Recent Development in Bioluminescence Tomography

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Abstract: Bioluminescence tomography (BLT) is a new molecular imaging tool. Using a modality fusion approach, we built the first BLT prototype that combines BLI data and micro-CT and micro-MRI images for proof of concept, established a theoretical framework for BLT, and reported encouraging preliminary results. In this overview, we highlight our key results and discuss further directions.

Keywords: Bioluminescence imaging, Bioluminescence tomography, Finite Element Method, Monte Carlo approach, Molecular optical simulation environment, Multi-spectral signal separation.

INTRODUCTION

Bioluminescence tomography (BLT) is a rapidly developing area for molecular imaging [1-15]. The introduction of BLT [1] relative to planar bioluminescent imaging (BLI) [16] can be in a substantial sense compared to the development of X-ray CT based on radiography. Without BLT, bioluminescent imaging is primarily qualitative. With BLT, quantitative and localized analyses on a bioluminescent source distribution become feasible inside a living mouse, which reveal molecular and cellular signatures [17].

In the March 2005 issue of the Molecular Imaging Outlook (http://www.diagnosticimaging.com/molecularimagingoutlook/2005mar/02.jhtml), Contag mentioned that BLI arose out of the frustration with sampling limitations of the standard assay techniques. Since the genes are duplicated with the cell division, BLI is more sensitive than other techniques such as nuclear imaging, in which the radioactive signal will be reduced with the cell division. In the same article, Piwnica-Worms emphasized that BLI could be applied to study disease, in small animal models.

In the following two sections, we will describe the BLT system architecture and the theory the BLT is based on.

SYSTEMS

Our first BLT system uses a CCD camera (Princeton Instruments VA 1300B, Roper Scientific, Trenton, NJ) [1, 9]. To collect bioluminescent signals around a mouse, a stage is vertically rotated under computer control and horizontally moved by a transport to match the focal length of the camera. A holder maintains the position of the mouse, and clamps into the stage. A light-tight enclosure has an entry hatch to accommodate wires and minimize light leaking. Typically for a given orientation, two images are obtained with and without light. Marks are placed on the skin for registration with a Micro-CT (MRI or another) volume of the same mouse.

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The second BLT system is being developed with major functional enhancements, Fig. **1**. In Fig. **1**, the multi-view mirror module includes a mounting plate, four mirror stages, and four mirrors. Without the use of the multi-spectral components, the mirror-based system can be a standalone multi-view system. To enhance the multi-view system into a multi-view multi-spectral system, we employ dichroic mirrors with interleaving filter bands to separate bioluminescent light into different spectral channels of interest. In this system, two different filters are placed on the side surface of the mouse holder.

THEORY

In BLT, target cells encoded by luciferase enzymes are implanted into a living mouse. The luciferase substrate luciferin is then injected into the mouse. The target cells that express the luciferase transgene hence emit bioluminescent photons in about 600nm wavelength in presence of oxygen and ATP. The resultant light intensity is directly correlated to the number of luciferase molecules and the luciferin concentration. The bioluminescence signal covers a red region of the spectrum, permitting a significant penetration depth. Therefore, a sufficiently large number of bioluminescent photons escape the attenuating environment, reach the mouse body surface and are detected using a highly sensitive CCD camera.

Let Ω be the region of mouse body in R^3, q a light source function in Ω, and $u(x,\theta,t)$ the radiance in $\theta \in S^2$ (unit sphere in R^3) at $x \in \Omega$. The radiative transfer equation (RTE) is as follows [18, 19]:

$$\frac{1}{c}\frac{\partial u}{\partial t} + \theta \cdot \nabla_x u + \mu u = \mu_s \iint_{S^2} \eta(\theta \cdot \theta') u(x,\theta',t)\, d\theta' + q,$$

where c denotes the photon speed, $\mu = \mu_a + \mu_s$ with μ_a and μ_s being the absorption and scattering coefficients, and the scattering kernel η satisfying $\iint_{S^2} \eta(\theta \cdot \theta')\, d\theta' = 1$.

