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Semi-automated Image Processing for Preclinical Bioluminescent Imaging

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Abstract

Objective—Bioluminescent imaging is a valuable noninvasive technique for investigating tumor dynamics and specific biological molecular events in living animals to better understand the effects of human disease in animal models. The purpose of this study was to develop and test a strategy behind automated methods for bioluminescence image processing from the data acquisition to obtaining 3D images.

Methods—In order to optimize this procedure a semi-automated image processing approach with multi-modality image handling environment was developed. To identify a bioluminescent source location and strength we used the light flux detected on the surface of the imaged object by CCD cameras. For phantom calibration tests and object surface reconstruction we used MLEM algorithm. For internal bioluminescent sources we used the diffusion approximation with balancing the internal and external intensities on the boundary of the media and then determined an initial order approximation for the photon fluence we subsequently applied a novel iterative deconvolution method to obtain the final reconstruction result.

Results—We find that the reconstruction techniques successfully used the depth-dependent light transport approach and semi-automated image processing to provide a realistic 3D model of the lung tumor. Our image processing software can optimize and decrease the time of the volumetric imaging and quantitative assessment.

Conclusion—The data obtained from light phantom and lung mouse tumor images demonstrate the utility of the image reconstruction algorithms and semi-automated approach for bioluminescent image processing procedure. We suggest that the developed image processing approach can be applied to preclinical imaging studies: characteristics of tumor growth, identify metastases, and potentially determine the effectiveness of cancer treatment.

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Keywords

Bioluminescent imaging; Tomography; CCD cameras; Light diffusion; 3D Iterative reconstruction; Lung cancer model

Introduction

Small animal bioluminescence imaging (BLI) is used to observe noninvasively and in real time the presence or activation of specific biological process at the molecular level [1]. With a multi-camera BLI device capable of tomographic studies [2–5], the 3D spatial distribution and temporal dynamics of luciferase expressing cells within the animal can be measured in great detail. The goal of BLI volumetric tumor investigations is to perform quantitative studies where intensity is a linear function of the number of light-emitting cells [2]. Since the intensity of light emitted from luciferase expressing cells depends on both the type of injection and the time from injection to imaging [2], it appears necessary to account for this variability, and a BLI tomography system with multiple cameras can account for varied time courses [3].

We have completed tests of the bioluminescent tomography system (BLT) with multiple rotating, high sensitivity CCD cameras to obtain 3D reconstructed images. These tests with different phantoms verify the utility of the approach and the accuracy of the light sources iterative reconstruction algorithm.

An important part of BLT is an automation of image processing from the data acquisition to obtaining 3D images. *In vivo* studies using a novel approach with multiple rotating cameras that, when combined with image reconstruction software [6], can provide the desired representation of point source metastases and small tumors.

The immediate purpose of this work was to develop, evaluate and optimize BLI image processing procedures (algorithms), to improve the quality of the BLT images. To achieve these goals we developed for internal BLI sources an iterative EM-like deconvolution method [7] for already binned data similar to the blind deblurring algorithm [8]. We describe its performance in using the diffusion approximation and balancing the internal and external intensities on the boundary of the media. We determine an initial order approximation for the photon fluence, and subsequently apply an iterative method to obtain a final reconstruction result. For phantom calibration tests and mouse surface reconstruction we used the Maximum Likelihood Expectation-Maximization (MLEM) method [9] to evaluate our reconstruction technique. In order to optimize the BLI image procedure we evaluated a semi-automated Linux based approach with multi-modality image handling environment.

Light diffusion in tissue

The factors that affect BLI quantitation are resolution, light absorption, scattering and interaction at major interfaces. The solution uniqueness for BLI [10–11] can be strengthened by incorporation of a priori information [12–15] and by using an appropriate simplified model of light transport in turbid media: bioluminescence photon scattering predominates over absorption in object tissue; steady-state diffusion [16–18]; partial current boundary

condition for a semi-infinite space [14]; an initial order approximation to the photon fluence [7]; the fluence rate data represented by a linear operator in terms of the internal source [12] or source function expressed by a linear combination of solid balls of known quantity and position and where each ball's total energy can be estimated [11]. With such a model the study of light source intensity and localization inside the phantom media can help assess and improve reconstruction algorithms for the BLI signals.

