begin{bmatrix}
\eta(\lambda_1) G(\lambda_1) \\
\vdots \\
\eta(\lambda_s) G(\lambda_s)
\end{bmatrix}
\begin{bmatrix}
x_1 \\
\vdots \\
x_N
\end{bmatrix},
\]

(5)

where \( J(\lambda_i) \) and \( G(\lambda_i) \) are the measurements and system matrix extended from Eq. (4) at wavelength \( \lambda_i \), and \( \eta(\lambda_i) \) is the relative spectral weight caused by the source spectrum, filters and CCD quantum efficiency at different wavelengths. A modified version of the open source NIRFAST software [35, 36] was used to calculate \( G(\lambda_i) \).

The BLT reconstruction is ill-posed and underdetermined with fewer measurements than unknowns, so directly solving Eq. (5) is impossible. With the sparse characteristic of the BLT solution, Eq. (5) is usually transformed to the basis pursuit problem

\[
\min_x \|x\| : Gx = J,
\]

(6)
where $\|x\|$ is the L1 norm of $x$, which determines an L1-minimal solution. Yin et al. [37] introduced a linearized Bregman method and demonstrated that it can efficiently and stably solve the basis pursuit problem by transferring Eq. (6) to an unstrained optimization problem
\[
\min_x \left\{ \|x\| + \frac{1}{2\alpha} \|x\|^2 : Gx = J \right\},
\]
(7)
where $\alpha$ is a non-negative smooth parameter, $\|x\|$ is the Euclidean norm of $x$. Whenever the smooth parameter $\alpha$ is appropriately chosen, Eq. (7) will converge to an exact solution of the basis pursuit problem. After the imaged object from CT was discretized into a 3D mesh, finite element method was used to numerically build the system matrix. The optical properties of different organs [38, 39] are shown in Table 1. The anisotropic parameter $g$ is set to 0.9 in all the simulation and in vivo experiments. A threshold of 10% the maximum source strength is applied for display of the BLT results shown in this work.

To quantitatively analyze and compare the reconstruction results, the size ratio, the Dice coefficient and the intensity ratio of the reconstructed source were estimated, respectively. The size of the reconstructed source was calculated by counting all the voxels that the intensities were larger than 10% of the maximum intensity. The Dice coefficient, Dice, takes two volumes and their overlap into account, and is defined as follow:
\[
\text{Dice} = 2 \frac{V_{\text{rec}} \cap V_{\text{true}}}{V_{\text{rec}} + V_{\text{true}}},
\]
(8)
where $V_{\text{rec}}$ and $V_{\text{true}}$ are the reconstructed volume and truth volume, respectively.

Table 1. Optical properties of different organs at different wavelengths [39]

| Organ  | $\mu_s$ ($\text{mm}^{-1}$) 590 nm | $\mu_s$ ($\text{mm}^{-1}$) 610 nm | $\mu_s$ ($\text{mm}^{-1}$) 630 nm | $\mu_s$ ($\text{mm}^{-1}$) 650 nm | $\mu_s'$ ($\text{mm}^{-1}$) 590 nm | $\mu_s'$ ($\text{mm}^{-1}$) 610 nm | $\mu_s'$ ($\text{mm}^{-1}$) 630 nm | $\mu_s'$ ($\text{mm}^{-1}$) 650 nm |
|--------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Heart  | 0.483                           | 0.094                           | 0.047                           | 0.033                           | 1.16                            | 1.10                            | 1.05                            | 1.01                            |
| Lung   | 1.336                           | 0.219                           | 0.105                           | 0.071                           | 2.33                            | 2.29                            | 2.25                            | 2.21                            |
| Liver  | 2.897                           | 0.566                           | 0.283                           | 0.197                           | 0.78                            | 0.75                            | 0.72                            | 0.70                            |
| Kidney | 0.541                           | 0.106                           | 0.053                           | 0.037                           | 2.73                            | 2.60                            | 2.47                            | 2.36                            |
| Soft tissue | 0.033                         | 0.007                           | 0.004                           | 0.003                           | 1.53                            | 1.46                            | 1.40                            | 1.35                            |

*The $\mu_s$ for adipose tissue were chosen from Ref [39]. The $\mu_s'$ were calculated from Eq. (1) and Table 2 in Ref [38].