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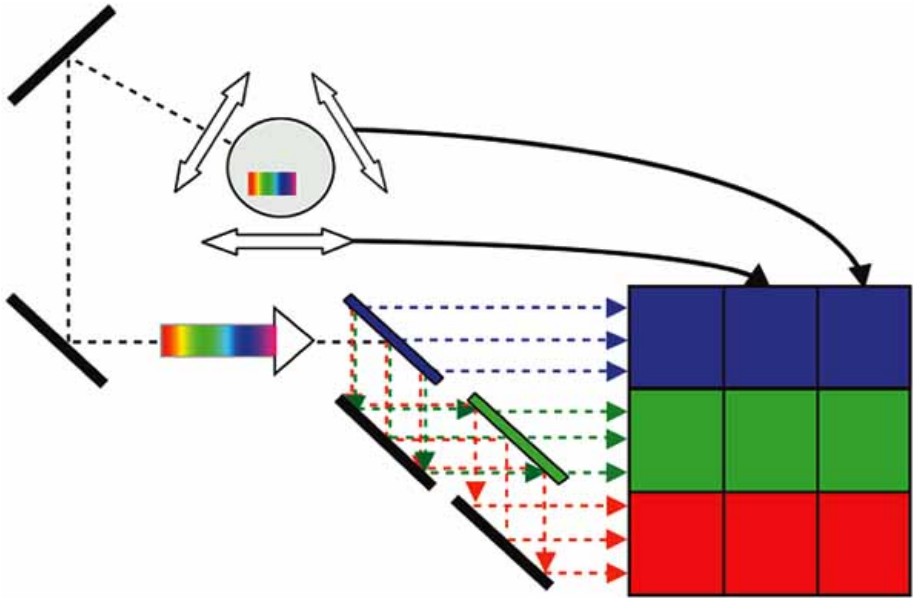

Fig. (1). One of our conceptual designs for a mirror and filter based multi-spectral signal separation (Provisional patent application pending). The system includes a specially designed mouse holder, highly reflective mirrors, dichroic mirros, collimated lens and extra-sensitive CCD camera.

Mathematically, BLT is the source inversion problem that recovers q from optical measurement on the domain boundary Γ (complete or partial $\partial\Omega$), utilizing detailed knowledge on the optical properties of Ω. Note that obtaining the individualized spatially variant optical properties is critical for BLT to work effectively.

Because the RTE is difficult to handle and in the range of ~600nm photon scattering outperforms absorption in a mouse, the following boundary value problem (BVP) is derived from the diffusion approximation:

$$-\quad (D\quad u_0)+\mu_a u_0 = q_0,\ x\quad \Omega,$$

$$u_0(x)+2D\frac{\partial u_0(x)}{\partial \nu} = g^-,\ x\quad \Gamma,$$

where $u_0(x)=\iint_{S^2} u(x,\theta)d\theta$, g^- is the inward flux on Γ (typically, zero), $D=1/[3(\mu_a+\mu_s')]$, $\mu_s=(1-\eta)\mu_s$, $\eta=\iint_{S^2}\theta\ \theta'\eta(\theta\ \theta')d\theta'$. The optical measurement is

$$g(x)=-D(x)\frac{\partial u_0}{\partial \nu}(x),\quad x\quad \Gamma.$$

Then, the BLT problem becomes to find a source q_0 given g^- and g such that

$$-\quad (D\quad u_0)+\mu_a u_0 = q_0,\ x\quad \Omega,$$

$$u_0(x)+2D\frac{\partial u_0(x)}{\partial \nu} = g^-,\ x\quad \Gamma,$$

$$g=-D\frac{\partial u_0(x)}{\partial \nu},\ x\quad \Gamma.$$

Despite that the solution to this inverse problem is not unique in general, we proved that if the source function can be expressed by a linear combination of solid/hollow balls and g is consistent to q_0, the number and positions of the source balls can be accurately identified, and the total energy in each ball can be estimated in terms of a moment preserving property [4, 10].

