Monte Carlo method for bioluminescence tomography

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Bioluminescence imaging plays an important role in the areas of cancer biology, cell biology, gene therapy, and so on. The 2D planar bioluminescent imaging has been transformed into a 3D framework by bioluminescence tomography (BLT) that enables bioluminescent source reconstruction in a mouse using a modality fusion approach. To solve this BLT problem, a geometrical model of the mouse is usually built from a CT/micro-CT/micro-MRI scan, which facilitates the assignment of optical parameters to various anatomical regions in the model. This optical model is then used to facilitate BLT. The forward model is based on Monte Carlo simulation to calculate the diffuse light flux on the surface of the mouse. The forward model data are used to define the imaging system and perform the BLT reconstruction. In this paper, we report the reconstruction of sources inside a heterogeneous highly scattering physical phantom to demonstrate the feasibility of this Monte Carlo based BLT method.

Keywords: Absorption, Bioluminescence tomography (BLT), CT/micro-CT, Monte Carlo simulation.

The phenomenon of living creatures producing light is known as bioluminescence. It results from the conversion of chemical energy to bioluminescent photons. As the charged coupled device (CCD) technology leads to ultra low light detection, bio- and chemi-luminescence has been exploited to sense chemicals present well below nanomolar levels in biological samples. The enzymes and other proteins associated with bioluminescence have been developed as markers or reporters of other biochemical processes in biomedical research. Small animal bioluminescence imaging systems help perform noninvasive assays to monitor the progression of diseases and biological processes. Thus, bioluminescence imaging is a unique tool for investigating numerous physiological and pathological events at cellular and molecular levels. Since bioluminescence does not have an external source, these systems generally produce higher signal to noise ratios than fluorescence imaging systems.

Bioluminescence tomography (BLT) was invented at University of Iowa, and is used to find the source location and strength inside the animal. The BLT is an extremely ill-posed problem, since photons undergo multiple scattering. The solution non-uniqueness behavior can be handled using a multi-modality approach, which utilizes a blending procedure and requires prior knowledge gained by the other imaging tools and in the application domain. For example, the animal can be scanned by X-ray CT, MRI, and/or optical diffuse tomography (DOT). The resultant images are then segmented, which enables the assignment of optical parameters to various regions. The segmented mouse helps create a geometrical model. The optical parameter labeled geometric model is a mouse optical model. The created optical model serves as the input into the BLT program. The numerical computation can be based on the diffusion theory or the Monte Carlo (MC) method, which can offer solutions for flux distributions inside the animal and on its surface. The inverse computation determines the bioluminescent source flux and the source distribution (especially, the location and energy). These techniques are model based and employ a forward model that provides prediction of the detector readings. The predicted detector readings can be compared with experimental data using an appropriately defined objective function. The true source(s) can be estimated iteratively.

Since diffusion approximation fails in various circumstances such as highly absorbing and weakly scattering regions, inhomogeneous conditions, etc., the Monte Carlo method is preferred to build light propagation models for the calculation of flux. The MC models show an excellent agreement with radiative transfer calculations. In this paper, we describe a BLT method that avails the data bank to

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reconstruct the sources. The data bank is generated using the Monte-Carlo method that facilitates to build the optical model for light propagation. A physical phantom luminescence experiment with embedded light sources, and relevant issues are also reported.

**Methodology**

**Monte Carlo Method**

The Monte Carlo (MC) method solves various mathematical and physical problems by simulation of random quantities. Modeling the path of a photon through tissue is a typical random walk problem. Photon interaction with matter via scattering and absorption is stochastic in nature and can be described using the Monte Carlo method by appropriately weighting absorption and scattering events. Diffuse emission of light from the animal/phantom surface due to embedded bioluminescent sources has been effectively simulated using the MC method. The TracePro (Lambda Research Corporation, Littleton, MA) is based on the MC method, which is commercial software, and used to simulate photon propagation in tissue medium.

The TracePro is a ray-tracing program and useful for optical analysis of solid models. This technique allows us to launch rays into a model without making any specific assumptions on the model geometry. At each intersection, individual rays are subject to absorption, reflection, refraction, diffraction and scatter. As the rays propagate along different paths throughout the solid model, TracePro keeps track of the optical flux associated with each ray.

The first step in TracePro is to build or import a geometrical model representing the system to analyze. The second step is to define properties of the different components in the solid model. Material properties representing desired attributes such as reflection, refraction, absorption and scattering are defined for the geometrical model. Once the properties are defined, the properties can be applied onto the appropriate objects in the model. The next step is ray tracing. A variety of analysis options are available to determine the location, extent and distribution of the resultant flux.

