

# BIOLUMINESCENCE TOMOGRAPHY: BIOMEDICAL BACKGROUND, MATHEMATICAL THEORY, AND NUMERICAL APPROXIMATION\*

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**Dedicated to Professor Junzhi Cui on the occasion of his 70th birthday**

## Abstract

Over the last couple of years molecular imaging has been rapidly developed to study physiological and pathological processes *in vivo* at the cellular and molecular levels. Among molecular imaging modalities, optical imaging stands out for its unique advantages, especially performance and cost-effectiveness. Bioluminescence tomography (BLT) is an emerging optical imaging mode with promising biomedical advantages. In this survey paper, we explain the biomedical significance of BLT, summarize theoretical results on the analysis and numerical solution of a diffusion based BLT model, and comment on a few extensions for the study of BLT.

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*Key words:* Biomedical imaging, Bioluminescence tomography (BLT), Inverse problem, Regularization, Numerical approximation, Error analysis.

## 1. Introduction

Tomography is an important branch of imaging science and technology which targets image reconstruction from indirect measurement of an object under consideration. Among its numerous applications, tomography has been the driving force in biomedical imaging. As cornerstones of modern hospitals and clinics, x-ray computed tomography (CT), magnetic resonance imaging (MRI), nuclear and ultrasound imaging are widely applied for spatial and temporal reconstructions of anatomical and functional features, generated tremendous healthcare benefits over the past decades.

Guided by the so-called NIH Roadmap, molecular imaging has been rapidly developed to study biological processes *in vivo* at the cellular and molecular levels [27, 29]. While some classic microscopic and spectroscopic techniques do reveal information on micro-structures of the tissues, only recently have molecular probes been utilized along with imaging technologies to detect and image molecular targets sensitively, specifically, and non-invasively. Among molecular imaging modalities, optical imaging is most attractive because of its unique advantages, especially performance and cost-effectiveness [8, 20, 30]. Fluorescent and bioluminescent probes

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are commonly used for optical molecular imaging in preclinical studies of mice and rats as models of various human diseases, as well as to a limited extent in clinical research. In this context, fluorescence molecular tomography (FMT) [21] and bioluminescence tomography (BLT) [26,28] are emerging as complementary optical molecular tomography modes.

Given the fast pace of the development in the BLT area and the major needs for more mathematical work, we present this survey as a reference for those mathematicians who are interested in solving cutting edge inverse problems for biomedical applications. In the following, first we explain the biomedical significance of BLT in Section 2. Then, we summarize theoretical results on the analysis and numerical solution of a diffusion-approximation based BLT model in Section 3. Finally, we discuss a few extensions of BLT in Section 4.

## 2. Biomedical Background

In the post-genomic era, great efforts are being made to associate genes to phenotypes for development of systems medicine that are predictive, preventive and personalized. An important aspect of this perspective is small animal imaging that allows *in vivo* studies at anatomical, functional, cellular and molecular levels. In molecular/cellular imaging, small animal features of interest are labeled with molecular probes [18,30]. A molecular probe has a high affinity for attaching itself to a target molecule and a tagging ability with a marker molecule that can be tracked outside a living body. Optical imaging methods include fluorescence molecular tomography (FMT) [21] and bioluminescent imaging (BLI) [22], which are most promising because of their performance and cost-effectiveness, and already successfully used to investigate tumorigenesis, cancer metastasis, cardiac diseases, cystic fibrosis, gene therapies, drug designs and so on. Particularly, bioluminescent imaging has unique capabilities in probing molecular and cellular processes, and produces superior signal-to-noise ratios with little background auto-fluorescence. In the March 2005 issue of the *Molecular Imaging Outlook*<sup>1)</sup>, 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 is reduced with the cell division. Piwnicka-Worms underlined in the same report that BLI could be applied to study almost all diseases in every small animal model.

Dr. Wang's group conceptualized and developed the first bioluminescence tomography (BLT) prototype which compensates for heterogeneous scattering properties of a mouse and performs quantitative 3D reconstruction of internal sources from bioluminescent views measured on the external surface of the mouse [7, 26, 28]. BLT has now become a rapidly developing area for optical molecular imaging. The introduction of BLT relative to planar bioluminescent imaging (BLI) 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 analysis on a bioluminescent source distribution become feasible inside a living mouse

The pre-requisites for BLT are bioluminescent probes, corresponding substrates, and subsequent signal collection. Naturally-occurring luciferases exhibit emission maxima between 480 nm and 635 nm. In principle, we may use luciferases with different spectral properties to sense various biological events. Recent results in the luciferase technology have confirmed spectrally-shifted signals from luciferases in various species and/or by mutagenesis. Among

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<sup>1)</sup> <http://www.diagnosticsimaging.com/molecularimagingoutlook/2005mar/02.jhtml>

the current options, combining firefly (*Photinus pyralis*) luciferase ( $\lambda_{\max} = 562$  nm) and click beetle (*Pyrophorus plagiopthalmus*) ( $\lambda_{\max} = 615$  nm) seems attractive because they utilize the same non-toxic substrate. There are also areas for further development of bioluminescence reporters that could expand the utility of bioluminescent imaging. These include isolation of novel luciferases, mutation of known luciferases, luminescence-resonance energy transfer to red-emitting fluorescent proteins, and development of luciferase substrate analogs with different emission properties. Coincidentally, the latest development in the cooled-CCD camera technology has reached the point that allows us to detect very weak optical signals such as bioluminescent signals on the mouse body surface.

