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Uniqueness Theorems in Bioluminescence Tomography

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Abstract

Motivated by bioluminescent imaging needs for studies on gene therapy and other applications in the mouse models, a bioluminescence tomography (BLT) system is being developed in the University of Iowa. While the forward imaging model is described by the well-known diffusion equation, the inverse problem is to recover an internal bioluminescent source distribution subject to Cauchy data. The primary goal of this paper is to establish the solution uniqueness for BLT under practical constraints despite the illposedness of the inverse problem in the general case. After a review on the inverse source literature, we demonstrate that in the general case the BLT solution is not unique by constructing the set of all the solutions to this inverse problem. Then, we show the uniqueness of the solution in the case of impulse sources. Finally, we present our main theorem that solid/hollow ball sources can be uniquely determined up to non-radiating sources. For better readability, the exact conditions for and rigorous proofs of the theorems are given in the appendices. Further research directions are also discussed.

Index Terms

Bioluminescence tomography (BLT), diffusion equation, inverse source problem, solution uniqueness.

I. Introduction

Small animals, particularly genetically enineered mice, are of increasing importance for development of the modern medicine. Small animal imaging offers a major opportunity to understand pathophysilogical and therapeutic processes at anatomical, functional, cellular and molecular levels. For example, gene therapy is a recent breakthrough, which promises to cure diseases by modifying gene expression. A key for development of gene therapy is to monitor the gene transfer and evaluate its efficacy in the living mouse model. Traditional biopsy methods are insensitive, invasive, and limited in the extent. To depict the distribution of the administered gene, reporter genes such as those producing luciferase are used to generate light signals within a mouse *in vivo*. These signals can be externally measured by a highly sensitive CCD camera [1]. Such a 2D bioluminescent view can be superimposed onto a

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photograph of the mouse for localization of the reporter gene activity. In addition to its application in gene therapy, this new imaging tool has great potentials in various other biomedical applications as well [2]–[6]. However, the single view based bioluminescent imaging, like the traditional radiography, takes only a 2D image, and is incapable of tomographic reconstruction of internal features of interest, that is, the 3D distribution of the bioluminescent source inside the mouse.

Supported by the National Institutes of Biomedical Imaging and Bioengineering (USA), our team is developing bioluminescence tomography (BLT) as a new modality for molecular imaging, initially of living mice [7], [8]. The novel concept is to collect emitted photons from multiple 3D directions with respect to a living mouse marked by bioluminescent reporter luciferases, and reconstruct an internal bioluminescent source distribution based on *both* the outgoing bioluminescent signals and a pre-scanned tomographic volume, such as a CT/micro-CT volume, of the same mouse.

Traditionally, optical tomography utilizes incoming visible or near infra-red light to probe a scattering object, and reconstructs the distribution of internal optical properties, such as one or both of absorption and scattering coefficients. In contrast to this active imaging mode, BLT reconstructs an internal bioluminescent source distribution, generated by luciferase induced by reporter genes, from external optical measures. In BLT, the complete knowledge on the optical properties of anatomical structures of the mouse is established from an independent tomographic scan, such as a CT/micro-CT scan, by image segmentation and optical property mapping. That is, we can segment the CT/micro-CT image volume into a number of structures, and assign optical properties to each structure using a database of the optical properties compiled for this purpose.

The outline of this paper is as follows. In § II, we present the basics for BLT, including the diffusion approximation for the radiative transfer equation, or Boltzmann equation, and formulate the BLT problem. In § III, we review known theoretical results relevant to the solution uniqueness of BLT. In § IV, we present the main results on the solution uniqueness of BLT. In § V, we discuss related issues and future work, and conclude the paper. Because an accurate presentation of our results requires rather mathematical terms, in the main text we only summarize our results as three theorems in engineer-friendly terms, then we give their complete conditions and proofs in the appendices. All the theorems in the main text are referenced by the roman numbers, while those in the appendices are indexed by the roman letters.

II. Problem Statement

Let Ω be a domain in the three dimensional Euclidean space $\mathbf{R}^3$ that contains the object to be imaged. Let $u(x,\theta)$ be the light flux in direction $\theta \in S^2$ at $x \in \Omega$, where S^2 is the unit sphere. A general model for light migration in a random medium is given by the radiative transfer equation, or Boltzmann equation [9]–[11]:

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

for $t > 0$, and $x \in \Omega$, where c denotes the particle speed, $\mu = \mu_a + \mu_s$ with μ_a and μ_s being the absorption and scattering coefficients respectively, the scattering kernel η is normalized such that $\int\limits_{S^2} \eta(\theta \cdot \theta')d\theta' = 1$, and q is the internal light source. The initial condition for u is formulated as

$$u(x, \theta, 0) = 0, \quad \text{for } x \in \Omega \text{ and } \theta \in S^2, \tag{2}$$

while the boundary condition for u represents the incoming flux g^{-1}:

$$u(x, \theta, t) = g^-(x, \theta, t), \quad \text{for } t > 0, \text{ and } x \in \Gamma,\ \theta \in S^2 \text{ such that } \nu(x) \cdot \theta \leq 0, \tag{3}$$

where $\nu(x)$ is the exterior normal at x on the boundary Γ of Ω. Then, we want to reconstruct the internal light source q from measurements of the outgoing radiation, i.e., the escaping energy through a unit area at $x \in \Gamma$ perpendicular to the exterior normal $\nu(x)$ on Γ [10], [11]

$$g(x, t) = \int_{S^2} \nu(x) \cdot \theta u(x, \theta, t) d\theta, \quad t > 0 \text{ and } x \in \Gamma. \tag{4}$$

Reconstruction of the light source q is quite complex based on the measurement g and above initial-boundary conditions with the radiative transfer equation (1) as the governing equation, mainly due to the difficulty in computing the flux u as the forward problem (1), (2) and (3). Then, we seek an approximation to simplify the radiative transfer equation (1). Because the mean free path of the particle is between 0.005 mm and 0.01 mm in biological tissues, which is very small compared to a typical object in this context, the predominant phenomenon is scattering instead of transport [11]. Hence, we can approximate the the radiative transfer equation (1) with a much simpler equation, the diffusion equation, which has already been widely used in optical tomography [10], [11]. Let u_0 be the average photon flux in all directions, i.e., the diffusion approximation,

$$u_0(x, t) = \frac{1}{4\pi} \int_{S^2} u(x, \theta, t) d\theta. \tag{5}$$

and q_0 be defined similarly

$$q_0(x, t) = \frac{1}{4\pi} \int_{S^2} q(x, \theta, t) d\theta. \tag{6}$$

It can be shown that u_0 satisfies the following initial-boundary value problem [10], [11]

$$\frac{1}{c}\frac{\partial u_0}{\partial t} - \nabla \cdot (D \nabla u_0) + \mu_a u_0 = q_0, \quad t > 0 \text{ and } x \in \Omega, \tag{7}$$

$$u_0(x, t) + 2D(x)\frac{\partial u_0}{\partial \nu}(x, t) = g^-(x, t), \quad t > 0 \text{ and } x \in \Gamma, \tag{8}$$

$$u_0(x, t = 0) = 0, \quad x \in \Omega, \tag{9}$$

where

$$D(x) = \frac{1}{3(\mu_a(x) + \mu_s'(x))}. \tag{10}$$

[1]Although we have $g^- = 0$ in a typical BLT case, we prefer keeping g^- here for generality of the formulation. For example, if we perform BLT of two mice simultaneously, the outgoing flux of one mouse would be partially intercepted by the other mouse as its incoming flux.