To estimate the position and intensity of a bioluminescence source one can describe photon propagation through tissue by the diffusion approximation to Radiative Transfer Equation [16]:

$$\frac{1}{c_n} \frac{\partial \varphi(r, t)}{\partial t} = -D \nabla^2 \varphi(r, t) - \mu_a \varphi(r, t) + S(r, t) \quad (1)$$

where $\varphi(r, t)$ is the isotropic fluency rate; D and μ_a are the diffusion and absorption coefficients, respectively; $S(r, t)$ is the internal light source distribution and $r(x, y, z)$ is the distance away from the source in time t . For steady-state diffusion, according to the dipole source model with extrapolated boundary [19], the photon fluence rate at the physical boundary can be expressed as a sum of contributions from the positive internal source $\varphi(r_s)$ and its negative image source above the tissue $\varphi(r_i)$:

$$\phi(r) = \phi(r_s) - \phi(r_i) = S_0 e^{-\mu_{eff} r_s} \frac{(4\pi D)^{-1}}{r_s} - S_0 e^{-\mu_{eff} r_i} \frac{(4\pi D)^{-1}}{r_i} \quad (2)$$

where $r_s = (z_s^2 + r^2)^{1/2}$ and $r_i = (z_i^2 + r^2)^{1/2}$ is the distance from the source and its image to point on the boundary, $\mu_{eff} = (\mu_a / D)^{1/2}$ is an effective attenuation coefficient, The surface radiance on the physical boundary is:

$$L(r) = \frac{1}{4\pi} \varphi(r) + \frac{3}{4\pi} [\varphi(r_s) \Delta_s + \varphi(r_i) \Delta_i]$$

$$\text{where } \Delta_\alpha = \frac{z_\alpha}{r_\alpha^2} (1 + r_\alpha \mu_{eff}), \text{ for } \alpha = s, i \quad (3)$$

The reflectance of photons at the tissue-air interface that will reduce the fluence rate out of the tissue:

$$R(r) = R(r_s) - R(r_i) = \varphi(r_s) \Lambda_s - \varphi(r_i) \Lambda_i;$$

$$\text{where } \Lambda_\alpha = \frac{z_\alpha}{r_\alpha^2} (1 + r_\alpha \mu_{eff}), \text{ for } \alpha = s, i \quad (4)$$

The diffusion approximation is very useful for estimating the surface photon intensity and the light spot size depth-dependence for BLI experiments [20], especially for in vivo small

animal studies. Figure 1 shows the parameter Full Width Half Maximum FWHM (the spot size) for different concentrations of Intralipid: higher concentrations of Intralipid give lower photon intensity level, but spatial resolution is better for depths ~1–1.5 cm. This depth is considered sufficient for the small animal BLI.

Materials and Methods

Image processing BLT system

The BLT system with multiple rotating, high sensitivity cooled Charged-Coupled Devices (CCD cameras) record simultaneously multiple views of the small-animal in order to assess the spatial distribution of the emitted light [1–5]. To identify the bioluminescence source distribution location within a small-animal and strength we use the light flux detected by CCD cameras on the surface of the imaged object (Figure 2). Each image was acquired in 1 min following administration of D-luciferin subcutaneously in the back fore-neck region.

We have chosen the back-illuminated CCDs (Scientific Imaging Technologies Inc., OR) because of their high quantum efficiency over a broad range of wavelengths. The quantum efficiency of the CCD is greater than 85% from 400 to 750 nm [4,5].

The nude mouse (BALBc/nu/nu), Figure 3 (the same mouse as on Figure 2b and 2c) was implanted with 10^6 A549-LUC human lung tumor cells IV via tail vein. An externally illuminated image of the mouse (Figure 3a) was obtained for co-registration at each camera position. The image of the animal surface is reconstructed in the same system of coordinates as bioluminescent image (Figure 3b) and is crucial to the process of 3D image reconstruction in non-contact imaging system.

All cameras in the system are calibrated in absolute units so that the resulting images can be digitally combined by the reconstruction algorithm.