### 3. Study of a Diffusion Based BLT Model

We use the symbol  $\Omega \subset \mathbb{R}^3$  for the domain occupied by a biological medium under consideration. The boundary of  $\Omega$  is denoted by  $\Gamma$ , which is assumed to be at least Lipschitz continuous. Thus, the unit outward normal vector  $\boldsymbol{\nu}$  exists almost everywhere (a.e.) on  $\Gamma$ .

Light propagation in the biological medium is described by the radiative transfer equation (RTE) [2, 19]. Denote by  $\mathbb{S}^2$  the unit sphere, and let  $\mu_a = \mu_a(\mathbf{x})$  and  $\mu_s = \mu_s(\mathbf{x})$  be the absorption and scattering coefficients of the medium. The steady state RTE is

$$\boldsymbol{\theta} \cdot \nabla_{\mathbf{x}} \phi + \mu_a \phi = \mu_s \int_{\mathbb{S}^2} k(\boldsymbol{\theta} \cdot \boldsymbol{\theta}') \phi(\mathbf{x}, \boldsymbol{\theta}') d\boldsymbol{\theta}' + q, \quad (3.1)$$

where  $\phi = \phi(\mathbf{x}, \boldsymbol{\theta})$  represents the expected number of photons per unit volume at location  $\mathbf{x} \in \Omega$  with a velocity in the direction  $\boldsymbol{\theta} \in \mathbb{S}^2$ , and  $q = q(\mathbf{x}, \boldsymbol{\theta})$  is a light source function. The scattering kernel function  $k$  is non-negative and is normalized by the condition

$$\int_{\mathbb{S}^2} k(\boldsymbol{\theta} \cdot \boldsymbol{\theta}') d\boldsymbol{\theta}' = 1.$$

In applications, Henyey-Greenstein scattering kernel function is widely used:

$$k_{HG}(s) = \frac{1}{4\pi} \frac{1 - g_a^2}{(1 + g_a^2 - 2g_a s)^{3/2}}, \quad -1 \leq s \leq 1.$$

Here the parameter  $g_a \in (-1, 1)$  is a measure for anisotropy, with  $g_a = 0$  corresponding to isotropic scattering.

The RTE (3.1) is to be supplemented by appropriate boundary value conditions. The forward model, namely the problem of determining the function  $\phi$  from the RTE and the boundary value condition with a known light source function  $q$ , has been theoretically studied extensively in the literature; see, e.g., [9] for results on existence and uniqueness of solutions.

Mathematically, BLT is the source inversion problem to recover  $q$  from optical measurement on the domain boundary  $\Gamma$ , utilizing detailed knowledge on the optical properties of  $\Omega$ . Note that knowledge of the individualized spatially variant optical properties is critical for BLT to work effectively.

The RTE is highly dimensional and presents a serious challenge for its accurate numerical simulations given the current level of development in computer software and hardware. However, since in the range of around 600 nm photon scattering outperforms absorption in a mouse, usually a diffusion approximation of the RTE is employed [2, 19]. The diffusion approximation of the RTE (3.1) is the following equation:

$$-\operatorname{div}(D\nabla u) + \mu_a u = q_0 \quad \text{in } \Omega, \quad (3.2)$$

where

$$u(\mathbf{x}) = \frac{1}{4\pi} \int_{\mathbb{S}^2} \phi(\mathbf{x}, \boldsymbol{\theta}) d\boldsymbol{\theta}, \quad q_0(\mathbf{x}) = \frac{1}{4\pi} \int_{\mathbb{S}^2} q(\mathbf{x}, \boldsymbol{\theta}) d\boldsymbol{\theta}$$

are the averaged quantities for  $\phi$  and  $q$  in all the directions. Here,  $D = 1/[3(\mu_a + \mu'_s)]$ ,  $\mu'_s = (1 - \bar{k})\mu_s$  is the reduced scattering coefficient with

$$\bar{k} = \frac{1}{4\pi} \int_{\mathbb{S}^2} \boldsymbol{\theta} \cdot \boldsymbol{\theta}' k(\boldsymbol{\theta} \cdot \boldsymbol{\theta}') d\boldsymbol{\theta}',$$

which is independent of  $\boldsymbol{\theta}$ . The equation (3.2) is to be supplemented by the boundary condition

$$u + 2AD \frac{\partial u}{\partial \nu} = g^- \quad \text{on } \Gamma, \quad (3.3)$$

where  $g^-$  is the incoming flux on  $\Gamma$ , and the differential operator  $\partial/\partial\nu$  denotes the outward normal derivative on  $\Gamma$ . The appearance of the parameter  $A$  in the boundary condition (3.3) is to incorporate diffuse boundary reflection arising from a refractive index mismatch between the body  $\Omega$  and the surrounding medium. Discussion of the value of the parameter can be found in [1, 5]. Usually, this parameter is computed by the formula

$$A = (1 + R)/(1 - R)$$

with a directionally varying refraction parameter

$$R = -1.4399\eta^{-2} + 0.7099\eta^{-1} + 0.6681 + 0.0636\eta$$

for some refractive index  $\eta$ . In BLT applications, the measurement is

$$g = -D \frac{\partial u}{\partial \nu} \quad \text{on } \Gamma \text{ or part of } \Gamma. \quad (3.4)$$