To overcome the ill-posedness of the inverse problem, we recently reformulated the BLT problem through regularization. For any q_0 $L^2(\Omega)$, the problem

$$\int_\Omega (D\quad u_0\quad v+\mu_a u_0 v)dx =$$

$$\int_\Omega q_0 v dx-\int_{\partial\Omega} g v ds,\quad v\quad H^1(\Omega)$$

has a unique solution $u_0 = u_0(q_0)$ $H^1(\Omega)$. Denote $g_0 = g^- + 2g$. For any $\varepsilon \geq 0$, let $J_\varepsilon(q_0)=\|u_0(q_0)-g_0\|_{L^2(\partial\Omega)}+\varepsilon\|q_0\|_{L^2(\Omega)}$. Suppose that we seek the source function in a closed convex subset Q $L^2(\Omega)$. Then, the reformulated BLT problem is to find p_ε Q such that $p_\varepsilon = \arg\inf_{q0\ Q} J\varepsilon(q_0)$. We have proved that for any $\varepsilon > 0$, the problem has a unique solution p_ε Q, and the solution depends continuously on the data. When $\varepsilon = 0$, for a practical Q, the problem has solutions, and the solution set S_0 is closed and convex. As $\varepsilon \to 0$, $p_\varepsilon \to p_0$, and p_0 S_0 is the minimal L^2 norm solution in S_0.

Then, stable and convergent numerical methods can be developed. For finite element analysis, we introduce a regular family of triangulations $\{T_h\}$ (h: mesh size) of $\bar{\Omega}$.

For each T_h, let $V^h \subset H^1(\Omega)$ be a linear element space. For $q_0 \in L^2(\Omega)$, the problem

$$\int_\Omega (D \nabla u_0^h \cdot \nabla v^h + \mu_a u_0^h v^h) dx = \int_\Omega q_0 v^h dx - \int_{\partial\Omega} g v^h ds, \quad v^h \in V^h$$

has a unique solution $u_0^h = u_0^h(q_0) \in V^h$. We let $Q_1 \subset Q$ be a subset for the approximate source function solution, e.g. Q_1 can be constructed using piecewise constants, and let $J_\varepsilon^h(q_0) = \|u_0^h(q_0) - g_0\|_{L^2(\partial\Omega)} + \varepsilon \|q_0\|_{L^2(\Omega)}$. Then, the discrete problem is to find $p_\varepsilon^h \in Q_1$ such that $p_\varepsilon^h = \arg\inf_{q_0 \in Q_1} J_\varepsilon^h(q_0)$. For $\varepsilon > 0$, we have a unique solution p_ε^h. Error bounds for $\|p_\varepsilon - p_\varepsilon^h\|^2_{L^2(\Omega)}$ and $\|u_0(p_\varepsilon) - u_0^h(p_\varepsilon^h)\|^2_{L^2(\partial\Omega)}$ can be derived, which imply the convergence $p_\varepsilon^h \to p_\varepsilon$ as $h \to 0$, as well as $p_\varepsilon^h \to p_0$ as $\varepsilon, h \to 0$. More details can be found in [26].

INTERACTIVE METHOD

Using the Monte Carlo approach, we developed a Molecular Optical Simulation Environment (MOSE) for studies on bioluminescent imaging [6]. Compared to existing Monte Carlo optical simulation programs, MOSE has three features. First, our object model is more complex. Besides 2D/3D building blocks, CT/MRI images can be input to construct a real anatomy based simulation environment. Second, MOSE is much faster than some popular programs. Third, MOSE is equipped with experimental setting tools and GUI-driven engines for interactive BLT reconstruction. This approach combines the human intervention and computing power. We are working to accelerate the speed of MOSE by using a combination of the Monte Carlo simulation, diffusion approximation, finite element analysis, and parallel computing.