The measurement equation (4) after the diffusion approximation reads [10], [11],

$$g(x,t) = -D(x)\frac{\partial u_0}{\partial \nu}(x,t), \quad t > 0 \text{ and } x \in \Gamma. \tag{11}$$

The above diffusion approximation procedure is also called the P_1-approximation [10], [11].

Because the internal bioluminescence distribution induced by reporter genes is relatively stable, we can use the stationary version of equations (7) — (9) as the forward model for BLT. By discarding all the time dependent terms and equation (9), the stationary forward model is

$$-\nabla \cdot (D\nabla u_0) + \mu_a u_0 = q_0, \quad x \in \Omega, \tag{12}$$

$$u_0(x) + 2D(x)\frac{\partial u_0}{\partial t}(x) = g^-(x), \quad x \in \Gamma, \tag{13}$$

and the stationary measurement equation (11) reads

$$g(x) = -D(x)\frac{\partial u_0}{\partial \nu}(x) \quad x \in \Gamma. \tag{14}$$

Given the measurement (14), it follows that the boundary value of $u_0(x)$ can be obtained according to (13) as follows:

$$u_0(x) = g^-(x) + 2g(x), \quad x \in \Gamma. \tag{15}$$

Hence, u_0 satisfies the following Cauchy condition on the boundary Γ [12]:

$$u_0(x) = g^-(x) + 2g(x), \quad x \in \Gamma, \tag{16}$$

$$D(x)\frac{\partial u_0}{\partial v}(x) = -g(x), \quad x \in \Gamma. \tag{17}$$

Therefore, BLT is equivalent to reconstruct the source q_0 of equation (12) from given $u_0(x)$ and $\frac{\partial u_0}{\partial \nu}(x)$ for $x \in \Gamma$, under the governing diffusion equation (12).

In summary, the BLT problem can be stated as follows: *Given the incoming flux $g^-(x)$ and outgoing flux $g(x)$ for $x \in \Gamma$, find a source q_0 with one corresponding photon flux u to satisfy*

$$\textbf{(BLT)} \begin{cases} -\nabla \cdot (D\nabla u_0) + \mu_a u_0 = q_0, \quad x \in \Omega \\ u_0(x) + 2D(x)\dfrac{\partial u_0}{\partial \nu}(x) = g^-(x), \quad x \in \Gamma \\ D(x)\dfrac{\partial u_0}{\partial \nu}(x) = -g(x), \quad x \in \Gamma, \end{cases} \tag{18}$$

or, equivalently,

$$\textbf{(BLT)} \begin{cases} -\nabla \cdot (D\nabla u_0) + \mu_a u_0 = q_0, \quad x \in \Omega \\ u_0(x) = g^-(x) + 2g(x), \quad x \in \Gamma \\ -D(x)\dfrac{\partial u_0}{\partial \nu}(x) = g(x), \quad x \in \Gamma. \end{cases} \tag{19}$$

The optical parameters D and μ_a can be established point-wise from a pre-requisite tomographic scan, such as a CT/micro-CT scan [7], [8]. In this paper, we assume that Ω is a bounded smooth domain of R^N, although the case of our main interest is $N=3$. We always assume that the parameters $D > D_0 > 0$ for some positive constant D_0 and that $\mu_a \geq 0$ are bounded functions in this work. We further assume that D is sufficiently regular near Γ, e.g., D is equal to a constant near Γ.

In practice, it is difficult to obtain all the measurement along the boundary Γ. We consider the case in which the measurement can only be taken on a portion $P_0 \subset \Gamma$. The BLT problem then becomes

$$\textbf{(BLT(}P_0\textbf{))} \quad \begin{cases} -\nabla \cdot (D\nabla u_0) + \mu_a u_0 = q_0, \quad x \in \Omega \\ u_0(x) + 2D(x)\dfrac{\partial u_0}{\partial \nu}(x) = g^-(x), \quad x \in P_0 \\ D(x)\dfrac{\partial u_0}{\partial \nu}(x) = -g(x), \quad x \in P_0. \end{cases} \tag{20}$$

III. Literature Review

BLT as formulated above is for reconstruction of an internal source from Cauchy data, which is called the inverse source problem of partial differential equations [13]. There are several theoretical studies relevant to the uniqueness of the solution to this type of problems. Although they do not provide a satisfactory answer to the solution uniqueness of BLT, these results do form a background for us to establish the uniqueness theorems under practical constraints for BLT. For a detailed historical survey, please refer to [13] and the references therein.

In [13], when the domain Ω is a bounded Lipschitz domain in the n-dimensional Euclidean space $\mathbf{R}^n$, the source $q_0 = \alpha q_1 + q_2$ with $\frac{\partial q_i}{\partial x_n} = 0$ for $i = 1, 2$ and $\frac{\partial \alpha}{\partial x_n} \geq 0$, where α is given, and the coefficient D does not depend on x_n and $\mu_a \geq 0$, then q_0 is uniquely determined by the Cauchy data (16) and (17).

In [14], Ω is a cylindrical domain $\Omega = \Omega' \times \Omega''$, $\Omega' \subset R^{n'}$, $\Omega'' \subset R^{n''}$. The governing equation is the Poisson equation

$$-\Delta u_0 = q_0 \tag{21}$$

i.e., $D = 1$ and $\mu_a = 0$. The source is assumed to be cylindrical

$$q_0(x) = b(x')h(x''), x = (x', x''). \tag{22}$$

If q_0 is with one known factor and a positive height-part, then it is uniquely determined by Cauchy data (16) and (17). In the standard case of $n'' = 1$, b and h are referred to as the base and height of the source, respectively.

In [15], Ω is a bounded Lipschitz domain in a two-dimensional Euclidean space $\mathbf{R}^2$. The governing equation is the Helmholtz equation

$$\Delta u_0 + k^2 u_0 = q_0 \tag{23}$$

i.e., $D = 1$ and $\mu_a = -k^2$. The source is assumed of either the form:

$$q_0(x) = \rho(x)\chi_B(x) \tag{24}$$

where B is an open subset of Ω, χ_B is the characteristic function of B[2], or the form

$$q_0(x) = \text{div}\left[\rho(x)\chi_B(x)a\right], \tag{25}$$

where a is a non-zero constant vector. Under some additional technical conditions, the convex hull of the source support B can be uniquely reconstructed given Cauchy data (16) and (17).

