

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 to combine BLI data and micro-CT 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.

1. INTRODUCTION

Bioluminescence tomography (BLT) is a rapidly developing area for molecular imaging [1-18]. The introduction of BLT [1] relative to planar bioluminescent imaging (BLI) [19] 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 [20].

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. Also, 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. Piwnica-Worms underlined in the same article that BLI could be applied to study almost all diseases in every small animal model.

2. 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 light on and off. Marks are placed on the mouse skin for registration with a CT (MRI or another) volume of the same mouse.

Our second BLT system is being developed with major functional enhancements, including components for diffuse optical tomography (DOT), computational optical biopsy (COB) [11], mirror and filter based multi-spectral signal separation (Fig. 1), and so on. The COB system consists of a source fiber, a detector fiber, a position tracking device, a laser light source, a photon counting detector, and a computer. COB is capable of sensing the tissue properties and/or the source parameters *in vivo*, and can be used alone or in combination with BLT.

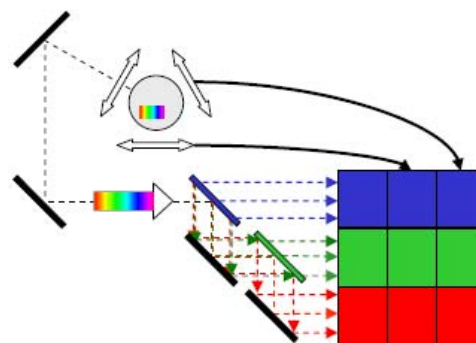


Fig. 1. One of our conceptual designs for a mirror and filter based multi-spectral signal separation (Provisional patent application pending).

3. THEORY

Let Ω be a domain in R^3 , q a light source function in Ω , and $u(x, \theta, t)$ the radiance in $\theta \in S^2$ at $x \in \Omega$. The radiative transfer equation (RTE) is as follows [21, 22]:

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

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 $\iint_{S^2} \eta(\theta \cdot \theta') d\theta' = 1$.

Mathematically, BLT is the source inversion problem that is to recover 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, we use the diffusion approximation to have the following boundary value problem (BVP):

$$\begin{aligned} -\nabla \cdot (D\nabla u_0) + \mu_a u_0 &= q_0, \quad x \in \Omega, \\ u_0(x) + 2D \frac{\partial u_0(x)}{\partial \nu} &= g^-, \quad x \in \Gamma, \end{aligned}$$

where $u_0 = \iint_{S^2} u(x, \theta, t) d\theta$, g^- is the inward flux on Γ (typically, zero), $D = 1/[3(\mu_a + \mu'_s)]$, $\mu'_s = (1 - \bar{\eta})\mu_s$, $\bar{\eta} = \iint_{S^2} \theta \cdot \theta' \eta(\theta \cdot \theta') d\theta'$. The outward measurement is

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

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

$$\begin{aligned} -\nabla \cdot (D\nabla u_0) + \mu_a u_0 &= q_0, \quad x \in \Omega, \\ u_0(x) + 2D \frac{\partial u_0(x)}{\partial \nu} &= g^-, \quad x \in \Gamma, \\ g &= -D \frac{\partial u_0(x)}{\partial \nu}, \quad x \in \Gamma. \end{aligned}$$

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 [17]. For any $q_0 \in L^2(\Omega)$, the problem

$$\begin{aligned} \int_{\Omega} (D\nabla u_0 \cdot \nabla v + \mu_a u_0 v) dx &= \\ \int_{\Omega} q_0 v dx - \int_{\partial\Omega} g v ds, \quad \forall v \in H^1(\Omega) \end{aligned}$$

has a unique solution $u_0 = u_0(q_0) \in H^1(\Omega)$. Denote $g_0 = g^- + 2g$. For any $\varepsilon \geq 0$, let

$J_\varepsilon(q_0) = \|u_0(q_0) - g_0\|_{L^2(\partial\Omega)}^2 + \varepsilon \|q_0\|_{L^2(\Omega)}^2$. Suppose that we seek the source function in a closed convex subset $Q \subset L^2(\Omega)$. Then, the reformulated BLT problem is to find $p_\varepsilon \in Q$ such that $p_\varepsilon = \inf_{q_0 \in Q} J_\varepsilon(q_0)$. We have proved that for any $\varepsilon > 0$, the problem has a unique solution $p_\varepsilon \in Q$, 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 $\varepsilon \rightarrow 0$, $p_\varepsilon \rightarrow p_0$, and $p_0 \in S_0$ 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

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

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 $J_\varepsilon^h(q_0) = \|u_0^h(q_0) - g_0\|_{L^2(\partial\Omega)}^2 + \varepsilon \|q_0\|_{L^2(\Omega)}^2$. Then, the discrete problem is to find $p_\varepsilon^h \in Q_1$ such that $p_\varepsilon^h = \inf_{q_0 \in Q_1} J_\varepsilon^h(q_0)$. For $\varepsilon > 0$, we have a unique solution p_ε^h . Error bounds for $\|p_\varepsilon - p_\varepsilon^h\|_{L^2(\Omega)}^2$ and $\|u_0(p_\varepsilon) - u_0^h(p_\varepsilon^h)\|_{L^2(\partial\Omega)}^2$ can be derived, which imply the convergence $p_\varepsilon^h \rightarrow p_\varepsilon$ as $h \rightarrow 0$, as well as $p_\varepsilon^h \rightarrow p_\varepsilon$ as $\varepsilon, h \rightarrow 0$.