3. Experiments and results

Simulations and in vivo experiments were conducted to assess the feasibility of using the statistical mouse atlas for BLT reconstruction, as shown in the following sections.
3.1 Organ localization through registration of a statistical mouse atlas

The 3D Digimouse [25] with manually segmented organs, as shown in Fig. 2(a), is regarded as the accurate organ localization which serves as the ‘ground truth’. As illustrated in Fig. 1, the mouse surface and high-contrast organs (bone and lung) are registered by fitting the statistical mouse atlas. Then the other organs, such as liver, heart and kidney, are subsequently estimated from the registered statistical mouse atlas. In this study, only the torso region was analyzed. Figure 2(b) shows the estimated organs which is overlapped with the ground truth, while Figs. 2(c-e) are the coronal, sagittal and transverse sections.

As shown in Fig. 2, the estimated organs through the statistical mouse atlas are not completely accurate with the manually segmented organs (ground truth). This may affect the following BLT reconstruction accuracy. Several numerical experiments were conducted to evaluate the influence of estimated organs on BLT reconstruction. In all the scenarios, the multispectral bioluminescence measurements (590, 610, 630 and 650nm) on the mouse surface were generated from the Digimouse with accurate organ localization which was discretized into approximately 290,000 tetrahedral elements and 52,000 nodes, as shown in Fig. 2(f). All the multispectral measurements were added with 10% of Gaussian noises. The parameter $\alpha$ was set to 5 times of the maximum measurements.

3.2 Scenario 1: light source in the lung

In this scenario, a light source (a sphere with 3mm in diameter) is localized in the lung which is accurately registered, as shown in Figs. 3(a1-a3). After organ estimation, an ROI was cropped from the mouse and was discretized for BLT reconstruction. The reconstruction mesh has approximately 113,000 tetrahedral elements and 20,000 nodes, as shown in Fig. 3(b), and the multispectral bioluminescent signals are then mapped on the mesh surface. The source distribution is then reconstructed using the proposed method and the BLT result is shown in Fig. 3(c). The fusions of the BLT reconstructed source with the CT image are shown in Figs. 3(e1-e2). For comparison, the BLT reconstruction with accurate organs (ground truth) is shown in Fig. 3(d), and the overlap of BLT with CT image is shown in Figs. 3(f1-f2). The center of mass (CoM) of the reconstructed source is shown in Table 2, where the 3D offset between the true source center and the BLT-reconstructed CoM is calculated and shown as well. The size ratio, Dice coefficient and intensity ratio of the reconstructed source are shown in Table 3.
3.3 Scenario 2: light source in the liver with accurate organ registration

In this scenario, the light source localizes in the liver where the estimated and accurate liver are well registered, as shown in Figs. 4(a1-a3). The reconstruction mesh (~152,000 elements and 27,000 nodes) mapped with multispectral bioluminescent signals on the surface is shown in Fig. 4(b). The reconstructed source distribution using the proposed method with the statistical mouse atlas is shown in Fig. 4(c). The fusions of the reconstructed source overlapped with the CT image are shown in Figs. 4(e1-e3). In contrast, the BLT reconstruction with accurate organ localization (ground truth) is shown in Fig. 4(d), while the
fusions of BLT with CT image are shown in Figs. 4(f1-f2). The BLT reconstructed source and the 3D offset between the true source center and the BLT-reconstructed CoM are shown in Table 2. The size ratio, Dice coefficient and intensity ratio of the reconstructed source are shown in Table 3.