In [16], Ω is a bounded domain of $\mathbf{R}^n$ with sufficiently regular boundary and partitioned into connected sub-domains coated in layers (see [16] for a precise presentation). The governing equation is

$$-\nabla \cdot (D\nabla u_0) = q_0 \tag{26}$$

i.e., $\mu_a = 0$. The coefficient D is constant in each sub-domain. The source distribution is assumed of the form

$$q_0 = \sum_{k=1}^{m} \chi_{\omega_k} \tag{27}$$

where χ_{ω_k} is the characteristic function of a ball ω_k with center S_k and radius r_k. The centers must be distinct but the balls may overlap each other. It was proved that the number m of balls ω_k and their parameters S_k and r_k can be uniquely determined by Cauchy data. Note that these sources are assumed to have identical intensity values; otherwise, the uniqueness does not hold. There is a counterexample in [16] that $q_0 = \lambda_i\chi_{\omega_i}$ with different λ_i and ω_i for $i = 1, 2$ such that

$$-\Delta u = \lambda_i \chi_{\omega_i}, \tag{28}$$

$$u_i = f, \quad \text{on } \Gamma, \tag{29}$$

$$\frac{\partial u_i}{\partial \nu} = g, \text{on } \Gamma, \tag{30}$$

with the same f and g. To that effect, it suffices to set the parameters for both ω_i such that

$$S_1 = S_2, \quad \text{and} \quad \lambda_1 r_1^2 = \lambda_2 r_2^2. \tag{31}$$

It is interesting that the solution uniqueness holds for the equation (26) assuming a combination of mono and dipolar sources of the following form [16]:

$$q_0(x) = \sum_{k=1}^{m_1} \lambda_k \delta_{S_k} + \sum_{j=1}^{m_2} p_j \nabla \delta_{C_j} \tag{32}$$

where m_1 and m_2 are positive integers, S_k and C_j are points in Ω_0, λ_k and p_j are respectively scalar and vector quantities, δ_{S_k} and $\nabla\delta_{C_j}$ are a δ-function and the the gradient of a δ-function at S_k and C_j, respectively.

The counterexample given in (28) – (31) shows the non-uniqueness of the solution to inverse source problems. Reconstructing sources of the form $q_0 = \lambda\chi_\omega$ with the Poisson equation (21) as the governing equation is related to the inverse gravimetry problem in geophysics, where the uniqueness does not hold unless the source support

[2]The characteristic function of any set B is deinfed as, $\chi_B(x) = 1$ for $x \in B$ and $\chi_B(x) = 0$ for $x \notin B$.

is star-shaped or convex [13]. For the Helmholtz equation, the inverse source problem does not admit a unique solution because of the possible *nonradiating sources* within the source support Ω [17]–[19]. In [14], with the Poisson equation as the governing equation it was proved that all the solutions to the inverse source problem with Cauchy data can be expressed as

$$q_0 = q_1 + q_2, \tag{33}$$

here q_1 is the minimal L^2-norm solution of the inverse source problem satisfying $-\Delta q_1 = 0$ and $q_2 = -\Delta h$ for some h with zero Cauchy data. Hence, q_1 is unique. The q_2 part corresponds to the nonradiating sources.

For clarity, our literature overview is summarized in Table I. As shown in Table I, the solution uniqueness results are not available for BLT, in which the diffusion equation assumes spatially variable optical properties μ_α and D, and its Cauchy data are measured on the domain boundary.

TABLE I

SUMMARY OF KNOWN RESULTS

Reference	Domain	Equation	μ_a	D	Source	Uniqueness of q_0
[13]	general	diffusion (12)	arbitrary	$\frac{\partial D}{\partial x_n} = 0$	$q_0 = \alpha q_1 + q_2$, known α; $\frac{\partial q_i}{\partial x_n} = 0$, $\frac{\partial \alpha}{\partial x_n} \geq 0$;	yes
[14]	cylindrical	Poisson (21)	0	$D = 1$	$b(x')h(x'')$, one known factor	yes
[15]	general	Helmholtz (23)	0	negative constant	$\rho(x)\chi_B(x)$	convex hull of B
[16]	general	diffusion (26)	0	piecewise constant	$\sum_{k=1}^{m} \chi_{\omega_k}$	yes
[16]	general	diffusion (26)	0	piecewise constant	$q_0(x) = \sum_{k=1}^{m_1} \lambda_k \delta_{S_k} + \sum_{j=1}^{m_2} p_j \delta_{C_j}$	yes
[16]	general	Poisson (21)	0	$D = 1$	$q_0(x) = \lambda \chi_S$	no
[14]	general	Poisson (21)	0	$D = 1$	arbitrary	no ($q_0 = q_1 + q_2$)

IV. RESULTS

Given its physical meaning, BLT must have at least one solution. Therefore, in this section we will not discuss the existence of the BLT solution, and primarily focus on the solution uniqueness of BLT. To convey our main points clearly we will just present our three theorems in a manner easily accessible to physicists and engineers while giving rigorous statements and proofs in the appendices.

The first result is about the solution structure of the BLT problem (18), which is a generalization of (33) in [14]. Let L be the following differential operator

$$L[v] = -\nabla \cdot (D \nabla v) + \mu_a v, \tag{34}$$

we have

Theorem IV.1. *Assume that the BLT problem is solvable. There is one special solution q_H for the* **BLT** *problem (18), which is of the minimal L^2-norm among all the solutions. All the solutions can be expressed as $q_0 = q_H + L[m]$, for any $m \in H_0^2(\Omega)$, which is the closure of all smooth functions in $\bar{\Omega}$ vanishing on Γ up to order one. (cf. Theorem B.2.)*

Given the difficulty that there is no unique solution to BLT in the general case by Theorem IV.1, we must restrict the solution space to a sub-space of bioluminescent source distributions so that the solution uniqueness may be established in that specific case. For example, we can study source distributions in a certain parameterized form to remove the ambiguity in the BLT solution.

In the following, we first consider the case of a linear combination of bioluminescent impulses

$$q_0(y) = \sum_{i=1}^{m} a_i \delta(y - y_i) \tag{35}$$

where each a_i is a constant coefficient, and y_i the location of a point source inside Ω, for $i = 1, \cdots, m$. We have

Theorem IV.2. *Assume that the conditions in Theorem D.4 (Appendix D) hold. If $q_0(y) = \sum\limits_{i=1}^{m} a_i \delta(y - y_i)$ and $Q_0(y) = \sum\limits_{j=1}^{M} A_j \delta(y - Y_j)$ are two solutions to the BLT problem (18), then $m = M$ and there is a permutation τ of $[1, m]$ such that $a_i = A_{\tau(i)}$ and $y_i = Y_{\tau(i)}$.*

Then, let us consider a linear combination of solid/hollow ball sources

$$q_0(y) = \sum_{i=1}^{m} \lambda_i \chi_{B_{r_0^i, r_1^i}}(x_i) \tag{36}$$

for the more general BLT(P_0) problem (20), which covers the BLT problem as a special case. To present our finding in this case, we need the following notations. For each $0 \leq r_0 < r_1 < \infty, x_0 \in R^N$, let $B_{r_0, r_1}(x_0)$ denote a hollow ball specified by $r_0 < |x - x_0| < r_1$ for $r_0 > 0$ and a solid ball specified by $|x - x_0| < r_1$ for $r_0 = 0$. To study the solution uniqueness, we need some practical assumptions on the domain Ω. Another assumption is that the coefficients D and μ_a must be piecewise constants, which is also reasonable in practice. Please see Theorem D.4 (Appendix D) to find the exact conditions for the following theorem.