Phantoms, light source calibration and 3D surface reconstruction

The main objective of phantom tests is to quantify the reconstruction capabilities of the tomographic device for the light sources at different tissue depths. We fabricated phantoms and light sources following procedures from published articles [21–23], but used a novel practical method to calibrate the phantom [24]. Specifically, tomographic data were obtained from a phantom without filler material to find corrections (space shifting) for any geometrical corrections were then used for 3D reconstruction of sources with unknown locations in a turbid medium filled phantom. The main details of our light emission phantom are: the dimensions approximate the size of a mouse; the light source is point-like, isotropic and reproducible within the phantom; the peak wavelength of the light source matches that of the firefly luciferase [22] *in vivo* spectrum; single and multiple light sources are possible; the phantom is solid and can be rotated through 2π degrees; the phantom material is a mixture of distilled water, agarose (Sigma A0169) and Intralipid. By changing the concentration of Intralipid from 0.25% to 2% the value of μ'_s can be varied from 2.5 cm^{-1} to 15 cm^{-1} without any significant change to the absorption coefficient μ_a

[21]. The reduced scattering coefficients μ'_s match those of typical mouse tissues. India ink can be used to increase the absorption coefficient to appropriate values.

Our 3D image reconstruction techniques both for interior light sources in phantom calibration tests and the object surface reconstruction (external diffuse illumination) are based on iterative MLEM algorithm. This approach permits not only a good estimation for irregular boundaries, but reconstructs location and intensity with a good resolution of any light sources emitted in air (in this case we can neglect correction for light diffusion).

The MLEM algorithm [9] starts with an initial estimate of emission light intensity and proceeds with the simple iterative procedure for obtaining a new one (image update). The object containing photon emitters is subdivided into voxels. Using the MLEM algorithm we can generate a sequence of image updates for the each iteration.

After the phantom was calibrated, the imaging procedure and reconstruction algorithms were verified using a light source 1 mm in diameter with phantom media both using single and dual source configurations. The number of iterations (image updates) in source reconstruction procedure was chosen to get a good agreement between source size resolution and light intensity.

Figures 4a show the example of X-profiles (Y and Z positions of sources were in the center of system coordinate) for the calibration tests. After MLEM reconstruction (example on Figure 4b) each profile was fitted with a Gaussian function, and the FWHM values of the distribution were taken as the reconstructed source sizes and are in a good agreement with physical dimensions. The position of the sources (10.16 mm) was correctly identified by the reconstruction algorithm (9.98 mm). The total reconstructed signal in air (10.44×10^7 photons/s) and Intralipid (13.9×10^7 photons/s) differed by only about 3%. Figure 4c shows the example of MLEM surface (skin) reconstruction for an externally illuminated living nude mouse. This image demonstrates that MLEM algorithm for surface reconstruction can provide necessary information about irregular boundaries.

3D BLI source reconstruction inside the biological tissue

We developed a novel iterative 3D reconstruction algorithm, which could constitute a good base for localizing small sources in preclinical imaging in the multi-scattering regime. This reconstruction approach consists of two main steps: 3D object surface reconstruction used to determine the geometry of turbid media boundary, followed by iterative reconstruction (estimation) of source position and intensity inside the tissue based on diffusion equations (1–4) for light emitting sources. A brief description of the algorithm for source reconstruction inside turbid media follows three steps:

Step 1 Estimate the source location by back-projecting the experimental data into the volume subdivided into voxels, calculate the intensity of reflection on boundary for each voxel j , and subtract its contribution from the transmitted (obtained from the CCD data) part of intensity. The diffusion equation is used to determine the balance intensities for internal sources N_S for the surface element in a single voxel. If the position of the light source exceeds the maximum detectable depth (Z_{det} –see

Flowchart on Figure 5) we need to use a more sophisticated method to obtain source coordinates estimates (FEM [25], Monte Carlo [18]), or use a priori information obtained from another imaging system (CT, MRI or OPET [26]).

Step 2 For each voxel j determines an initial order approximation φ_j^0 (i.e. initial guess as an input image, which contains all diffuse light information) using the solution for photon fluence:

$$\phi_j^0 = (\rho \otimes \phi + \hat{n})_j \cong \frac{1}{\sum_{i=1}^I x_{ij} p_i^t} \sum_{i=1}^I x_{ij} p_i^0 \sum_{s=1}^{s=N_s} S_o' \exp(-\mu_{eff} r_{js}) / r_{js} \quad (5)$$

where $\mu_{eff} = (\mu_a / D)^{1/2}$. The initial photon fluence φ_j^0 depends upon the pathlength from source in voxel i to voxel j on the surface, intensities in the detectors from source in medium ρ_i^t , intensity estimates ρ_i^o (in air), the steady-state source power S_o^r , and the optical properties of the medium D and μ_a , the diffusion and absorption coefficients respectively. Here we have utilized the basis function from the semi-infinite geometry as a first approximation to the diffuse photon propagator.