FINITE ELEMENT METHODS

Let us define two boundary operators $\gamma_0[u_0] = u_0|_{\partial\Omega}$ and $\gamma_1[u_0] = D \frac{\partial u_0}{\partial \nu}\Big|_{\partial\Omega}$. Then, we have the Dirichlet-to-Neumann map $N(f) \equiv \gamma_1(w_1)$ such that

$$-\nabla \cdot (D \nabla w_1) + \mu_a w_1 = 0, \quad x \in \Omega,$$
$$\gamma_0(w_1) = f, \quad x \in \partial\Omega.$$

We also define $\Lambda(q_0) \equiv -\gamma_1(w_2)$ such that

$$-\nabla \cdot (D \nabla w_2) + \mu_a w_2 = q_0, \quad x \in \Omega,$$
$$\gamma_0(w_2) = 0, \quad x \in \partial\Omega.$$

We proved that q_0 is a BLT solution if and only if $\Lambda(q_0) = b$, where $b = N(g^-) + 2g$ [4, 7, 10].

To solve this equation, we can seek $p_\varepsilon^{\cdot\cdot}$ [8, 9] or a maximum likelihood solution using an EM-type algorithm (or a penalized EM variant) [7, 10]. More details can be found in [26]:

$$q_0^{(n+1)} = \frac{1}{\Lambda^*(1)} q_0^{(n)} \Lambda^*\left[\frac{b}{\Lambda[q_0^{(n)}]}\right]$$

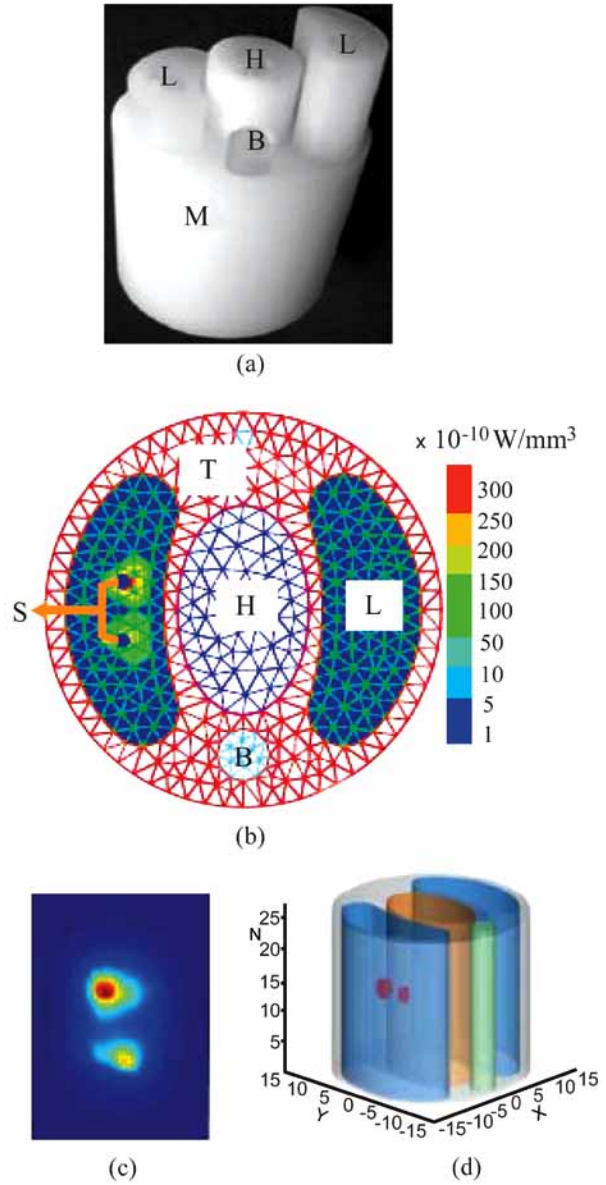

Fig. (2). Validation experiment with a "mouse" (M) phantom: **(a)** The phantom containing bone (B), heart (H), lung (L), and body tissues (T); **(b)** a BLT reconstruction using the regularized finite-element method, with the true positions of two luminescent source (S) shown as blue dots [8, 9]; and corresponding reconstructions using **(c)** an EM method and **(d)** a boundary element method. For details on **(c)** and **(d)**, please see [7, 10, 12, 16].