Theorem IV.3. *Assume that the conditions in Theorem D.4 (Appendix D) hold. If $q_1(y) = \sum\limits_{i=1}^{m} \lambda_i \chi_{B_{r_0^i, r_1^i}}(x_i)$ and $q_2(y) = \sum\limits_{j=1}^{M} \Lambda_j \chi_{B_{R_0^i, R_1^i}}(X_i)$ are two solutions to the BLT(P_0) problem (20), then $m = M$ and there exist a permutation τ of $[1, m]$ and a map $C : [1, m] \to [1, I]$ such that $x_i = X_{\tau(i)} \in \Omega_{C(i)}$ and*

$$\lambda_i \int_{r_0^i}^{r_1^i} r^{N-1} \varphi_{C(i)}(r)\, dr = \Lambda_{\tau(i)} \int_{R_0^{\tau(i)}}^{R_1^{\tau(i)}} r^{N-1} \varphi_{C(i)}(r)\, dr, \quad \textit{for } i = 1, ..., I, \tag{37}$$

where φ_j is the unique solution of

$$-D_j \left(\varphi_j'' + \frac{N-1}{r} \varphi_j' \right) + \mu_j \varphi_j = 0, \tag{38}$$

$$\varphi_j(0) = 1, \quad \varphi_j'(0) = 0. \tag{39}$$

V. Discussions and Conclusion

Theorem IV.1 reveals a fundamental feature of BLT. That is, without incorporation of effective *priori* knowledge on the source distribution there would be no hope to determine a unique solution. Actually, no matter how many higher order derivatives are measured, the uniqueness of the solution cannot be claimed without use of additional constraints on the source. For example, if ζ_0 is a solution and $m(x)$ is any smooth function with compact support in Ω and $D^\alpha m|_\Gamma = 0$ for all α, then it is straightforward to prove that $\varphi = \zeta_0 + aL[m]$ is also a solution to the BLT problem. Physically speaking, no matter how many measures are taken in an open band around the boundary of the domain Ω, we will not be able to find the solution uniquely without utilization of adequate *priori* knowledge. In other words, Theorem IV.1 suggests that one must utilize all possible information on the source distribution to achieve the best possible reconstruction for BLT.

Theorem IV.2 is not only theoretically inspiring but also practically useful. As a modality for molecular imaging, BLT is often intended for detection of small pathological events and changes such as for cancer screening. In this context, a combination of bioluminescent impulses may model the early stage of tumor development very well. With increasingly more imaging probes and smart drugs available, the solution uniqueness in that case would definitely facilitate early diagnosis and better treatment of the cancer in general.

Theorem IV.3 is our main result in this paper. Interestingly, if we only consider solid ball sources and assume that their intensities are known, it can be readily shown that the solution to the BLT problem is unique. Practically, the source intensity is closely related to the strength of the molecular/cellular activity, such as gene expression. Hence, it is often reasonable to take the intensity or its parametric form as known to find the unique solution.

Our uniqueness results are instrumental for reconstruction of a bioluminescent source distribution. For sources as parameterized in Theorem IV.3, once a solution is found, any other solution can be easily constructed by adjusting a limited number of source related parameters (intensity λ_i and so on) according to the relationships given in Theorem IV.3, subject to any other available anatomical and physiological constraints. Note that since a practical source function can be approximated by a linear combination of solid/hollow ball sources as parameterized in Theorem IV.3, our uniqueness results cover a quite general class of source distributions, spanned by those solid/hollow ball sources.

We emphasize that BLT as defined in this paper is a new area, and there remain many theoretical, numerical and experimental issues to be resolved. Theoretically, we would like to relax the assumptions on the properties of the scattering media and enrich the family of parametric source distributions. The solution uniqueness with some additional internal measurement, such as endoscopic measurement, may improve the well-posedness of BLT. The stablity of the BLT solution is also an important problem to be addressed. The perspective for multi-spectral and dynamic BLT should be even more challenging. While the continuous domain formulation is important, various digital algorithms must be designed for practical BLT. However, development and evaluation of these algorithms are beyond the scope of this theoretical paper. Currently, we are developing our BLT prototype with an initial emphasis on mouse models of various lung diseases [8].

While we were in the stage of finalizing this paper, it came to our attention that some similar work was performed at Xenogen as reported in a SPIE paper [20] and the company website (http://www.xenogen.com/). Some 3D imaging systems have been recently released to a few test sites, which take multiple views around a mouse or rat. A diffuse luminescent imaging tomography algorithm is used to reconstruct an internal source, coupled with a *homogeneous* scattering-media assumption. Clearly, this approach may reveal subcutaneous depth information, but satisfactory reconstruction of a bioluminescent source distribution (both geometric and power) cannot be achieved in general without compensation for the *heterogeneous* anatomy of the mouse.

In conclusion, we have determined the set of the solutions to BLT in the general case to demonstrate that the generic BLT problem is not uniquely solvable. Then, we have established the solution uniqueness in the cases of (i) impulse sources, and (ii) solid/hollow ball sources (up to non-radiating sources) assuming that the scattering media are piece-wise constant in terms of D and μ_α. It has been emphasized that by introducing the *priori* knowledge on the bioluminescent source structure the BLT problem becomes well defined. Therefore, the BLT is feasible for localization and quantification of the bioluminescent source distribution. We believe that BLT will grow into an important molecular imaging modality, and play a significant role in development of molecular medicine.

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APPENDIX A
MATHEMATICAL PRELIMINARIES

A. Notations

The following function spaces [21]–[23] are used in the proofs below:

$$L^2(\Omega) = \left\{ u : \int_\Omega |f(x)|^2 \, dx < \infty \right\} \tag{A.2}$$

with the inner product defined by

$$\langle u, v \rangle_{L^2(\Omega)} = \int_\Omega u(x) v(x) \, dx. \tag{A.3}$$

We need the Sobolev spaces

$$H^1(\Omega) = \left\{ u \in L^2(\Omega) : \nabla u \in L^2(\Omega) \right\} \tag{A.4}$$

where ∇u is the derivative in the sense of distribution, with the inner product defined by

$$\langle u, v \rangle_{H^1(\Omega)} = \langle u, v \rangle_{L^2(\Omega)} + \langle \nabla u, \nabla v \rangle_{L^2(\Omega)}, \tag{A.5}$$

and

$$H^2(\Omega) = \left\{ u \in H^1(\Omega) : \nabla^2 u \in L^2(\Omega) \right\} \tag{A.6}$$

with the inner product defined by

$$\langle u, v \rangle_{H^2(\Omega)} = \langle u, v \rangle_{H^1(\Omega)} + \langle \nabla^2 u, \nabla^2 v \rangle_{L^2(\Omega)}. \tag{A.7}$$

The subspaces $H_0^1(\Omega)$ and $H_0^2(\Omega)$ of $H^1(\Omega)$ and $H^2(\Omega)$ are the closure of smooth functions with compact support inside Ω in $H^1(\Omega)$ and $H^2(\Omega)$ with the associated norms, respectively. In fact, there is a family of Sobolev spaces, denoted by $H^s(\Omega)$, for $s \in \mathbf{R}$. Similarly, we can define $H_0^s(\Omega)$.