Step 3 Apply the iterative deconvolution Expectation-Maximization method and Finite Element analysis [14] to obtain a final reconstruction result φ_j^{n+1}

$$\varphi_j^{n+1} = \varphi_j^n \left\{ \hat{\rho} \otimes \left(\frac{\varphi_j^0}{\rho \otimes \varphi_j^n} \right) \right\} \quad (6)$$

where $\otimes$ denotes convolution procedure, ρ is a Gaussian deconvolution kernel, and n denotes the iteration number.

Semi-automated image-processing approach

An important part of BLT is an automation of image processing from the data acquisition to obtaining 3D reconstruction results. A semi-automation of 3D volumetric bioluminescence imaging [27] combines five main parts: data acquisition; initial data processing; creating input files for BLI sources and objects surface in the file format for 3D reconstruction; 3D reconstruction for BLI sources based on Radiative Transfer Equation [10] and Finite Element analysis [25]; 3D surface reconstruction based on the MLEM method [9]; image management for visualization purposes. A simplified structure with basic program flow is shown in Figure 6.

In order to optimize this procedure Linux based software with a Multi-Modality Image Handling Environment was developed [28]. User-derived formats, such as Finite Element Meshes or Color maps, are easily incorporated. Series graphical applications which operate in an object-oriented or data-driven fashion are also available.

The software is structured in a modular way (the main functions are contained in discrete sections of their own) and consists of four discrete parts. The first part is the file format conversion, which converts files to the format used in the object viewer. The second part is

the programs for BLI sources and surface reconstruction. The third part is based on a statistical analysis and histogram package [29,30] for preparing data for image manipulation (rotation, chosen volume determination size, etc.) and the fourth the Multi-Modality Image Handling programs (perform linear or sigmoid interpolation; create Maximum Intensity Projection (MIP) matrices for 3D rotation movie) [31].

To support diverse imaging data, the original TR3 file format and associated software were designed [31]. The format is simple and incorporates the ability to add new modalities and data sources. The TR3 file format consists of three parts. A fixed-length header contains the most important information about the data, which immediately follows. Finally there is a variable length list of attached, pertinent information, known as a tag list. An extensive library of software and command line programs has been created to allow the user and programmer to interact with the TR3 file format, including data visualization.

In [31] the TR3 file format described in more detail and programming examples using the library provided along with snapshots of the output from typical commands. All data and descriptive information is in the same file. A fixed size header occupies the first thirty six bytes of the file. This header contains the most important parameters about the data, such as dimensionality and base data type. Immediately following the header comes the data, in the format through which it was acquired or generated from other data. Immediately following the data comes any extra information useful to other TR3 programs or users. Thus the TR3 file format is partly tagged in that some information is found in a fixed place in the file and some in variable locations. Figure 7 shows the format of the TR3 file. All software was developed in a Linux environment with the aim of using it on any Unix-like computer platform.

Results and Discussion

The goal of this study was to develop and assess the potential utility of optimal image processing procedure for preclinical BLI studies to better understand the effects of human disease in animal models. This approach based on the iterative procedure for source distribution function (which includes main diffusion effects) is very useful for source estimation and localization in BLI.

We have completed several tests (phantoms and living mice) with high sensitivity multi-camera BLI device to obtain 3D reconstructed images. We consider a tissue phantom with optical properties, which match those for typical mouse tissues, as a useful part of light diffusion studies in biological media in order to get important parameters for any reconstruction technique. Solid tissue phantoms are particularly useful for the study of light-tissue interaction and are still an actively used in BLI investigation [13,24]. Tests verify the utility of BLT system and the approaches for both surface and light sources reconstruction algorithms and source calibration procedure.

We find that the BLT system with multiple rotating, high sensitivity CCD cameras offers imaging of BLI sources within living mice and phantoms with appropriate spatial resolution. To identify and analyze bioluminescence source location and strength we used the light flux

detected on the surface of imaged object by CCD cameras. The dynamic range of the CCD is allowing quantitative detection of both high (large tumor) and very low (small metastasis) signals, simultaneously. A computer-controlled rotating gantry allows imaging at intermediate angular position to give the extended angular coverage necessary for 3D reconstruction.