TracePro is designed to provide an easy-to-use graphical interface for viewing the model; adding solid objects to it; and applying material properties, surface properties and source properties. The generated solid model that was used for ray tracing is shown in Fig. 1. TracePro made use of variance reduction techniques to reduce the number of rays required to obtain a reliable result. In MC ray tracing, scattering was treated as a random process, and the ray tracing was done in two ways, i.e., ray splitting ON and OFF. Ray splitting means that rays are split into different components, and each component carries a fraction of the incident flux. For example, when a ray meets a surface, TracePro creates up to four rays (specular R and T, scattered R and T) leaving the surface. The sum of the flux of the four rays is equal to the flux of the incident ray less any absorption at the surface. Ray trace is controlled through the flux threshold and the number of starting rays. The convergence is monitored by reviewing the lost flux column of the flux report.

**Bioluminescent tomography (BLT)**

The real mouse anatomy is very complex. In general, a finite element based mouse model is built using an X-ray CT/PET/MRI scan. The optical parameter assigned mouse model was used to calculate light flux by diffusion theory. In our approach, the commercial software TracePro was used to build the animal/phantom model for numerical calculation of flux and reconstruction of a bioluminescent source distribution. In doing so, the M detectors were distributed on the body surface of the mouse to receive the photon fluence rate. To overcome the ill-posed nature of BLT, a permissible source region $\Omega_p$ was specified based in reference to mouse bioluminescence images, and divided into N

Fig. 1—Heterogeneous phantom model generated by TracePro to calculate the escaping diffuse emission signal on the peripheral surrounding surface of the phantom. (B- bone, H- heart, L-lungs, M- muscle, S1 and S2- sources).
volume elements \( \{e_1, e_2, \cdots, e_N\} \). These elements were the potential light source positions, and their volume size was related to reconstruction resolution of the light source. We can compute the photon fluence rate \( \{a_{iN}, a_{2i}, \cdots, a_{Ni}\}^T \) at all the detectors on the surface of the mouse model from a unit density source on every source element \( \{i = 1, 2, \cdots, N\} \). After obtaining such the contributions to the detectors, we can construct a matrix \( A \), which reflects the photon fluence rate at every detector on the surface of the model from every unit elemental source. It can then be applied to the bioluminescence source reconstruction from measured bioluminescent data on the detectors.

In a BLT experiment, the measured data \( b \) on the mouse surface is captured with a CCD camera. The bioluminescence imaging process can be formulated as a linear system,

\[
AX = b
\]  

where \( X = \{x_1, x_2, \cdots, x_N\}^T \) and \( x_i \) is the photon density on the element \( e_i \).

Generally, due to the BLT ill-posedness it is not practical to solve the linear system (5) directly, an effective approach is to find a regularized solution by minimizing the following objective function:

\[
\min_{0 \leq X \leq U} \|AX - b\|_W^2 
\]

where \( W \) is a weighting matrix and norm \( \|X\|^2 = X^TWX \), \( U \) denotes an upper bound on \( X \) to be physically meaningful. A penalty term may be added to (6) as well. The flowchart for the source reconstruction is shown in Fig. 2.

**Results**

**Heterogeneous phantom luminescence experiment**

A cylindrical heterogeneous mouse chest phantom of 30 mm height and 30 mm diameter was designed and fabricated. It consisted of four different materials, high-density polyethylene, nylon 6/6, delrin and polypropylene (McMaster-Carr Supply Company, Chicago, IL, US) to represent muscle (M), lungs (L), heart (H) and bone (B), respectively (Fig. 3). Two small holes of 0.86 mm diameter and 2 mm height were drilled in the phantom with their centers at (1.5, -9.0, 15.0) and, (-1.5, -9.0, 15.0) in the left lung region of the phantom, respectively. Two red luminescent liquid filled catheter tubes of 1.9 mm height and 0.90 mm diameter were placed inside the two small holes, respectively. The total power of the red luminescent liquid filled polythene tubes was measured with the CCD camera. They were 105.1 nano-Watts (86.99 nano-Watts/mm²) and 97.4 nano-Watts.
Watts (80.62 nano-Watts/mm³), respectively. The calculated absorption coefficient (μₐ) and reduced scattering coefficient (μ′ₕ) at 650nm of these different materials used in the mouse chest phantom are given in Table 1⁹,¹².

The heterogeneous mouse chest phantom containing the two light sources was placed on a sample holder in front of the CCD camera. The experimental setup was placed in a totally dark environment. At every 15° rotation of the holder, the flux density was recorded with the CCD camera on the cylindrical surface of the phantom and captured images along four radial directions separated by 90 degrees are shown in Fig. 4.