To solve the boundary value problems of partial differential equations, we need functions on Γ. We can define the space $L^2(\Gamma)$ on Γ similarly. Definitions for the Sobolev spaces $H^t(\Gamma)$ on Γ involve tedious specifics [21]–[23], and are skipped here. For a smooth function u, its boundary value is defined by restriction of u to Γ: $u|_\Gamma(x) = u(x)$, for $x \in \Gamma$. For a Sobolev space, it can be established that there is a unique map τ from $H^s(\Omega)$ to $H^{s-\frac{1}{2}}(\Gamma)$ such that: (1) $\tau[u] = u|_\Gamma$ for a smooth u; (2) τ is continuous and onto. τ is called the trace operator. Hence, for example, the space for characterizing the boundary values of functions in $H^1(\Omega)$ is naturally $H^{\frac{1}{2}}(\Gamma)$. It can be proved that $u \in H_0^s(\Omega)$ if and only if $\tau[u] = 0$. It is well-known that $L^2(\Omega)$, $H^s(\Omega)$ and $H^t(\Gamma)$ are Hilbert spaces with the norms induced from the corresponding inner products.

We need the following notations from functional analysis [24]. Let A be a linear operator from a Banach space X to a Banach space. The kernel or null space of A is defined as $\mathcal{N}[A] = \{x \in X : A[x] = 0\}$, and the range of A is $\mathcal{R}[A] = \{y \in Y : y = A[x] \text{ for some } x \in X\}$. For a subspace M of a Hilbert space H, $M^\perp$ is the set of all $y \in H$, such that $\langle y, x \rangle = 0$ for all $x \in M$.

B. Dirichlet-to-Neumann Map

To make the presentation concise, we introduce the following notations. Let γ_0 and γ_1 be the boundary value maps

$$\gamma_0[u] = u|_\Gamma, \quad \text{and} \quad \gamma_1[u] = D\frac{\partial u}{\partial \nu}\bigg|_\Gamma. \tag{A.8}$$

Let $L[u]$ be the differential operator

$$L[u] = -\nabla \cdot (D\nabla u) + \mu_a u. \tag{A.9}$$

Then, the forward model can be written as

$$L[u] = q_0, \text{ in } \Omega, \tag{A.10}$$

$$\gamma_0[u] + 2\gamma_1[u] = g^-, \text{ on } \Gamma. \tag{A.11}$$

Given $f \in H^{\frac{1}{2}}(\Gamma)$, let $w_1 \in H^1(\Omega)$ be the solution of the following boundary value problem [22], [25]

$$L[w_1] = 0, \text{ in } \Omega, \tag{A.12}$$

$$\gamma_0[w_1] = f, \text{ on } \Gamma. \tag{A.13}$$

We define a linear operator $\mathcal{N}$ from $H^{\frac{1}{2}}(\Gamma)$ to $H^{-\frac{1}{2}}(\Gamma)$ by

$$\mathcal{N}[f] = \gamma_1[w_1]. \tag{A.14}$$

$\mathcal{N}$ is the well-known Dirichlet-to-Neumann (or Steklov-Poincaré) map [13].

On the other hand, for $q_0 \in L^2(\Omega)$, we consider the problem

$$L[w_2] = q_0, \text{ in } \Omega, \tag{A.15}$$

$$\gamma_0[w_2] = 0, \text{ on } \Gamma, \tag{A.16}$$

and define another linear operator Λ by

$$\Lambda[q_0] = \gamma_1[w_2]. \tag{A.17}$$

From the regularity theory for second order elliptic partial differential equations, $w_2 \in H^2(\Omega) \bigcap H_0^1(\Omega)$ and $\gamma_1[w_2] \in H^{\frac{1}{2}}(\Gamma)$ [22], [25].

In terms of γ_0 and γ_1, the BLT problem is to find q_0 such that

$$L[u] = q_0, \text{ in } \Omega, \tag{A.18}$$

$$\gamma_0[u] + 2\gamma_1[u] = g^-, \text{ on } \Gamma, \tag{A.19}$$

$$\gamma_1[u] = -g, \text{ on } \Gamma, \tag{A.20}$$

given the observed g and assumed g^-, where u is unknown. Assume that such a source q_0 exists. Then, we can find u by solving the following boundary value problem

$$L[u] = q_0, \text{ in } \Omega, \tag{A.21}$$

$$\gamma_0[u] = g^- + 2g, \text{ on } \Gamma. \tag{A.22}$$

Let w_1 be defined as in (A.12) – (A.13) with $f = g^- + 2g$, and w_2 be defined as in (A.15) – (A.16). It follows that $u = w_1 + w_2$. The measurement equation implies that

$$-g = \gamma_1[u] = \gamma_1[w_1] + \gamma_1[w_2] = N[g^- + 2g] + \Lambda[q_0], \tag{A.23}$$

i.e.,

$$\Lambda[q_0] = N[g^- + 2g] - g. \tag{A.24}$$

Conversely, if there exists a q_0 satisfying (A.24), we can construct u as indicated above. It follows easily that u satisfies the forward model and the measurement equation. In summary, we have

Proposition A.1. *q_0 is a solution for the inverse problem (A.18) – (A.20) if and only it is a solution to (A.24).*

C. Green's Formula

For v and $p \in H^2(\Omega)$ the following Green's formula is well-known [22], [25]

$$\int_\Omega [v \cdot L[p] - p \cdot L[v]] \, dx = -\int_\Gamma [v\gamma_1[p] - p\gamma_1[v]] \, d\Gamma. \tag{A.25}$$

Let $F(x, y)$ be the fundamental solution of L on $\mathbf{R}^n$ with coefficients smoothly extended from Ω to $\mathbf{R}^n$ with the same properties, which tends to zero at ∞ for each fixed $x \in \mathbf{R}^n$ [26], i.e.,

$$L_y F(x, y) = \delta(y - x), \quad \lim_{y \to \infty} F(x, y) = 0, \; y \in R^N. \tag{A.26}$$

Then, we can apply Green's formula (A.25) to obtain a formula for the solution of the inverse problem (A.18) — (A.20). Let u be the solution satisfying (A.18) — (A.20). For any $x \in \Omega$, by Green's formula (A.25) with $v = F(x, y)$ and $p = u$, we have

$$\int_\Omega [F(x, y) \cdot L[u](y) - u(y) \cdot \delta(x - y)] \, dy = -\int_\Gamma [F(x, y)\gamma_1[u](y) - u(y)\gamma_1[F(x, y)]] \, d\Gamma_y. \tag{A.27}$$

Hence,

$$u(x) = \int_\Omega [F(x, y) \cdot q_0(y)] \, dy - \int_\Gamma \left[F(x, y)g(y) + (g^-(y) + 2g(y))\gamma_1[F(x, y)]\right] \, d\Gamma_y, \quad \forall x \in \Omega. \tag{A.28}$$

Note that $L[F(x, \cdot)] = 0$ if $x \in \mathbf{R}^N \setminus \overline{\Omega}$. We obtain, by Green's formula again,

$$0 = \int_\Omega [F(x, y) \cdot q_0(y)] \, dy - \int_\Gamma \left[F(x, y)g(y) + (g^-(y) + 2g(y))\gamma_1[F(x, y)]\right] \, d\Gamma_y, \quad \forall x \in \mathbf{R}^N \setminus \overline{\Omega}. \tag{A.29}$$