The BLT system [5] uses innovative analysis and reconstruction software capable of providing 3D reconstruction and tomographic imaging for bioluminescent tumors that are effectively point sources or small spheres. Accuracy of reconstructed images varies with the number of angular positions used and the optical quality of CCD data.

The main idea of this study is to find a simple reconstruction method and conditions (depth, media optical properties) where we could use the light diffusion approach in BLI for good and fast estimations. An iterative reconstruction of source position and intensity inside the tissue based on diffusion equations (1–6) for light emitting BLI sources consists of several necessary steps:

1. 3D surface reconstruction used to determine the geometry of boundary
2. The interior of the object is subdivided into voxels
3. Determination the detectable depth- estimates for source location
4. Taking into account reflection on boundary
5. Balance intensities for internal sources for the surface element in a single voxel
6. Correction near light source
7. Initial order approximation for the photon fluence
8. 3D source reconstruction inside the tissue by using iterative deconvolution algorithm

Briefly, using the diffusion approximation and balancing of intensities (internal and external) on the boundary of the media, we determine an initial estimate of the photon fluence and subsequently apply the iterative deconvolution EM-like method to obtain a final reconstruction. For complex surface topographies, the boundary of the object is derived from images obtained with an external light source. If the source is sufficiently bright, it may also be possible to map the surface topography using only the surface of last scattering, but care must be taken to avoid artifacts. This reconstruction method can provide both volumetric and tomographic representations, and could potentially provide better quantification of the light emission than simple planar imaging techniques [32].

A set of 20 images (18° apart) was obtained using 1 min exposure starting 3 min post-injection of *D-luciferin*. An externally illuminated image of the mouse was obtained for co-registration at each camera position (Figure 3). In total 20 images were acquired for the most quantitative 3D image reconstruction (Figure 8). For the performance aspects of the algorithm, we draw additional attention to our results in coronal cross-sections of BLI data showing A549-LUC tumor in mouse lungs (Figure 9) demonstrates the utility of novel iterative method for semi-automated image processing to provide a realistic 3D model of the

lung tumor. We also performed a reconstruction using fewer angular views (8, 10 and 12 views) and found a similar pattern, though with lower signal to background ratio. Predicted lung surface radiance based on the voxel populations in the 3D reconstruction is shown in Figure 10. Pathologic studies after sacrifice confirmed the presence of A549-LUC human tumor cells dispersed throughout the lobes of the lungs with multiple metastatic foci [5].

We find that about 20 image projections are an optimal number of CCD views to provide a quantitative image reconstruction.

We find that BLI spatial resolution is better for depths ~1–1.5 cm. This depth was considered for small animal BLI sources reconstruction.

The imaging procedure and reconstruction algorithms were verified using a light source 1 mm in diameter with phantom media both using single and dual source configurations. The number of iterations (image updates) in source reconstruction procedure was chosen to get a good agreement between source size resolution and light intensity.

Some numerical BLT algorithms have already been reported, including the Finite Element Method [15,16], boundary element based algorithm [19], EM reconstruction technique [7,17], BLT in combination with PET [26] and multispectral BLT [12]. The Finite Element Method has been widely used by several groups as the forward solver for iterative reconstruction. There are only a handful of publications available about EM iterative reconstruction of this difficult problem [16,32].

We find that our main results for bioluminescence source location, strength and resolution are in a good agreement with other image reconstruction techniques. In [15] two sources with ~10 mm separation were reconstructed using Finite Element algorithm. We present 3D reconstruction results for 1% Intralipid-filled dual source phantom with separation 10.16 mm (Figure 11) using our novel algorithm. After reconstruction each source profile was fitted with a Gaussian function and the FWHM values of the distribution is taken as the reconstructed sources sizes and are in a good agreement with physical dimensions. The both positions of the sources (0 mm and 10 mm) were correctly identified by the reconstruction algorithm (−0.25 mm and 10.27 mm). The total light intensity in air and Intralipid differed by less than 8% - intensity in air 9.6×10^9 photon/sec versus 8.8×10^9 photons/sec in Intralipid.