The BLT problem we study is then to find a source function  $q_0$  given  $g^-$  and  $g$  such that (3.2), (3.3) and (3.4) are satisfied. Inverse source problems in such a pointwise formulation are the subject of numerous references. A recent reference is [10], where the objective is to identify the source function as a linear combination of monopolar and dipolar sources. We comment in passing that there is a related but different problem, the so-called diffuse optical tomography (DOT), also based on the diffusion approximation, where the aim is to find optical properties (absorption and reduced scattering coefficients) of an object from diffuse signals generated by a controllable optical stimulation and measured on the external surface of the object. Some theoretical studies on the DOT problem are reported in [2, 3, 13, 24].

It is helpful to incorporate as much known information as possible in the problem formulation so as to reconstruct the source function more accurately. We call a subset of  $\Omega$  the support of the light source if the light source function is nonzero in the subset and is zero outside the subset. In applications, usually a rough bound on the support of the light source is available. Thus, we suppose  $\Omega_0 \subset \Omega$  is a region that contains the light source support. The set  $\Omega_0$  is known as the permissible region in the literature. It is desirable to have  $\Omega_0$  exactly the light source support. But even if  $\Omega_0$  is larger than the light source support, knowledge of a known  $\Omega_0$  is still helpful in reconstruction of the light source. Accordingly, the differential equation (3.2) is written in the following more precise form:

$$-\operatorname{div}(D\nabla u) + \mu_a u = p \chi_{\Omega_0} \quad \text{in } \Omega. \quad (3.5)$$

Here  $\chi_{\Omega_0}$  denotes the characteristic function of  $\Omega_0$ , i.e., its value is 1 in  $\Omega_0$ , and is 0 in  $\Omega \setminus \Omega_0$ .

To avoid complicated notation, we express the BLT problem as the determination of a source function  $p$  in the differential equation (3.5) from two boundary conditions:

$$u + 2AD \frac{\partial u}{\partial \nu} = g_1 \quad \text{on } \Gamma, \tag{3.6}$$

$$AD \frac{\partial u}{\partial \nu} = g_3 \quad \text{on } \Gamma, \tag{3.7}$$

i.e., we use the symbol  $g_1$  for  $g^-$ ,  $g_3$  for  $-Ag$ , and assume the measurement (3.4) is available on the entire boundary  $\Gamma$ . We can also consider the case where the measurement is available only on a part of the boundary. Note that the influx  $g_1$  is zero in a typical BLT problem where the experiment is done in a dark environment. Combining (3.6) and (3.7) we obtain a third possible boundary condition

$$u = g_2 \equiv g_1 - 2g_3 \quad \text{on } \Gamma. \tag{3.8}$$

Only two of the three boundary conditions (3.6)-(3.8) are independent. To determine the source function  $p$ , we may associate one of the three boundary conditions (3.6), (3.7) or (3.8) with the differential equation (3.5) to form a boundary value problem, and choose one of the remaining boundary conditions to form the inverse problem for  $p$ . To be definite, in the rest of the section, we choose (3.6) as the boundary condition for the boundary value problem, and use (3.8) for the recovery of the source function  $p$ . In other words, we study the following problem, in pointwise form.

**Problem 3.1.** *Given suitably smooth functions  $D > 0$ ,  $A > 0$ ,  $\mu_a \geq 0$ ,  $g_1$  and  $g_2$ , find a source function  $p$  such that the solution of the boundary value problem*

$$-\operatorname{div}(D\nabla u) + \mu_a u = p \chi_{\Omega_0} \quad \text{in } \Omega, \tag{3.9}$$

$$u + 2AD \frac{\partial u}{\partial \nu} = g_1 \quad \text{on } \Gamma \tag{3.10}$$

satisfies

$$u = g_2 \quad \text{on } \Gamma. \tag{3.11}$$

It is pointed out in [14] that Problem 3.1 is ill-posed: (1) in general, there are infinite many solutions; (2) when the form of the source function is specified, generally there are no solutions; and (3) the source function does not depend continuously on the data (instability). Since the BLT problem has to be solved through numerical means, lack of solution stability prevents the direct use of the pointwise formulation for practical simulations. We will study the BLT problem through minimizing the mismatch between predictions from the BVP and available measurements coupled with a regularization for stabilization.