3.4 Scenario 3: light source in the liver with inaccurate organ estimation

In this scenario, the light source localizes in the liver region where the estimated and the accurate liver are not well registered, as shown in Figs. 5(a1-a3). This scenario aims to estimate the influence of the inaccurate estimated organs on the BLT reconstruction, i.e., the reconstruction mesh is generated from the estimated liver but its localization is not accurately revealed from the statistical mouse atlas. An ROI is cropped and the estimated organs is discretized for BLT reconstruction. The reconstruction mesh (the same as that in Scenario 2) mapped with multispectral bioluminescent signals on the surface is shown in Fig. 5(b). The reconstructed source distribution with statistical mouse atlas is shown in Fig. 5(c), and the corresponding fusions of BLT and CT images are shown in Figs. 5(e1-e2). In contrast, the BLT reconstruction with accurate organ localization (ground truth) is shown in Fig. 5(d), while the fusions of BLT with CT image are shown in Figs. 5(f1-f2). The BLT reconstructed source and the 3D offset between the true source center and the BLT-reconstructed CoM are shown in Table 2. The size ratio, Dice coefficient and intensity ratio of the reconstructed source are shown in Table 3.
3.5 Scenario 4: light source in the kidney with inaccurate organ estimation

In this scenario, the light source localizes in the kidney region where the estimated kidney and the ground truth are not well registered, as shown in Figs. 6(a1-a3). The reconstruction mesh is generated from the estimated organs with inaccurate kidneys. The multispectral bioluminescent signals mapped on the mesh (~175,000 elements and 31,000 nodes) surface are shown in Fig. 6(b). The BLT reconstructions using the statistical mouse atlas and accurate organ localization (ground truth) are shown in Fig. 6(c) and (d), respectively. The corresponding fusions of BLT results and CT images are shown in Figs. 6(e1-e2) and (f1-f2), respectively. The BLT reconstruction results are shown in Table 2. The size ratio, Dice coefficient and intensity ratio of the reconstructed source are shown in Table 3.

3.6 Scenario 5: double sources in the lung

In this scenario, the capability for revealing double sources is evaluated. Two light sources are assigned in the lungs, as shown in Figs. 7(a1-b2). The reconstruction mesh (the same as that in Scenario 1, ~113,000 elements and 20,000 nodes) mapped with multispectral bioluminescent signals on the surface are shown in Fig. 7(c). The BLT reconstructions with the statistical mouse atlas and accurate organ localization (ground truth) are shown in Fig. 7(d) and (e), respectively. The corresponding BLT images overlapped with CT images are shown in Figs. 7(f1-f4) and (g1-g4), respectively. The BLT reconstruction results are shown in Table 2. The size ratio, Dice coefficient and intensity ratio of the reconstructed source are shown in Table 3.
Fig. 6. Scenario 4: Light source in the kidney, and the estimated organ is not accurately registered. (a1-a3) The coronal, sagittal and transverse sections of the sphere source. The estimated organs are fused with the ground truth. (b) The BLI is mapped on the mesh surface (only 610nm is shown). BLT reconstruction with statistical mouse atlas (c) and accurate organ segmentation (d). (e1-e2) Fusion of BLT reconstructed with statistical mouse atlas with CT images. (f1-f2) Fusion of BLT reconstructed with accurate organ segmentation with the CT images.

Fig. 7. Scenario 5: Two light sources in the lung. (a1-a2) The transverse and coronal sections of source 1 (s1). (b1-b2) The transverse and coronal sections of source 2 (s2). The estimated organs are fused with the ground truth. (c) The BLI is mapped on the mesh surface (only 610nm is shown). BLT reconstruction with statistical mouse atlas (d) and accurate organ segmentation (e). (f1-f4) Fusion of BLT reconstructed with statistical mouse atlas with CT images corresponding to s1 and s2. (g1-g4) Fusion of BLT reconstructed with accurate organ segmentation with the CT images.

3.7 In vivo experiments

Three nude mice (BALB/C, ~8 weeks old, ~20g weight) were used in the following CT and BLT study in accordance with the guidelines from the Xidian University Animal Care and Use Committee. After the mouse was anesthetized with Pentobarbital (50mg/kg, 0.1ml, IP injection), a transversal incision was made in the abdomen. Then the liver lobe was gently lifted and a self-illuminated source (2mm diameter × 6mm length, trigalight; mb-microtec ag, Niederwangen, Switzerland) was placed in the abdomen. The incision was sutured with nylon 7/0 (AROSurgicale Instruments Inc., Newport Beach, CA). After the surgery, the mouse was
taped on the animal stage for imaging, as shown in Fig. 8(a). The depth of the implanted source was ~5 mm. The entire surgical procedure lasted approximately 10 min per mouse. After the experiments, the mice were euthanized.