APPENDIX B
PROOF OF THEOREM IV.1

By Proposition A.1, to study the uniqueness property of the BLT solution we should characterize the kernel $\mathcal{N}[\Lambda]$ of the operator $\Lambda : L^2(\Omega) \to H^{\frac{1}{2}} \subset L^2(\Gamma)$. We begin with determining the adjoint Λ^* of Λ, because $\mathcal{N}[\Lambda] = \mathcal{R}[\Lambda^*]^\perp$ [24]. Let $\psi \in H^{\frac{1}{2}}(\Gamma)$ and $\phi = T[\psi]$ as the unique solution in $H^1(\Omega) \subset L^2(\Omega)$ of the boundary problem

$$L[\phi] = 0, \text{ in } \Omega, \tag{A.30}$$

$$\gamma_0[\phi] = -\psi, \text{ on } \Gamma. \tag{A.31}$$

Then, by Green's formula (A.25), (A.16), (A.17) and (A.30),

$$\int_\Omega q_0 \cdot \phi \, dx = \int_\Omega L[w_2] \cdot \phi \, dx = -\int_\Gamma [-\psi\Lambda[q_0] - w_2\gamma_1[\phi]] \, d\Gamma + \int_\Omega w_2 L[\phi] \, dx = \int_\Gamma \psi\Lambda[q_0] \, d\Gamma.$$

Thus, for the operators $\Lambda : L^2(\Omega) \to H^{\frac{1}{2}}(\Gamma) \subset L^2(\Gamma)$ and $T : H^{\frac{1}{2}}(\Gamma) \subset L^2(\Gamma) \to L^2(\Omega)$,

$$\langle q_0, T[\psi] \rangle_{L^2(\Omega)} = \langle \Lambda[q_0], \psi \rangle_{L^2(\Gamma)}, \tag{A.32}$$

i.e.,

$$\Lambda^* = T. \tag{A.33}$$

Then, the kernel of Λ is

$$\mathcal{N}[\Lambda] = \mathcal{R}[\Lambda^*]^{\perp} = \mathcal{R}[T]^{\perp}. \tag{A.34}$$

We have the following proposition characterizing $\mathcal{R}[T]^{\perp}$:

Proposition B.1.

$$\mathcal{R}[T]^{\perp} = L[H_0^2(\Omega)]. \tag{A.35}$$

Proof. If $q \in L[H_0^2(\Omega)]$ with $q = L[p]$ for some $p \in H_0^2(\Omega)$, then for $v = T[\psi] \in \mathcal{R}[T]$, by Green's formula (A.25),

$$\langle q, v \rangle_{L^2(\Omega)} = \int_\Omega q \cdot v \, dx = \int_\Omega v \cdot L[p] \, dx = \int_\Gamma [p\gamma_1[v] - v\gamma_1[p]] + \int_\Omega L[v] \cdot p \, dx = 0,$$

because $\gamma_0[p] = 0$, $\gamma_1]p] = 0$ and $L[v] = 0$. Hence, $q \perp \mathcal{R}[T]$. Therefore, $L[H_0^2(\Omega)] \subset \mathcal{R}[T]^{\perp}$.

Conversely, assume that $q \in \mathcal{R}[T]^{\perp} = \mathcal{N}[\Lambda]$. We have, by (A.15) — (A.17), there exists w_2 such that

$$L[w_2] = q, \text{ in } \Omega,$$

$$\gamma_0[w_2] = 0, \text{ on } \Gamma,$$

$$\gamma_1[w_2] = 0, \text{ on } \Gamma.$$

We have $w_2 \in H^2(\Omega)$ by the regularity theory for second order elliptic partial differential equations [22], [25]. The above boundary conditions imply that $w_2 \in H_0^2(\Omega)$. Hence, $q = L[w_2] \in L[H_0^2(\Omega)]$. The conclusion follows immediately. $\square$

By Proposition A.1, all the solutions to the BLT problem form a convex set in $L^2(\Omega)$. There exists one unique solution of the minimal L^2-norm among those solutions [24], denoted as q_H. Then, all the solutions can be expressed as $q_H + \mathcal{N}[\Lambda]$. We summarize the above results into the following theorem.

Theorem B.2. *Assume that the BLT problem is solvable. For any couple (g^-, g) such that*

$$N[g^- + 2g] - g \in H^{\frac{1}{2}}(\Gamma), \tag{A.36}$$

there is one special solution q_H for the **BLT** *problem (18), which is of the minimal L^2-norm among all the solutions. Then, any solution can be expressed as $q_0 = q_H + L[m]$, for some $m \in H_0^2(\Omega)$.*

Remark B.3. *Naturally, the condition (A.36) for (g^-, g) is automatically satisfied when g is a normal trace $\gamma_1[u]$, where u is a solution of the forward model (A.10) and (A.11) for $q_0 \in L^2(\Omega)$.*

APPENDIX C
PROOF OF THEOREM IV.2

We present the exact conditions on Ω, D, μ_a and q_0 for Theorem IV.2, which are also part of conditions for Theorem IV.3.

C1: Ω is a bounded C^2 domain of R^N and partitioned into non-overlapping sub-domains Ω_i, $i = 1, 2, ..., I$;

C2: Each Ω_i is connected with piecewise smooth boundary P_i;

C3: D and μ_α are C^2 near the boundary of each sub-domain.

C4: $D > D_0 > 0$ for some positive constant D_0 is Lipschitz on each sub-domain; $\mu_a \geq 0$ and $\mu_\alpha \in L^p(\Omega)$ for some $p > N/2$;

Theorem C.1. *Assume the conditions C1 – C4 hold. If* $q_0(y) = \sum_{i=1}^{m} a_i \delta(y - y_i)$ *and* $Q_0(y) = \sum_{j=1}^{M} A_j \delta(y - Y_j)$ *are two solutions to the BLT problem (18), then* $m = M$ *and there is a permutation* τ *of* $[1, m]$ *such that* $a_i = A_{\tau(i)}$ *and* $y_i = Y_{\tau(i)}$.

Proof. For $x \in \mathbf{R}^N \setminus \overline{\Omega}$, let

$$b(x) = \int_\Gamma \left[F(x,y)g(y) + (g^-(y) + 2g(y))\gamma_1[F(x,y)]\right] d\Gamma_y. \tag{A.37}$$

If $q_0(y) = \sum_{i=1}^{m} a_i \delta(y - y_i)$ and $Q_0(y) = \sum_{j=1}^{M} A_j \delta(y - Y_j)$ are both solutions to the BLT problem (18), then we have, by (A.29),

$$\int_\Omega F(x,y) \left[\sum_{i=1}^{m} a_i \delta(y - y_i)\right] dy = b(x), \quad \forall x \in \mathbf{R}^N \setminus \overline{\Omega}, \tag{A.38}$$

or

$$\sum_{i=1}^{m} a_i F(x, y_i) = b(x), \quad \forall x \in \mathbf{R}^N \setminus \overline{\Omega}. \tag{A.39}$$

Similarly, we have

$$\sum_{j=1}^{M} A_j F(x, Y_j) = b(x), \quad \forall x \in \mathbf{R}^N \setminus \overline{\Omega}. \tag{A.40}$$