We will use standard function spaces such as  $V = H^1(\Omega)$ ,  $V_0 = H_0^1(\Omega)$ ,  $Q = L^2(\Omega_0)$ ,  $L^2(\Omega)$ ,  $L^\infty(\Omega)$ , and  $L^\infty(\Gamma)$ . For the given data, we assume  $D \in L^\infty(\Omega)$ ,  $D \geq D_0$  a.e. in  $\Omega$  for some constant  $D_0 > 0$ ;  $A \in L^\infty(\Gamma)$ ,  $A_1 \leq A \leq A_2$  for some constants  $A_2 \geq A_1 > 0$ ; and  $\mu_a \in L^\infty(\Omega)$ ,  $\mu_a \geq 0$  a.e. in  $\Omega$ . We also assume  $g_1 \in L^2(\Gamma)$  and  $g_2 \in L^2(\Gamma)$ .

Suppose we seek the source function  $p$  in a closed convex subset  $Q_{ad}$  of the space  $Q$ . A typical choice in BLT applications is

$$Q_{ad} = \{q \in Q \mid q \geq 0 \text{ a.e. in } \Omega_0\}.$$

We may also choose  $Q_{ad}$  to be the subset of non-negatively valued functions from a finite dimensional subspace of linear combinations of specified functions such as the characteristic functions of certain subsets of  $\Omega$ .

For any  $q \in Q$ , the following weak formulation of the boundary value problem (3.9)-(3.10)

$$\int_{\Omega} (D \nabla u \cdot \nabla v + \mu_a u v) dx + \int_{\Gamma} \frac{1}{2A} u v ds = \int_{\Omega_0} q v dx + \int_{\Gamma} \frac{1}{2A} g_1 v ds \quad \forall v \in V \quad (3.12)$$

has a unique solution  $u = u(q) \in V$  by an application of the Lax-Milgram Lemma [4, 12]. Following the idea of Tikhonov regularization (e.g., [11, 25]), we let

$$J_{\varepsilon}(q) = \frac{1}{2} \|u(q) - g_2\|_{L^2(\Gamma)}^2 + \frac{\varepsilon}{2} \|q\|_Q^2, \quad \varepsilon \geq 0$$

and introduce the following problem which is similar to the one studied in [14].

**Problem 3.2.** Find  $p_{\varepsilon} \in Q_{ad}$  such that  $J_{\varepsilon}(p_{\varepsilon}) = \inf \{J_{\varepsilon}(q) : q \in Q_{ad}\}$ .

We have the following results concerning Problem 3.2.

- For any  $\varepsilon > 0$ , Problem 3.2 has a unique solution  $p_{\varepsilon} \in Q_{ad}$ . Moreover, the solution  $p_{\varepsilon} \in Q_{ad}$  is characterized by a variational inequality

$$(u(p_{\varepsilon}) - g_2, u(q) - u(p_{\varepsilon}))_{L^2(\Gamma)} + \varepsilon (p_{\varepsilon}, q - p_{\varepsilon})_Q \geq 0 \quad \forall q \in Q_{ad}.$$

When  $Q_{ad} \subset Q$  is a subspace, the variational inequality is reduced to a variational equation

$$(u(p_{\varepsilon}) - g_2, u(q) - u(0))_{L^2(\Gamma)} + \varepsilon (p_{\varepsilon}, q)_Q = 0 \quad \forall q \in Q_{ad}.$$

- The solution  $p_{\varepsilon}$  of Problem 3.2 depends continuously on all the data.
- Assume the solution set  $S_0$  for Problem 3.2 with  $\varepsilon = 0$  is nonempty (this is valid if e.g.,  $Q_{ad}$  is bounded). Then it is closed and convex. Moreover,

$$p_{\varepsilon} \rightarrow p_0 \text{ in } Q, \text{ as } \varepsilon \rightarrow 0,$$

where  $p_0 \in S_0$  is the unique element with minimal  $Q$ -norm among the solutions of Problem 3.2 for  $\varepsilon = 0$ :

$$\|p_0\|_Q = \inf_{q \in S_0} \|q\|_Q.$$

- If  $S_0 = \{p\}$ , then we have the convergence

$$p_{\varepsilon} \rightarrow p \text{ in } Q, \text{ as } \varepsilon \rightarrow 0.$$

For a numerical approximation of Problem 3.2, we use the finite element method to solve the boundary value problem (3.12). Let  $\{\mathcal{T}_h\}_h$  ( $h$ : meshsize) be a regular family of finite element partitions of  $\bar{\Omega}$  such that each element at the boundary  $\Gamma$  has at most one non-straight face (or at most one curved side when we consider a two-dimensional analogue of the BLT problem). For each triangulation  $\mathcal{T}_h$ , let  $V^h \subset H^1(\Omega)$  be the corresponding linear element space. For any  $q \in Q$ , denote  $u^h = u^h(q) \in V^h$  the finite element solution of the problem (3.12) defined by the relation

$$\begin{aligned} & \int_{\Omega} (D \nabla u^h \cdot \nabla v^h + \mu_a u^h v^h) dx + \int_{\Gamma} \frac{1}{2A} u^h v^h ds \\ &= \int_{\Omega_0} q v^h dx + \int_{\Gamma} \frac{1}{2A} g_1 v^h ds \quad \forall v^h \in V^h. \end{aligned} \quad (3.13)$$

Corresponding to the functional  $J_\varepsilon(\cdot)$ , let

$$J_\varepsilon^h(q) = \frac{1}{2} \|u^h(q) - g_2\|_{L^2(\Gamma)}^2 + \frac{\varepsilon}{2} \|q\|_Q^2, \quad \varepsilon \geq 0.$$

The admissible source function set  $Q_{ad}$  may or may not need to be discretized. In general, let  $\tilde{Q}_{ad} \subset Q_{ad}$  be non-empty, closed and convex. Later in the section, we will consider two possible choices of  $\tilde{Q}_{ad}$ . We then introduce the following discretization of Problem 3.2.