Multispectral bioluminescence images at 590, 615, 625 and 643 nm were acquired with 5s exposure time per wavelength and 2 × 2 binning (0.3 mm/pixel) with a scientific EMCCD camera (iXon 888, Andor Technology, Belfast, UK) mounted with a 25mm f/0.95 lens (Xenon, Schneider, Germany). After bioluminescence imaging, 360 X-Ray projections were acquired for CBCT. The CBCT volume was reconstructed at a 512 × 512 × 512 matrix with a voxel size of 0.156 × 0.156 × 0.156 mm³. The coronal, transverse and coronal sections of the imbedded source are shown in Figs. 8(b1-b3). Due to the low-contrast of soft tissue, it is difficult to distinguish the organs such as liver and kidney. The mouse surface and high-contrast organs (bone and lung) were quickly segmented through thresholding and registered by fitting the statistical mouse atlas. Then the low-contrast organs were quickly estimated, as shown in Figs. 8(c1-c3). Figure 8(d) shows the 3D rendering of the estimated organs. A section of the torso was cropped from the CBCT image with estimated organs to generate a 3D tetrahedral mesh (~59,000 elements and 11,000 nodes) for BLT reconstruction. The multispectral bioluminescent signals were mapped on the mesh surface, as shown in Fig. 8(e). The relative spectral weights of the source were 0.87, 0.88, 1.00 and 0.89 at wavelengths 590, 615, 625 and 643 nm, respectively. Heterogeneous optical properties were assigned to the organs estimated from the statistical mouse atlas for BLT reconstruction. The reconstructed source distribution is shown in Fig. 8(f). The fusions of the BLT reconstructed source overlapped with the CBCT image are shown in Figs. 8(g1-g3). The reconstructed light source and the true source are in good agreement. The 3D offset between the BLT-reconstructed CoM and the true source center is 1.0 mm, which demonstrates that the proposed method has potential for practical applications.

4. Discussion

Previous studies have demonstrated that BLT reconstruction based on the heterogeneous model with appropriate optical properties can provide more accurate results in localization and quantification than the homogeneous model [9, 11, 14]. Many multimodal BLT systems use micro-CT to provide anatomical structure, however, the low soft tissue contrast makes the
segmentation of soft tissue organs from micro-CT images without contrast enhancement is a challenging problem. In this paper, we adopted the statistical mouse atlas to facilitate soft tissue organ estimation for mouse CT images. By registering the statistical mouse atlas to the high-contrast features (surface, bone and lung) of the CT images, the other low contrast organs could be simply and efficiently estimated, which provides anatomical priors to alleviate the ill-posedness of BLT reconstruction. As shown in Section 3.2, 3.3 and 3.6 (Figs. 3, 4 and 7), the bioluminescence sources imbedded in the liver and lung can be accurately reconstructed based on the statistical mouse atlas where the 3D offsets are 0.4-0.6mm from the true source center, which is comparative to the reconstruction result using accurate organ localization (ground truth), as shown in Table 2. Additionally, the computation time for the simulation and mouse experiments was all less than 3 minutes for the SP3 based BLT reconstruction using a 64-bit laptop with an Intel Core Xeon E3-1505M 2.8 GHz processor and 16 GB of memory, a time short enough to support in vivo applications [4, 5, 10].