We find that data reconstruction techniques successfully used the light transport approach and semi-automated image processing to provide a 3D model of the lung tumors and decrease the time of volumetric imaging and quantitative assessment.

The average processing time (reconstruction times to convergence) per image update for surface reconstruction was about 350 msec (Linux workstation) and for the deconvolution iterative procedure for BLI source reconstruction was a few minutes: depending on number of voxels, iterations and amount of experimental data from CCDs and upon the discrete Fast Fourier Transform algorithm optimization.

In order to optimize and automate bioluminescence image processing procedure, Linux based software with multi-modality image handling environment was developed and evaluated. This environment supports multiple user-derived data formats and object-oriented serial graphical applications. To support diverse imaging data, the TR3 file format and associated software was designed [31]. TR3 format is simple and incorporates the ability to add new modalities and data sources.

Conclusion

The data obtained on single/dual light sources and lung mouse tumors images demonstrate the utility of the iterative image reconstruction algorithms and semi-automated approach for BLI image processing procedure.

The reconstruction of three-dimensionally distributed light sources is the main goal of tomographic BLI studies. There are many difficulties in obtaining information about the irregular surfaces or optical properties of heterogeneous objects in the interior of an animal's body or phantom. In this case, BLI reconstruction could be strengthened by incorporation of a priori information on the animal anatomy and tissue properties to overcome this extremely difficult inverse problem. We have shown that while the inverse source problem for BLI tomography is far from completely solved, we have identified a useful fast reconstruction approach that can localize single sources as depth in small animal models. The iterative reconstruction method based on diffusion equation can be also useful for recently provided 3D tumor shape [35] and location [36] reconstruction from 2D bioluminescence images.

3D image reconstruction for turbid media can be difficult or even impossible if BLI information is missing due to incomplete data acquisition or sources with limited light propagation [32]. It may be possible to use neural networks method with input dependent weights (Self-Organizing Map) [33] combined with the EM algorithm for missing data [34] or Monte Carlo method [18] to overcome excessive attenuation and obtain the appropriate tool for BLI reconstruction procedure. The sensitivity function analysis of the optical heterogeneity, source separation distance, and source strength will be an important step in assessing the quantitative power of this 3D imaging method. Incorporation all of these above in consideration will further improves our results at the expense of imaging time.

The improved semi-automated BLI image processing method described above provides a solid base for further preclinical imaging studies: characteristics of tumor growth, identify metastases and potentially determine the effectiveness of cancer treatment.