4. INTERACTIVE METHOD

Using the Monte Carlo approach, we developed a Molecular Optical Simulation Environment (MOSE) for studies on bioluminescent imaging [6, 15]. 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, our simulation 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 the computing power. We are working to accelerate the MOSE speed by a combination of the Monte Carlo simulation, diffusion approximation, finite element analysis, and parallel computing.

5. 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

$$\begin{aligned} -\nabla \cdot (D\nabla w_1) + \mu_a w_1 &= 0, \quad x \in \Omega, \\ \gamma_0[w_1] &= f, \quad x \in \partial\Omega. \end{aligned}$$

We also define $\Lambda[q_0] \equiv -\gamma_1[w_2]$ such that

$$\begin{aligned} -\nabla \cdot (D\nabla w_2) + \mu_a w_2 &= q_0, \quad x \in \Omega, \\ \gamma_0[w_2] &= 0, \quad x \in \partial\Omega. \end{aligned}$$

We proved that q_0 is a BLT solution if and only if

$$\Lambda[q_0] = b,$$

where $b = N[g^- + 2g] + g$ [4, 7, 10].

To solve this equation, we can seek p_ϵ^h [8, 9, 17] or a maximum likelihood solution using an EM-type algorithm (or a penalized EM variant) [7, 10, 16]:

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

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.

6. 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 in 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%.

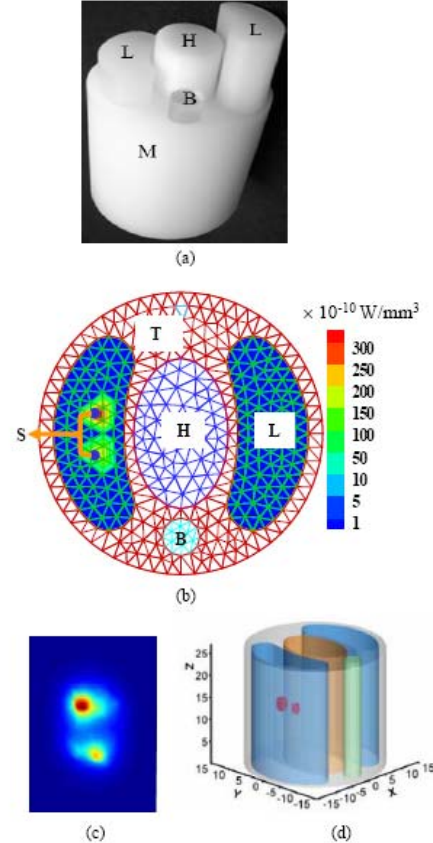


Fig. 2. Validation experiment with a “mouse” (M) phantom: (a) The phantom containing bone (B), heart (H), lung (L), and body tissues (T); (b) a BLT reconstruction using the regularized finite-element method, with the true positions of two luminescent source (S) shown as blue dots [8, 9]; and corresponding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

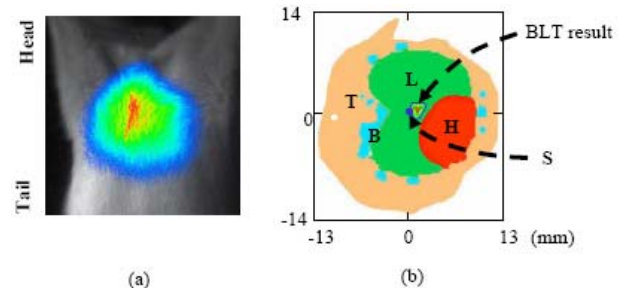


Fig. 3. Validation experiment with a living mouse. (a) A fused view showing the luminescent signal due to an embedded mm-sized source; and (b) the true and reconstructed sources superimposed on a segmented CT slice.