**Problem 3.3.** Find  $p_\varepsilon^h \in \tilde{Q}_{ad}$  such that  $J_\varepsilon^h(p_\varepsilon^h) = \inf \{J_\varepsilon^h(q) : q \in \tilde{Q}_{ad}\}$ .

Problem 3.3 has properties similar to those listed above for Problem 3.2.

For error estimation, we assume additionally that  $\Gamma \in C^{1,1}$ ,  $D \in C^{0,1}(\bar{\Omega})$ ,  $A^{-1} \in H^{1/2}(\Gamma)$ , and  $g_1 \in H^{1/2}(\Gamma)$ . We say the admissible set  $Q_{ad}$  and the boundary data  $g_1, g_2$  are compatible if for some  $p_1 \in Q_{ad}$ ,  $u(p_1) = g_2$  on  $\Gamma$ . The compatibility assumption is valid, e.g. where  $\Omega_0 = \Omega \in C^{1,1}$ ,  $g_2 \in H^{1/2}(\Gamma)$  and  $Q_{ad} = L^2(\Omega)$ . It is also valid when  $g_2$  is the trace of some solution of the boundary value problem (3.12).

We distinguish two cases regarding the choice of the set  $\tilde{Q}_{ad}$ . First, with the choice  $\tilde{Q}_{ad} = Q_{ad}$ , it can be shown that for some constant  $c > 0$  independent of  $\varepsilon$  and  $h$ ,

$$\begin{aligned} & \|u(p_\varepsilon) - u^h(p_\varepsilon^h)\|_{L^2(\Gamma)} + \varepsilon^{1/2} \|p_\varepsilon - p_\varepsilon^h\|_Q \\ & \leq c h^{3/4} \|u(p_\varepsilon) - g_2\|_{L^2(\Gamma)}^{1/2} \|p_\varepsilon - p_\varepsilon^h\|_Q^{1/2} + c h^{3/2} [\|p_\varepsilon\|_Q + \|g_1\|_{H^{1/2}(\Gamma)}]. \end{aligned}$$

Consequently, if  $Q_{ad}$  is a bounded set in  $L^2(\Omega)$ , then

$$\|u(p_\varepsilon) - u^h(p_\varepsilon^h)\|_{L^2(\Gamma)} + \varepsilon^{1/2} \|p_\varepsilon - p_\varepsilon^h\|_Q \leq c h^{3/4}.$$

And if  $Q_{ad}, g_1$  and  $g_2$  are compatible, then

$$\|u(p_\varepsilon) - u^h(p_\varepsilon^h)\|_{L^2(\Gamma)} + \varepsilon^{1/2} \|p_\varepsilon - p_\varepsilon^h\|_Q \leq c h^{3/2}.$$

Next, consider the case where  $\tilde{Q}_{ad}$  is constructed with a discretization of the set  $Q_{ad}$ . In addition to the regular family of finite element partitions  $\{\mathcal{T}_h\}_h$  of  $\bar{\Omega}$ , let  $\{\mathcal{T}_{0,H}\}_H$  be a regular family of finite element partitions of  $\bar{\Omega}_0$  such that each element at the boundary  $\partial\Omega_0$  has at most one non-straight face (at most one curved side for a two-dimensional version of the BLT problem). The partitions  $\mathcal{T}_h$  and  $\mathcal{T}_{0,H}$  do not need to be related; however,  $\mathcal{T}_h$  is allowed to be constructed based on  $\mathcal{T}_{0,H}$ . Let  $Q^H \subset Q$  be the piecewise constant space. Define  $\tilde{Q}_{ad} = Q_{ad}^H \equiv Q^H \cap Q_{ad}$ . We denote the solution of Problem 3.3 by  $p_\varepsilon^{h,H}$ . Denote by

$$E^H(p_\varepsilon) = \inf \{ \|p_\varepsilon - q^H\|_Q : q^H \in Q_{ad}^H \}$$

the best approximation error in  $Q$ -norm of  $p_\varepsilon$  by functions from  $Q_{ad}^H$ . Then, for some constant  $c > 0$  independent of  $\varepsilon, h$  and  $H$ ,

$$\begin{aligned} & \|u(p_\varepsilon) - u^h(p_\varepsilon^{h,H})\|_{L^2(\Gamma)} + \varepsilon^{1/2} \|p_\varepsilon - p_\varepsilon^{h,H}\|_Q \\ & \leq c \|u(p_\varepsilon) - g_2\|_{L^2(\Gamma)}^{1/2} \left[ H^{1/2} E^H(p_\varepsilon)^{1/2} + h^{3/4} \|p_\varepsilon - p_\varepsilon^{h,H}\|_Q^{1/2} \right] \\ & \quad + c H E^H(p_\varepsilon) + c h^{3/2} [\|p_\varepsilon\|_Q + \|g_1\|_{H^{1/2}(\Gamma)}]. \end{aligned}$$