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References

1. Wang GE, Cong W, Durairaj K, Qian X, Shen H, et al. In vivo mouse studies with bioluminescence tomography. *Opt Express*. 2006; 14:7801–7809. [PubMed: 19529149]
2. Paroo Z, Bollinger RA, Braasch DA, Richer E, Corey DR, et al. Validating bioluminescence imaging as a high-throughput, quantitative modality for assessing tumor burden. *Mol Imaging*. 2004; 3:117–124. [PubMed: 15296676]
3. Richer E, Slavine NV, Lewis MA, Tsyganov E, Gellert GC, et al. Three dimensional light emission tomography using multiple rotating CCD cameras. *Proceedings of Third Annual Meeting of the Society for Molecular Imaging*, St. Louis. 2004; 3:229–230.
4. Dikmen ZG, Gellert G, Dogan P, Mason R, Antich P, et al. A New diagnostic system in cancer research: Bioluminescent Imaging. *Turk J Med Sci*. 2005; 35:65–70.
5. Lewis MA, Richer E, Slavine NV, Kodibagkar VD, Soesbe TC, et al. A multi-camera system for bioluminescence tomography in preclinical oncology research. *Diagnostics*. 2013; 3:325–343.
6. Slavine, NV.; McColl, RW.; Richer, E.; Mason, R.; Antich, P. An automated 3D image-processing strategy for small animal bioluminescence cancer studies. *Proceedings of 5th Biotechnology and Bioinformatics Symposium (BIOT)*; Arlington, Texas, USA. 2008. p. 97-102.
7. Slavine NV, Lewis MA, Richer E, Antich PP. Iterative reconstruction method for light emitting sources based on diffusion equation. *Med Phys*. 2006; 33:61–68. [PubMed: 16485410]
8. Jiang M, Wang G. Development of blind image deconvolution and its applications. *J Xray Sci Technol*. 2003; 11:13–19. [PubMed: 22388094]
9. Shepp LA, Vardi Y. Maximum Likelihood reconstruction for emission tomography. *IEEE Trans Med Imaging*. 1992; 1:113–122. [PubMed: 18238264]
10. Ishimaru, A. Wave propagation and scattering in random media. 1st edtn. New York, USA: Wiley-IEEE Press; 1977.
11. Wang G, Li Y, Jiang M. Uniqueness theorems in bioluminescence tomography. *Med Phys*. 2004; 31:2289–2299. [PubMed: 15377096]
12. Dehghani H, Davis SC, Jiang S, Pogue BW, Paulsen KD, et al. Spectrally resolved bioluminescence optical tomography. *Optics Letters*. 2006; 31:365–367. [PubMed: 16480210]
13. Cong W, Kumar D, Liu Y, Cong A, Wang GE. A practical method to determine the light source distribution in bioluminescent imaging. *Proceedings of the SPIE*. 2004; 5535:679–686.
14. Kuo, C.; Xu, H.; Rice, B. Improved techniques in diffuse luminescent tomography on whole animal meshes; *Proceedings of the Society of Molecular Imaging, 5th Annual Meeting*; Hawaii, USA. 2006.
15. Gu X, Zhang Q, Larcom L, Jiang H. Three-dimensional bioluminescence tomography with model-based reconstruction. *Optics Express*. 2004; 12:3996–4000. [PubMed: 19483937]
16. Han W, Cong W, Wang GE. Mathematical theory and numerical analysis of bioluminescence tomography. *Inverse Probl*. 2006; 22:1659–1675.
17. Jiang M, Wang GE. Image reconstruction for bioluminescence tomography. *Developments in X-ray tomography IV*. *Proceedings of International Society for optics and photonics*. 2004; 5535
18. Kumar D, Cong WX, Wang G. Monte Carlo method for bioluminescence tomography. *Indian J Exp Biol*. 2007; 45:58–63. [PubMed: 17249328]
19. Hielscher AH, Jacques SL, Wang L, Tittel FK. The influence of boundary conditions on the accuracy of diffusion theory in time-resolved reflectance spectroscopy of biological tissue. *Phys Med Biol*. 1995; 40:1957–1975. [PubMed: 8587943]
20. Cong W, Wang LV, Wang G. Formulation of photon diffusion from spherical bioluminescent sources in an infinite homogeneous medium. *Biomed Eng Online*. 2004; 3:12. [PubMed: 15125780]
21. Cubeddu R, Pifferi A, Taroni P, Torricelli A, Valentini G. A solid tissue phantom for photon migration studies. *Phys Med Biol*. 1997; 42:1971–1979. [PubMed: 9364593]
22. Larossa, R. *Methods in Molecular Biology*. USA: Humana Press Inc.; 1998. *Bioluminescence Methods and Protocols*.