Now, let us define two functions w and W on $\mathbf{R}^N$ as follows,

$$w(x) = \sum_{i=1}^{m} a_i F(x, y_i), \tag{A.41}$$

and

$$W(x) = \sum_{j=1}^{M} A_j F(x, Y_j). \tag{A.42}$$

Since $F(x,y) = F(y,x)$, we have

$$-\nabla \cdot (D\nabla w) + \mu_a w = 0, \quad \text{in } \mathbf{R}^N \setminus \{y_1, ..., y_m\}, \tag{A.43}$$

$$-\nabla \cdot (D\nabla W) + \mu_a W = 0, \quad \text{in } \mathbf{R}^N \setminus \{Y_1, ..., Y_M\}, \tag{A.44}$$

and $w(x) \equiv W(x)$ in $\mathbf{R}^N \setminus \overline{\Omega}$ by (A.39) and (A.40). Then, by the unique continuation theory [27], we have

$$w(x) \equiv W(x), \quad \text{in } \mathbf{R}^N \setminus \{y_1, ..., y_m, Y_1, ..., Y_M\}. \tag{A.45}$$

Now since

$$-\nabla \cdot (D\nabla w) + \mu_a w = \sum_{i=1}^{m} a_i \delta(x - y_i), \quad \text{in } \mathbf{R}^N, \tag{A.46}$$

$$-\nabla \cdot (D\nabla W) + \mu_a W = \sum_{j=1}^{M} A_j \delta(x - y_i), \quad \text{in } \mathbf{R}^N, \tag{A.47}$$

and from (A.45)

$$\int_\Omega W(y)L[u](y)\, dy = \int_\Omega w(y)L[u](y)\, dy, \tag{A.48}$$

which implies that

$$\sum_{i=1}^{m} a_i u(y_i) = \sum_{j=1}^{M} A_j u(y_i), \tag{A.49}$$

for all rapidly decaying C^2 functions, it follows that w and W must possess the same singular point set, i.e., $\{y_1, ..., y_m\} = \{Y_1, ..., Y_M\}$ and their weights at each singular point be the same, which finishes this proof. □

APPENDIX D

PROOF OF THEOREM IV.3

Lemma D.1. *For any given source $q_0 \in L^2(\Omega)$ and any nontrivial C^2 patch $P \subset \Gamma$, the solution u_0 of the forward model is uniquely determined by the boundary values $u_0|_P$ of u_0 and $\frac{\partial u_0}{\partial \nu}\big|_P$ of $\frac{\partial u_0}{\partial \nu}$ on P.*

Proof. Because D and μ_a can be smoothly extended across P by our assumption, the conclusion follows easily from the unique continuation theory [27]. □

Lemma D.2. *For any constant $D > 0$, $\mu_\alpha \geq 0$, and any solution u_0 of $-\nabla \cdot (D\nabla u_0) + \mu_a u_0 = 0$ in $B_R(x_0)$, we have*

$$\int\limits_{r_0<|x-x_0|<r_1} u_0(x)\, dx = \left(\int\limits_{r_0}^{r_1} \omega_N r^{N-1} \varphi(r)\, dr \right) u_0(x_0), \tag{A.50}$$

where $0 \leq r_0 < r_1 < R$, ω_N is the surface area of the unit sphere in $\mathbf{R}^N$, and $\varphi(r)$ is the unique positive radial solution of

$$-D\Delta\varphi + \mu_a \varphi = -D\left(\varphi'' + \frac{N-1}{r}\varphi'\right) + \mu_a \varphi = 0 \tag{A.51}$$

with $\varphi(0) = 1$ and $\varphi'(0) = 0$.

Proof. Define $\bar{u}(r) \equiv \frac{1}{\omega_N r^{N-1}} \int_{\partial B_r(x_0)} u_0(x)\, ds_x$, we have

$$\bar{u}(0) = \lim_{r \to 0+} \bar{u}(r) = u(x_0), \tag{A.52}$$

and

$$-D(\bar{u}'' + \frac{N-1}{r}\bar{u}') + \mu_a \bar{u} = 0 \tag{A.53}$$

with $\bar{u}(0) = u(x_0)$ and $\bar{u}'(0) = 0$. Hence, by the uniqueness of the initial value problem,

$$\bar{u}(r) = u_0(x_0)\varphi(r). \tag{A.54}$$

Now,

$$\int\limits_{r_0<|x-x_0|<r_1} u_0(x)dx = \int_{r_0}^{r_1} dr \int\limits_{|x-x_0|=r} u_0(x)\, dS_x \tag{A.55}$$

$$= \int_{r_0}^{r_1} \omega_N r^{N-1}\bar{u}(r)dr = u(x_0)\int_{r_0}^{r_1} \omega_N r^{N-1}\varphi(r)dr. \tag{A.56}$$

□

Remark D.3. *We have, for $\mu_a = 0$,*

$$\varphi(r) = 1, \tag{A.57}$$

and for $\mu_a > 0$,

$$\varphi(r) = \begin{cases} \mathrm{BesselI}(0, \sqrt{\frac{\mu_a}{D}}r), & N = 2 \\ \frac{\sinh(\sqrt{\frac{\mu_a}{D}}r)}{\sqrt{\frac{\mu_a}{D}}r}, & N = 3, \end{cases} \tag{A.58}$$

where BesselI *is a Bessel function of the first kind.*

Note that $\varphi(r) \equiv 1$ for $\mu_\alpha = 0$ is equivalent to the mean value theorem for harmonic functions.

Now, we present the additional conditions on Ω, D, μ_a and q_0 for Theorem IV.3:

C4*: D and μ_α are piecewise constant in the sense that there exist constants $D_1, ..., D_I > 0$ and $\mu_1, ..., \mu_I \geq 0$ such that $D(x) \equiv D_i$ and $\mu_a(x) \equiv \mu_i$, $\forall x \in \Omega_i$.

Note that condition C4* is a special case of condition C4.

C5: There exists a C^2 patch P_0 of Γ;

C6: For each sub-domain Ω_m, there exists a sequence of indices $i_1, i_2, ..., i_k \in [1, I]$ with the following connectivity property: the intersection $P_0 \cap \Gamma_{i_1}$ contains a smooth C^2 open patch and $P_{i_j} \cap P_{i_{j+1}}$ contains a smooth C^2 open patch, for $j = 1, ..., k-1$, and $\Omega_{i_k} = \Omega_m$;

C7: q_0 is of the following form

$$q_0(y) = \sum_{i=1}^{m} \lambda_i \chi_{B_{r_0^i, r_1^i}}(x_i), \tag{A.59}$$

where each λ_i, $i = 1, ..., I$, is constant, and each source support $B_{r_0^i, r_1^i}(x_i) \subset\subset \Omega_k$[3] for some $k \in [1, I]$.

[3]This means that $B_{r_0^i, r_1^i}(x_i)$ is compactly included in Ω_k; that is, there is a positive distance from $B_{r_0^i, r_1^i}(x_i)$ to the boundary Γ_k of Ω_k.