Consequently, if  $Q_{ad}$  is bounded in  $Q$ , then

$$\|u(p_\varepsilon) - u^h(p_\varepsilon^{h,H})\|_{L^2(\Gamma)} + \varepsilon^{1/2} \|p_\varepsilon - p_\varepsilon^{h,H}\|_Q \leq c \left[ H^{1/2} E^H(p_\varepsilon)^{1/2} + h^{3/4} \right].$$

And when  $Q_{ad}$ ,  $g_1$  and  $g_2$  are compatible,

$$\begin{aligned} & \|u(p_\varepsilon) - u^h(p_\varepsilon^{h,H})\|_{L^2(\Gamma)} + \varepsilon^{1/2} \|p_\varepsilon - p_\varepsilon^{h,H}\|_Q \\ & \leq c \left[ h^{3/2} + H^{1/2} \varepsilon^{1/4} E^H(p_\varepsilon)^{1/2} + H E^H(p_\varepsilon) \right]. \end{aligned}$$

Numerical examples can be found in [14] showing the performance of the proposed numerical methods.

### 4. Extensions

In this section, we point out a few extensions of the BLT model studied in Section 3.

First, recall that the goal of BLT is to produce a quantitative reconstruction of a bioluminescent source distribution within a living mouse from bioluminescent signals measured on the body surface of the mouse. While in most BLT studies so far the optical parameters of the key anatomical regions are assumed known from the literature or diffuse optical tomography (DOT), these parameters cannot be very accurate in general. In [16], we propose and study a new BLT approach that optimizes optical parameters when an underlying bioluminescent source distribution is reconstructed to match the measured data. We prove the solution existence and the convergence of numerical methods. Also, we present numerical results to illustrate the utility of our approach and evaluate its performance.

Second, a two regularization parameter framework for the BLT problem is introduced and analyzed in [6]. Similar to the discussion in [6], for any  $q \in Q$ , we denote by  $u_1 = u_1(q) \in V$  the solution of the problem (3.12), and denote by  $u_2 = u_2(q) \in g_2 + V_0$  the solution of the problem

$$\int_{\Omega} (D\nabla u_2 \cdot \nabla v + \mu_a u_2 v) dx = \int_{\Omega_0} qv dx \quad \forall v \in V_0.$$

This is a weak formulation of the boundary value problem defined by (3.9) and (3.11). For fixed constants  $r_1, r_2 \geq 0$  with  $r_1 + r_2 = 1$ , we define the functional

$$J_{\varepsilon,r_1,r_2}(q) = \frac{r_1}{2} \|u_1(q) - g_2\|_{L^2(\Gamma)}^2 + \frac{r_2}{2} \|AD\partial_\nu u_2(q) - g_3\|_{L^2(\Gamma)}^2 + \frac{\varepsilon}{2} \|q\|_Q^2, \quad \varepsilon \geq 0,$$

and introduce the following problem.

**Problem 4.1.** Find  $p_{\varepsilon,r_1,r_2} \in Q_{ad}$  such that  $J_{\varepsilon,r_1,r_2}(p_{\varepsilon,r_1,r_2}) = \inf\{J_{\varepsilon,r_1,r_2}(q) : q \in Q_{ad}\}$ .

All the theoretical results presented in the previous section can be extended to the analysis of Problem 4.1 and its numerical approximations. Note that when  $r_2 = 0$ , Problem 4.1 reduces to Problem 3.2. Numerical results reported in [6] suggest that it is beneficial to choose the two regularization parameters  $r_1, r_2$  and the finite element mesh-size  $h$  such that  $r_2 = \mathcal{O}(r_1 h)$ .

Third, let us discuss at some length a general mathematical theory for the study of multi-spectral BLT. With simultaneous use of multiple optical reporters it becomes feasible to capture and decompose composite molecular and cellular signatures under *in vivo* conditions. That is, multispectral data can be measured in spectral bands on the body surface of a mouse, and the distributions of multiple biomarkers can be reconstructed in an integrated fashion using a sophisticated algorithm. In [17], a comprehensive mathematical framework for multispectral BLT is introduced and analyzed for the most general situation of using multiple bioluminescent

reporters whose spectral characteristics may be affected by their *in vivo* environment. In multi-spectral BLT, the spectrum is divided into certain numbers of bands, say  $i_0$  bands  $\Lambda_1, \dots, \Lambda_{i_0}$ , with

$$\Lambda_i = [\lambda_{i-1}, \lambda_i), \quad 1 \leq i \leq i_0 - 1, \quad \Lambda_{i_0} = [\lambda_{i_0-1}, \lambda_{i_0}].$$