23. Troy T, Jekic-McMullen D, Sambucetti L, Rice B. Quantitative comparison of the sensitivity of detection of fluorescent and bioluminescent reporters in animal models. *Mol Imaging*. 2004; 3:9–23. [PubMed: 15142408]
24. Slavine, NV.; Soesbe, T.; Richer, E.; Lewis, M.; Antich, PP. Construction, Calibration and Evaluation of a tissue phantom with reproducible optical properties for investigations in Light Emission Tomography. Vol. 1. Dallas, USA: Proceedings of the IEEE Workshop on Engineering in Medicine and Biology; 2007. p. 122-125.
25. Lun, L.; Zhu, T. Finite element modeling of light fluence distribution in prostate during photodynamic therapy; Proceedings of the COMSOL Multiphysics Conf; Boston, USA. 2005. p. 169-176.
26. Prout DL, Silverman RW, Chatziioannou A. Detector concept for OPET- A combined PET and optical imaging system. *IEEE Trans on Nucl Sci*. 2004; 51:752–756.
27. Slavine, NV.; McColl, RW. Semi-automated image-processing approach and software for small-animal Bioluminescence Cancer Studies. Vol. 1. USA: University of Texas Southwestern Medical Center at Dallas; 2011. p. 7
28. McColl, R.; Faber, T.; Opperman, R.; Reshock, R. TR3-A Simple multimodality image handling environment; Proceedings of the Tenth Conference on Computer Applications to Assist Radiology; Carlsbad, CA, USA. 1990. p. 457-462.
29. Couet, O. Physics Analysis Workstation, Reference Manual. Geneva, Switzerland: CERN; 1997.
30. Bran, R.; Rademakers, F.; Canal, P.; Antceva, PI.; Buskalic, D. User Guide 5.20. Geneva, Switzerland: CERN; 2008. ROOT an Object-oriented data analysis framework.
31. McColl, RW. TR3 software a technical report, Department of Radiology. USA: UT Southwestern Medical Center at Dallas; 2013. p. 1-21.
32. Wang GE, Cong W, Shen H, Qian X, Henry M, et al. Overview of bioluminescence tomography—A new molecular imaging modality. *Front Bioscience*. 2008; 2:1281–1293.
33. de Freitas JFG, Niranjana M, Gee AH. Dynamic learning with the em algorithm for neural networks. *J VLSI Signal Process Syst Signal Image Video Technol*. 2000; 26:119–131.
34. McLachlan, GJ.; Krishnan, T. The EM algorithm and extensions. New York, USA: John Wiley and Sons Inc.; 1997.
35. Huang, J.; Huang, X.; Metaxas, D.; Banerjee, D. 3D tumor shape reconstruction from 2D bioluminescence images; Proceedings of the IEEE Intern Sym Biomed Imaging; Arlington, Virginia, USA. 2006. p. 18-21.
36. Huang X, Metaxas D, Menon L, Mayer-Kuckuk P, Bertino J, et al. Recovering 3D tumor location from 2D bioluminescence images and registration with CT images. Fourth Annual Meeting of the Society for Molecular Imaging. 2005:1–9.

Figure 1. FWHM as a function of source depth calculated for different concentration of Intralipid (IL) by diffusion approximation with extrapolated boundary condition. The BLI spatial resolution is better for depths ~1–1.5 cm. This depth was considered for small animal BLI sources reconstruction.

Figure 2.
Light flux (arrows) detected by CCD camera on the surface of imaged animal with tumor (a) and nude mouse was implanted with 106A549-LUC human lung tumor cells on the detector face for the zero (b) and 90 (c) CCD rotation angles.

Figure 3. Digital images of mouse surface from reflected light (a) and BLI signals from mouse lungs (b) on the detector face for the zero CCD rotation angle. Two sets of imaging data for mouse surface and BLI sources are automatically involved for co-registration.

Figure 4.

2D histogram shows the maximum intensity for 1% Intralipid medium phantoms with different source positions on x-axis in inches (a), 3D-reconstructed image of 1% Intralipid-filled phantom displaced 10.16 mm (0.4 in) from the center of system coordinate by x-axis (b) and 3D surface reconstruction result for living mouse (skin) from reflected light (c). The voxel size is 0.5 mm^3 .

Figure 5.

The Flowchart description of 3D iterative BLI source reconstruction method based on the diffusion equation and finite element analysis.

Figure 6.

The Flowchart description of 3D iterative BLI source reconstruction method based on the diffusion equation and finite element analysis.

Figure 7.
The TR3 file layout.

Figure 8.

Coronal (a), Sagittal (b) and Transversal (c) slices through the 3D iterative reconstruction of the nude mouse with 106A549-LUC human lung tumor cells injected. Reconstructed lungs image co-registers with external light surface image reconstruction (compare with Figure 2 and 3). The voxel size is 0.5 mm^3 .

Figure 9.
Coronal slices through the 3D iterative reconstruction showing A549-LUC tumor in mouse lungs. The sections are 0.5 mm thick, 1 mm apart, and start dorsally (top left corner) ending ventrally (bottom right). The voxel size is 0.5 mm^3 .

Figure 10.

Correlation of the voxel populations (top) and surface radiance (bottom) with number of projections included in 3D iterative image reconstruction procedure: lungs reconstruction from 8 views (a), 12 views (b) and 20 views (c). The voxel size is 0.5 mm^3 .

Figure 11. 2D histogram shows the maximum intensity for 1% Intralipid medium phantom with dual source positions on x-axis with 10.16 mm apart (a) and 3D iterative construction result for the same dual source phantom (b). The voxel size is 0.5 mm³.