The estimated organs may be not completely accurate because of the complex shape and localization variation of the organs, such as liver and kidney shown in Fig. 2. This may affect the accuracy of BLT reconstruction. As illustrated in Sections 3.4 and 3.5, the bioluminescence source localizes in the organs where the estimations are not accurate. From Figs. 5 and 6 and Table 2, it is notable that the BLT results are getting worse when compared with the BLT results reconstructed with accurate organ localization, either with more artifacts (Fig. 5(c)) or with larger CoM deviation. However, the BLT reconstruction results (as shown in Section 3.4 and 3.5) demonstrate that, even though the estimated organs are not perfectly registered with the ground truth, the bioluminescence sources still can be reconstructed with acceptable accuracy (3D CoM offset < 1mm). These results are comparable with the conditions that using accurate organs distribution for BLT reconstruction, as illustrated in Table 2, and those of previous works [9, 11, 17]. From the quantitative comparison shown in Table 3, it is notable that the size of the reconstructed sources using the statistical mouse atlas is closer to the ground truth than reconstruction using accurate organ segmentation. Additionally, the size of the reconstructed sources is smaller than the ground truth when using the accurate organ segmentation, while larger than the ground truth when using the statistical mouse atlas with the linearized Bregman method. Next, the Dice coefficient reveals that the BLT reconstruction results with statistical mouse atlas are comparative to those results reconstructed with accurate organ segmentation, except scenario 3 in which the Dice is much smaller for the BLT reconstruction result with statistical mouse atlas because the CoM is far away from the ground truth (CoM offset = 0.9 mm). Last, the intensity of the reconstructed sources using the statistical mouse atlas is more approximate to the ground truth than the reconstruction using accurate organ segmentation. That is because the size of the reconstructed sources using the statistical mouse atlas is prone to be larger than those results reconstructed using accurate organ segmentation with the linearized Bregman method.

The BLT reconstruction is an ill-posed and underdetermined problem and has attracted wide attentions. In this paper, the linearized Bregman method was adopted to solve the BLT inverse problem by transferring it to an unconstrained minimization problem (Eq. (7)). The adopted Bregman method is a generalized form comparing to those previously used in BLT reconstruction [40, 41]. It has been analyzed that the linearized Bregman has the exact regularization property and will converge to the solution of the basis pursuit problem if the smooth parameter is greater than a certain value. The smooth parameter was empirically chosen by comparing the reconstruction results in simulation. The variations of the source CoM were not remarkable when it was set to 2 to 10 times of the maximal measurements. The reconstruction with 5 different random processes for scenarios 1 and 4 have been computed (data not shown), and the standard deviations of the CoM offset, size ratio, Dice and intensity ratio are very small. The results of BLT reconstruction under different scenarios indicate that the method can reveal the bioluminescent source with acceptable accuracy and robustness. The automatic parameter selection strategy will be concerned in the following study.
In the *in vivo* experiments, the reconstructed source distribution appears as a cluster of points which is not close to a cylindrical source. More efforts will concern on the reconstruction of the shape of the source. Additionally, there are many other forms of regularization such as the L1 norm regularization [42–44], Lp norm regularization [45], mixed L1/L2 norm regularization [46], joint L1/total-variation (TV) regularization [47]. Previous studies have demonstrated that these methods are feasible and potential for BLT applications *in vivo*. Combination of these regularization methods with the statistical-mouse-atlas-based method for BLT reconstruction will be implemented in further research.

In the *in vivo* study, the light source used allows to direct validation of the BLT reconstruction result by micro-CT, which would be not possible for a mouse with tumor model. Even though the true organ localization may not available, the simulation and *in vivo* results demonstrate that the BLT reconstruction based on statistical mouse atlas have the potential to accurately localize bioluminescent targets. The next step is to apply the statistical-mouse-atlas-based BLT method to the orthotopic tumor model, which will reveal a more realistic condition for human cancer research.

5. Conclusion

In this study, we presented a method for BLT reconstruction based on the statistical mouse atlas and assessed the feasibility by simulations and *in vivo* experiments. The internal organs of the low-contrast mouse CT images were efficiently estimated by registering to the statistical mouse atlas. The estimated organs were then used to establish heterogeneous model for mesh generation and system matrix assembly during BLT reconstruction. The BLT reconstruction results demonstrate that the proposed method is feasible to reconstruct the bioluminescent source effectively and accurately, even though the localization of the estimated organs may be not exactly accurate. This method could greatly benefit the bioluminescent light source localization for the hybrid BLT/CT system.

**Funding**

National Natural Science Foundation of China (61571076, 81401475, 81627807); National Key Research and Development Program of China (2016YFC0103802); the Liaoning Science & Technology Project (2015020040); Since and Technology Star Project Fund of Dalian City (2016RQ019); Xinghai Scholar Cultivating Funding of Dalian University of Technology (DUT14RC(3)066); Fundamental Research Funds for the Central Universities.

**Disclosures**

The authors declare that there are no conflicts of interest related to this article.