Here,  $\lambda_0 < \lambda_1 < \dots < \lambda_{i_0}$  is a partition of the spectrum range. Let there be  $j_0$  biomarkers with bioluminescent source distributions  $p_j \chi_{\Omega_j}$ ,  $1 \leq j \leq j_0$ . Here,  $\Omega_j$  is a measurable subset of  $\Omega$ , and  $\chi_{\Omega_j}$  is the characteristic function of  $\Omega_j$ . The set  $\Omega_j$  is the permissible region for the source  $p_j$ . For each biomarker, its bioluminescent source distribution within the band  $\Lambda_j$  is  $\omega_{ij} p_j \chi_{\Omega_j}$ ,  $1 \leq i \leq i_0$ , with the weights  $\omega_{ij} > 0$  satisfying  $\sum_{i=1}^{i_0} \omega_{ij} = 1$ , for any  $1 \leq j \leq j_0$ . Denote by  $p_{ij} = \omega_{ij} p_j$  the portion of the source function  $p_j$  in the band  $\Lambda_i$ . We allow variation of the source spectrum caused by the environment. Thus, we will reconstruct sources  $p_{ij}$  such that  $p_{ij} \approx \omega_{ij} p_j$  with  $p_j = \sum_{i=1}^{i_0} p_{ij}$ . For each spectral band  $\Lambda_i$ ,  $1 \leq i \leq i_0$ , we use the following diffusion equations to describe the photon density  $u_{ij}$  in  $\Lambda_i$ :

$$-\operatorname{div}(D_i \nabla u_{ij}) + \mu_{a,i} u_{ij} = p_{ij} \chi_{\Omega_j} \quad \text{in } \Omega. \tag{4.1}$$

Here,  $D_i(\mathbf{x}) = 1/[3(\mu_{a,i}(\mathbf{x}) + \mu'_{s,i}(\mathbf{x}))]$ ,  $\mu_{a,i}(\mathbf{x})$  and  $\mu'_{s,i}(\mathbf{x})$  are the absorption coefficient and the reduced scattering coefficient within the band  $\Lambda_i$ . The bioluminescent imaging experiments are usually performed in a dark environment so that the natural boundary condition takes the form

$$u_{ij} + 2AD_i \frac{\partial u_{ij}}{\partial \nu} = 0 \quad \text{on } \Gamma. \tag{4.2}$$

With the emission filters of bandpasses  $\Lambda_i$ , the measured quantities are the outgoing flux densities [23]:

$$\tilde{f}_i = -D_i \frac{\partial}{\partial \nu} \sum_{j=1}^{j_0} u_{ij}(q_{ij}) = \frac{1}{2A} \sum_{j=1}^{j_0} u_{ij}(q_{ij}) \quad \text{on } \Gamma_i, \quad 1 \leq i \leq i_0. \tag{4.3}$$

We assume that  $\Gamma_i$  is a non-trivial part of the boundary, i.e.,  $\operatorname{meas}(\Gamma_i) > 0$ . Thus, we allow the situation where the measurement of the outgoing flux densities is available only on parts of the boundary  $\Gamma$ .

Let us introduce some notations to simplify the exposition. The range of the index  $i$  is  $\{1, \dots, i_0\}$ , and that of  $j$  is  $\{1, \dots, j_0\}$ ; in particular,  $\sum_i$  stands for  $\sum_{i=1}^{i_0}$ , and  $\sum_j$  stands for  $\sum_{j=1}^{j_0}$ . Matrix  $(\mathbb{R}^{i_0 \times j_0})$  valued variables, as well as their row or column vectors, will be indicated by Euler Fraktur alphabets, e.g.,  $\mathbf{p} = (p_{ij})$ ,  $\mathbf{q} = (q_{ij})$ ,  $\mathbf{u} = (u_{ij})$ , and

$$\mathbf{q}_{*j} = (q_{1j}, \dots, q_{i_0j})^T, \quad \mathbf{q}_{i*} = (q_{i1}, \dots, q_{ij_0}).$$

Vector valued variables are indicated by boldface math fonts. We denote

$$S(\mathbf{q}_{*j}) = \sum_i q_{ij}, \quad \ell_i(\mathbf{q}_{*j}) = q_{ij} - \omega_{ij} S(\mathbf{q}_{*j}), \quad \boldsymbol{\ell}(\mathbf{q}) = (\ell_i(\mathbf{q}_{*j})),$$

$$U_i(\mathbf{q}_{i*}) = \sum_j u_{ij}(q_{ij}), \quad \mathbf{U}(\mathbf{q}) = (U_i(\mathbf{q}_{i*})).$$

Then the boundary measurement equation (4.3) can be written as

$$\tilde{f}_i = -D_i \frac{\partial U_i(\mathbf{q}_{i*})}{\partial \nu} = \frac{1}{2A} U_i(\mathbf{q}_{i*}) \quad \text{on } \Gamma_i.$$

For a vector valued variable with a subscript, we use “ $\cdot_j$ ” to indicate its  $j$ th component, e.g.,  $\mathbf{p}_\varepsilon = (p_{\varepsilon,j})$ . Similarly, for a matrix valued variable with a subscript, we use “ $\cdot_{ij}$ ” for its  $(i,j)$ th component, e.g.,  $\mathbf{p}_{\varepsilon M} = (p_{\varepsilon M,ij})$ .