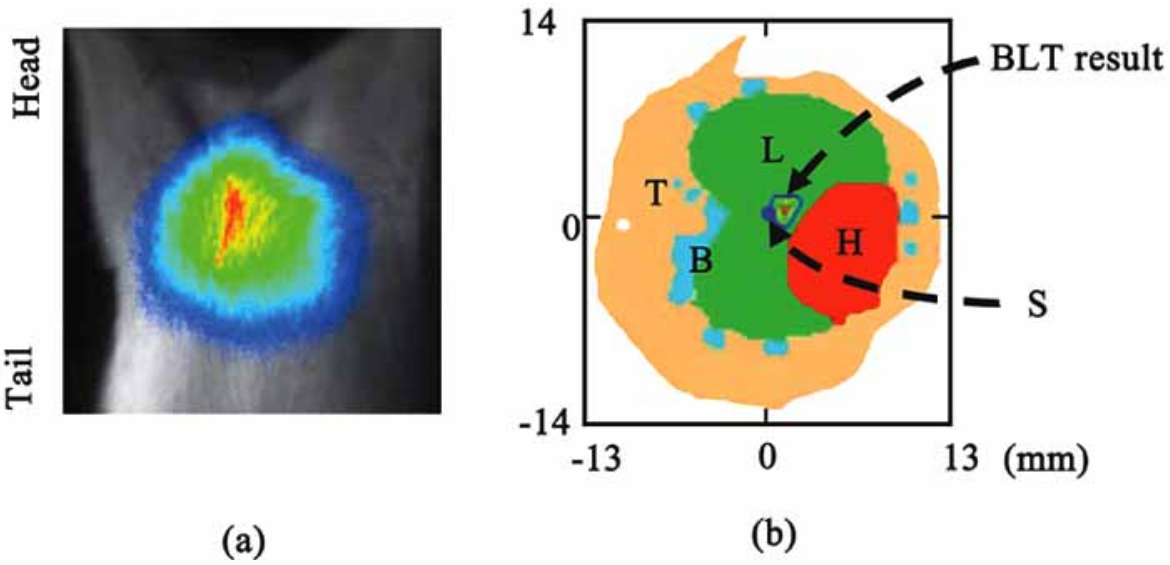

Fig. (3). Validation experiment with a living mouse. **(a)** A fused view showing the luminescent signal due to an embedded mm-sized source; and **(b)** the true and reconstructed sources superimposed on a segmented CT slice.

Moreover, instead of using volumetric elements, we developed a boundary element method for BLT [12]. This methodology primarily uses finite element meshes of structural boundaries. Hence, the computational complexity and reconstruction stability can be improved.

EXPERIMENTAL RESULTS

To demonstrate the BLT feasibility, we performed a series of experiments using a highly scattering heterogeneous physical phantom geometrically similar to the mouse chest. Two small luminescent sources 3mm apart were put into the phantom. We also embedded a small light source in a lung of a living mouse as a most realistic "phantom".

We coded the above BLT algorithms. To avoid the notorious "Inverse Crime", we employed different and adaptive finite elements (shape functions and meshes) in modeling and inversion. The reconstructed results were satisfactory in terms of source position and total energy (Figs. **2** and **3**). The differences between reconstructed and real source positions were about 2mm. The relative errors in source strength were less than 30%.

DISCUSSION AND CONCLUSIONS

In addition to our above work, significant results on or related to BLT were also reported by a number of groups over past several years [16, 17, 20-25]. Given the remarkable difficulties and high importance of this problem, BLT is attracting more and more researchers. Future directions include system optimization, theoretical characterization, algorithm improvement, systematic evaluation, biomedical applications, and so on.

In conclusion, we have gained a basic understanding of the BLT theory and methodology, and obtained pilot data. However, we need more biomedical studies using this imaging mode. Overall, BLT seems a powerful and universal tool, and has a promising future for development of individualized molecular medicine.

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