During each data acquisition, one luminescent view was taken by exposing the camera for 60 seconds. Furthermore, the recorded pixel gray levels of the luminescent views were transformed into corresponding light units according to the calibration relationship that we already established ¹¹-¹².

**Light source reconstruction**

To simulate the photon propagation in the phantom, a geometrical model of 30 mm diameter and 30 mm height with various compartments as in the physical phantom was established using TracePro. The optical properties of every element were assigned in reference to the optical parameters reported. The geometry of the embedded source inside the heterogeneous phantom was a cylinder of 0.5 mm radius and 2 mm height. On the surface of the geometric model corresponding to a middle section of

<table>
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<th>Optical Parameter (mm⁻¹)</th>
<th>Muscle</th>
<th>Lung</th>
<th>Heart</th>
<th>Bone</th>
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<tr>
<td>μₐ</td>
<td>0.007</td>
<td>0.01</td>
<td>0.011</td>
<td>0.001</td>
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<tr>
<td>μ′ₕ</td>
<td>0.631</td>
<td>0.600</td>
<td>1.096</td>
<td>0.060</td>
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**Fig. 3**—Mouse Chest phantom. (a) A heterogeneous mouse phantom consisting of bone (B), heart (H), lungs (L), and muscle (M); (b) a middle cross-section through two hollow cylinders for hosting luminescent sources in one lung.

**Fig. 4**—Luminescent views of the side surface covering the cylindrical phantom taken using a CCD camera in four directions 90 degrees apart. (a) Front view, (b) right view, (c) back view, and (d) left view.
the physical phantom, 16 circles, separated by about 1.75 mm, were selected, along each of which 64 detection locations were uniformly distributed. The measured photon density at each detector location was obtained from the CCD luminescent image using the calibration formula. The computed photon density at the corresponding detection point for possible unit source locations was obtained using TracePro. TracePro took about 8 hr to generate the surface flux datasets on an AMD Athlon™ MP 2800+2.13 GHz computer with 1 GB RAM for a point source at (0, -8.5, 15) in the cylindrical phantom. Then, the point source was moved to various locations within the permissible region, and the corresponding flux data sets were obtained. These data formed a data bank as the imaging system matrix for source reconstruction.

Then, the reconstruction method was applied to reconstruct the light source distribution in the heterogeneous phantom. The reconstructed results correctly revealed that there were two strong light sources in the phantom located at (2.5, -9.0, 15.0) with flux density 63.65 nano-Watts/mm³ and at (-2.5, -9.0, 15.0) with 63.65 nano-Watts/mm³, respectively. Note that the volumes of the reconstructed sources are different from the actual source volumes.

The differences between the reconstructed and real source positions were less than 1 mm for the two sources, respectively. The relative errors in the source strength were about 27 and 21%, respectively. The computed surface photon density measures based on the reconstructed light sources were in good agreement with the experimental counterparts, with the average relative error being about 20% as shown in Fig. 5.

Discussion and conclusion

A reconstruction procedure that uses MC method to identify a 3D bioluminescent source distribution by incorporating a priori knowledge has been developed. The use of the permissible source region is to enhance numerical stability and efficiency. More importantly, the data in physical phantom experiment were produced by real physical sources, and they are effectively free from the well-known “inverse crime”. The finite element method (FEM) based practical reconstruction method and a Born-type approximation method for BLT using diffusion...
approximation (DA) for light propagation may fail in these models, because propagating photons encounter highly absorbing region, weakly scattering and weakly absorbing regions and voids. On the other hand, the MC method can be used to preserve the effects of the various structures unfavorable to the DA and can be used to compare with the results obtained using different methods.

The shortcoming to use MC simulation is that it needs around 8 hr to track $10^6$ photons from the sources. Changing the source position to generate a data bank requires many such the trials that consume much more time. Once the data are numerically generated, the reconstruction algorithm that we have implemented can determine the source distribution. Our numerical simulation experiment and reconstruction algorithm for the reconstruction of bioluminescent point sources have indicated that the MC based procedure can be used in principle but it is not very efficient.

The reconstruction algorithm will be improved to estimate a larger source and multiple sources inside the medium. Dependency of the heavy computational overhead of the method obstructs the quick reconstruction process. However, the MC method is versatile and can be applied to a medium of any kind. One way to overcome the computational difficulty may be to use a hybrid MC\textsuperscript{23}. Another alternative is the PC cluster technology that we are developing.

In conclusion, we have developed a MC simulation based BLT method for reconstruction of a bioluminescent source distribution inside the medium, and demonstrated its feasibility in a heterogeneous physical phantom experiment. Further research directions include improvement of the algorithm and acceleration of the simulation. Mouse studies will be performed in the future as well.

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References