Theorem D.4. *Assume the conditions C1 – C4*, C5 – C7 hold. If* $q_1(y) = \sum_{i=1}^{m} \lambda_i \chi_{B_{r_0^i, r_1^i}}(x_i)$ *and* $q_2(y) = \sum_{j=1}^{M} \Lambda_j \chi_{B_{R_0^i, R_1^i}}(X_i)$ *are two solutions to the BLT(P_0) problem (20), then* $m = M$ *and there exist a permutation* τ *of* $[1, m]$ *and a map* $C : [1, m] \to [1, I]$ *such that* $x_i = X_{\tau(i)} \in \Omega_{C(i)}$ *and*

$$\lambda_i \int_{r_0^i}^{r_1^i} r^{N-1} \varphi_{C(i)}(r)\, dr = \Lambda_{\tau(i)} \int_{R_0^{\tau(i)}}^{R_1^{\tau(i)}} r^{N-1} \varphi_{C(i)}(r)\, dr, \quad \textit{for } i = 1, ..., I, \tag{A.60}$$

where φ_j *is the unique solution of*

$$-D_j \left(\varphi_j'' + \frac{N-1}{r} \varphi_j' \right) + \mu_j \varphi_j = 0, \tag{A.61}$$

$$\varphi_j(0) = 1, \quad \varphi_j'(0) = 0. \tag{A.62}$$

Proof. Let u_1 and u_2 be the solutions to (20) corresponding to q_1 and q_2, respectively. Let $w = u_1 - u_2$, then w is a solution of

$$-\nabla \cdot (D \nabla w) + \mu_a w = q_1 - q_2, \text{ in } \Omega, \tag{A.63}$$

$$w|_{P_0} = D \frac{\partial w}{\partial v}|_{P_0} = 0. \tag{A.64}$$

Based on the fact that the support G of $q_1 \cup q_2$ does not touch any part of Γ or Γ_i, for $i = 1, \cdots, I$, in the following we will show that $w|_{\Gamma_i} = D_i \frac{\partial w}{\partial \nu}|_{\Gamma_i} = 0$, $i = 1, \cdots, I$.

First, let Ω_j be any sub-domain such that $P_0 \cap \Gamma_j$ contains a C^2 open patch, we have

$$-\nabla \cdot (D_j \nabla w) + \mu_j w = 0, \quad \text{in } \Omega_j \setminus G, \tag{A.65}$$

$$w|_{P_0 \cap \Gamma_j} = D_j \frac{\partial w}{\partial \nu}\bigg|_{P_0 \cap \Gamma_j} = 0. \tag{A.66}$$

Then, there exists an open peripheral narrow band B_j of Γ_j: $B_j = \{x \in \Omega_j \setminus G : \text{dist}(x, \partial\Omega_j) < \varepsilon\}$[4] for a sufficiently small $\varepsilon > 0$. Clearly, B_j can be covered from $P_0 \cap \Omega_j$ by overlapped open balls in $\Omega_j \setminus G$. Then, our Lemma D.1 implies that $w|_{B_j} \equiv 0$. Hence, $w|_{\Gamma_j} = D_j \frac{\partial w}{\partial \nu}|_{\Gamma_j} = 0$.

Next, let us deal with other sub-domains. Let Ω_k be any adjacent sub-domain such that $\Gamma_j \cap \Gamma_k$ contains a C^2 open patch P_{jk}. Then, we have [25],

$$w|_{P_{jk}} = w|_{P_{jk}} \text{ and } D_k \frac{\partial w}{\partial \nu_k}\bigg|_{P_{jk}} + D_j \frac{\partial w}{\partial \nu_j}\bigg|_{P_{jk}} = 0 \tag{A.67}$$

where ν_k and ν_j are the exterior normals of Γ_k and Γ_j, respectively. That is, w satisfies

$$-\nabla \cdot (D_k \nabla w) + \mu_k w = 0, \quad \text{in } \Omega_k \setminus G, \tag{A.68}$$

$$w|_{P_{jk}} = D_k \frac{\partial w}{\partial \nu}\bigg|_{P_{jk}} = 0. \tag{A.69}$$

[4] Here, dist$(\cdot, \cdot)$ denotes a distance function.

Similarly, we can conclude that there is an open band B_k around Γ_k in $\Omega_k \backslash G$ such that $w|_{B_k} \equiv 0$. Our connectivity assumption C4 guarantees that the above propagation procedure works for all the sub-domains.

Now, we can proceed with the rest of the sub-domains and show that the conclusion of the theorem holds for each of those sub-domains. Without loss of generality, we may now assume that $G \subset\subset \Omega_1$. Let $F_1(x, y)$ be the fundamental solution of $-\nabla \cdot (D_1 \nabla u_0) + \mu_1 u_0$ with the Dirichlet condition at ∞, that is,

$$-\nabla \cdot (D_1 \nabla F_1(x, y)) + \mu_1 F_1(x, y) = \delta(x - y), \quad y \in \mathbf{R}^N. \tag{A.70}$$

Then, according to (A.29), we have

$$\int_{\Omega_1} F_1(x, y)(q_1(y) - q_2(y))\, dy = 0, \quad \forall x \in \mathbf{R}^N \setminus \bar{\Omega}_1. \tag{A.71}$$

Also, we have

$$-D_1 \Delta_y F_1(x, y)) + \mu_1 F_1(x, y) = 0, \quad \forall x \in \mathbf{R}^N \setminus \bar{\Omega}_1. \tag{A.72}$$

For $x \in \mathbf{R}^N$, let us define

$$W(x) = \int_{\Omega_1} F_1(x, y)(q_1(y) - q_2(y))\, dy. \tag{A.73}$$

Lemma D.2 implies that, for $x \in \mathbf{R}^N \setminus \bar{\Omega}_1$,

$$\begin{aligned}
W(x) &= \int_{\Omega_1} F_1(x, y)[\sum_{i=1}^{m} \lambda_i \chi_{B_{r_0^i, r_1^i}}(x_i) - \sum_{J=1}^{M} \Lambda \chi_{B_{R_0^j, R1}^{j}}(X_j)]dy \\
&= \sum_{i=1}^{m} \lambda_i \int_{r_0^i \le |y - x_i| \le r_1^i} F_1(x, y)dy - \sum_{J=1}^{M} \Lambda_j \int_{R_0^j \le |y - X_j| \le R_1^j} F_1(x, y)dy \\
&= \sum_{i=1}^{m} \lambda_i (\int_{r_0^i}^{r_1^i} w_n r^{N-1} \varphi_1(r) dr) F_1(x, x_i) - \sum_{j=1}^{M} \Lambda_j (\int_{R_0^j}^{R_1^j} w_n r^{N-1} \varphi_1(r) dr) F_1(x, X_j) = 0.
\end{aligned} \tag{A.74}$$

Since

$$-D_1 \Delta W + \mu_1 W = 0, \quad \text{on } \mathbf{R}^N \setminus \left\{ \bigcup_{i=1}^{m} \{x_i\} \cup \bigcup_{j=1}^{M} \{X_j\} \right\}, \tag{A.75}$$

the unique continuation theory [27] implies that $W \equiv 0$ in $R^N \setminus \left\{ \bigcup_{i=1}^{m} \{x_i\} \cup \bigcup_{j=1}^{M} \{X_j\} \right\}$, which immediately leads to our theorem. □

Remark D.5. *Actually, the solid/hollow ball sources assumed in Theorem D.4 can be generalized to any radial weight functions with radial supports, such as*

$$q_0(y) = \sum_{i=1}^{m} \lambda_i G(y - x_i, \sigma_i) \chi_{B_{\alpha\sigma^i, \beta\sigma^i}(x_i)}(y) \tag{A.76}$$

where $B_{\alpha\sigma^i,\beta\sigma^i}(y_i) \subset\subset \Omega_k$ for some $k \in [1, I]$, $\alpha < \beta$ are two fixed constants, and $G(x, \sigma)$ denotes the 3D radial Gaussian distribution of zero mean. The conclusion of Theorem D.4 can be similarly derived but the proof is omitted here for brevity.

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