7. DISCUSSIONS AND CONCLUSION

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 [19, 20, 23-29]. Given the remarkable difficulties and high importance of this problem, BLT is attracting more and more researchers. The 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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REFERENCES

1. Wang G, Hoffman EA, McLennan G, Wang LV, Suter M, Meinel J, *et al.*: Development of the first bioluminescent CT scanner: *Radiology* 229(P):566, 2003
2. Wang, G., Hoffman EA, McLennan G: Systems and methods for bioluminescent CT reconstruction. Patent disclosure filed in July 2002; US provisional patent application filed in March 2003; US patent application filed in March 2004
3. Lavery A: First bioluminescent CT prototype is "a new imaging modality". *Clinica World Medical Device & Diagnostic News*, Page 2, PJB Publications Ltd., Richmond, Surrey, UK, December 17, 2003
4. Wang G, Li Y, Jiang M: Uniqueness theorems in bioluminescence tomography. *Med. Phys.* 31:2289-2299, 2004
5. Cong WX, Wang LH, Wang G: Formulation of photon diffusion from spherical bioluminescent sources in an infinite homogeneous medium. *Biomed. Eng. Online* 3:12, 2004
6. Li H, Tian J, Zhu FP, Cong WX, Wang LV, Hoffman EA, Wang G: A mouse optical simulation environment (MOSE) to investigate bioluminescent phenomena with the Monte Carlo method. *Acad. Radiology* 11:1029-1038, 2004
7. Jiang M, Wang G: Image reconstruction for bioluminescence tomography. *Proc. SPIE* 5535:335-351, 2004
8. Cong WX, Kumar D, Liu Y, Cong A, Wang G: A practical method to determine the light source distribution in bioluminescent imaging. *Proc. SPIE* 5535:679-686, 2004
9. Cong WX, Wang G, Kumar D, Liu Y, *et al.*: Practical reconstruction method for bioluminescence tomography. *Opt. Exp.* 13:6756-6771, 2005
10. Jiang M, Li Y, Wang G: Inverse problems in bioluminescence tomography. In *Frontier and Prospect of Contemporary, Higher Education Press (Beijing) and World Scientific, 2005*
11. Li Y, Jiang M, Wang G: Computational optical biopsy. *Biomed. Eng. Online* 4:36, 2005 (Provisional patent filed)
12. Cong WX, Wang G: A boundary integral method for bioluminescence tomography. To appear in *J. Biomed. Opt.*
13. Cong WX, Kumar D, Wang LV, Wang G: A Born-type approximation method for bioluminescence tomography. To appear in *Med. Phys.*
14. Cong A, Wang G: Multi-spectral bioluminescence tomography: Methodology and simulation. To appear in *Int'l J. of Biomed. Imaging*
15. Li H, Tian J, Luo J, Lv YJ, Cong WX, Hoffman EA, Wang G: Development of a molecular optical simulation environment. To appear in *Int'l J. of Pattern Recognition & Artificial Intelligence*
16. Jiang M, Zhou T, Cheng JT, Cong WX, Wang G: An EM-type algorithm for bioluminescence tomography. In review
17. Han WM, Cong WX, Wang G: Theory and numerical analysis of bioluminescence tomography. In review
18. Ntziachristos V, Ripoll VJ, Wang LV, Weissleder R: Looking and listening to light: the evolution of whole-body photonic imaging. *Nature Biotechnology* 23:313-320, 2005
19. Rice W, Cable MD, Nelson MB: *In vivo* imaging of light-emitting probes. *J. Biomed. Opt.* 6:432-440, 2001
20. Contag C, Bachmann MH: Advances in Bioluminescence imaging of gene expression. *Annu. Rev. Biomed. Eng.* 4:235-260, 2002
21. Ishimaru A: Wave propagation and scattering in random media. Oxford, Oxford University Press, 1997
22. Natterer F, Wübbeling F: *Mathematical methods in image reconstruction*. Philadelphia, Society for Industrial and Applied Mathematics, 2001
23. Coguz O, Troy TL, Jekic-MSMullen D, Rice BW: Determination of depth of in-vivo bioluminescent signals using spectral imaging techniques. *Proc. of SPIE* 4967:37-45, 2003
24. Gu X, Zhang Q, Larcom L, Jiang H: Three-dimensional bioluminescence tomography with model-based reconstruction. *Opt. Express* 12:3996-4000, 2004
25. Kuo C, Coquoz O, Troy T, Zwarg D, Rice B: Bioluminescent tomography for in vivo localization and quantification of luminescent sources from a multiple-view imaging system. *Molecular Imaging* 4:370, 2005
26. Alexandrakis G, Rannou FR, Chatziioannou AF: Tomographic bioluminescence imaging by use of a combined optical-PET (OPET) system: a computer simulation feasibility study. *Phys. Med. Biol.* 50:4225-4241, 2005
27. Chaudhari AJ, Darvas F, Bading JR, Moats RA, Conti PS, *et al.*: Hyperspectral and multispectral bioluminescence optical tomography for small animal imaging. *Phys. Med. Biol.* 50: 5421-5441, 2005
28. Slavine NV, Lewis MA, Richer E, Antich PP: Iterative reconstruction method for light emitting sources based on the diffusion equation. *Med. Phys* 33:61-69, 2006
29. Dehghani H, Davis S, Jiang SD, Pogue B, Paulsen K, Patterson M: Spectrally-resolved bioluminescence optical tomography. To appear in *Optics letters*, 2006