Let  $Q_j = L^2(\Omega_j)$ ,  $G_i = L^2(\Gamma_i)$ . Denote by  $Q_{ad,j}$  the admissible set for  $p_{ij}$ . We assume  $Q_{ad,j}$  is a closed convex subset of the space  $Q_j$ . Let

$$\mathfrak{Q} = \{\mathbf{q} = (q_{ij}) : q_{ij} \in Q_j\}$$

with the inner product and norm:

$$(\mathbf{p}, \mathbf{q})_{\mathfrak{Q}} = \sum_{i,j} w_{ij}(p_{ij}, q_{ij})_{Q_j}, \quad \|\mathbf{q}\|_{\mathfrak{Q}} = (\mathbf{q}, \mathbf{q})_{\mathfrak{Q}}^{1/2}$$

for some positive weighting constants  $w_{ij}$ . We seek the unknown source field  $\mathbf{p} = (p_{ij})$  of the multispectral BLT problem in

$$\mathfrak{Q}_{ad} = \{\mathbf{q} \in \mathfrak{Q} : q_{ij} \in Q_{ad,j}\}.$$

With possibly different positive weighting constants  $w_{\ell,ij}$ , we let

$$(\ell(\mathbf{p}), \ell(\mathbf{q}))_{\mathfrak{Q}_\ell} = \sum_{i,j} w_{\ell,ij} (\ell_i(\mathbf{p}_{*j}), \ell_i(\mathbf{q}_{*j}))_{Q_j}, \quad |\ell(\mathbf{q})|_{\mathfrak{Q}_\ell} = (\ell(\mathbf{q}), \ell(\mathbf{q}))_{\mathfrak{Q}_\ell}^{1/2}.$$

We also need the space  $\mathbf{G} = G_1 \times G_2 \times \dots \times G_{i_0}$ , endowed with the inner product and norm

$$(\mathbf{f}, \mathbf{g})_{\mathbf{G}} = \sum_i w_i(f_i, g_i)_{G_i}, \quad \|\mathbf{g}\|_{\mathbf{G}} = (\mathbf{g}, \mathbf{g})_{\mathbf{G}}^{1/2}$$

with positive constants  $w_i$ .

We assume  $\Omega \subset \mathbb{R}^d$  ( $d \leq 3$ ) is a non-empty, open, bounded set with a Lipschitz boundary  $\Gamma$ ,  $A(\mathbf{x}) \in [A_l, A_u]$  for some constants  $0 < A_l \leq A_u < \infty$ ,  $D_i \in L^\infty(\Omega)$ ,  $D_i \geq D_0$  a.e. in  $\Omega$  for some constant  $D_0 > 0$ ,  $\mu_{a,i} \in L^\infty(\Omega)$ ,  $\mu_{a,i} \geq 0$  a.e. in  $\Omega$ ,  $\tilde{f}_i \in L^2(\Gamma_i)$ .

For any  $q \in Q_j$ , define  $u_{ij}(q) \in V$  to be the unique solution of the problem

$$\int_{\Omega} [D_i \nabla u_{ij}(q) \cdot \nabla v + \mu_{a,i} u_{ij}(q) v] dx + \int_{\Gamma} \frac{1}{2A} u_{ij}(q) v ds = \int_{\Omega_j} q v dx \quad \forall v \in V. \quad (4.4)$$

Write  $f_i = 2A\tilde{f}_i$ ,  $\mathbf{f} = (f_i)$ . Let  $\varepsilon \geq 0$ ,  $M > 0$ , and define a penalized Tikhonov regularization functional

$$J_{\varepsilon M}(\mathbf{q}) = \frac{1}{2} [\|\mathbf{U}(\mathbf{q}) - \mathbf{f}\|_{\mathbf{G}}^2 + \varepsilon \|\mathbf{q}\|_{\mathfrak{Q}}^2 + M |\ell(\mathbf{q})|_{\mathfrak{Q}_\ell}^2].$$

We then introduce the following problem.

**Problem 4.2.** Find  $\mathbf{p}_{\varepsilon M} \in \mathfrak{Q}_{ad}$  such that  $J_{\varepsilon M}(\mathbf{p}_{\varepsilon M}) = \inf \{J_{\varepsilon M}(\mathbf{q}) : \mathbf{q} \in \mathfrak{Q}_{ad}\}$ .

We have the following results for the problem.

• Problem 4.2 with  $\varepsilon > 0$  has a unique solution  $\mathbf{p}_{\varepsilon M} \in \mathfrak{Q}_{ad}$ , and the solution  $\mathbf{p}_{\varepsilon M} \in \mathfrak{Q}_{ad}$  is characterized by a variational inequality

$$\begin{aligned} & (\mathbf{U}(\mathbf{p}_{\varepsilon M}) - \mathbf{f}, \mathbf{U}(\mathbf{q} - \mathbf{p}_{\varepsilon M}))_{\mathbf{G}} \\ & + \varepsilon (\mathbf{p}_{\varepsilon M}, \mathbf{q} - \mathbf{p}_{\varepsilon M})_{\mathfrak{Q}} + M (\ell(\mathbf{p}_{\varepsilon M}), \ell(\mathbf{q} - \mathbf{p}_{\varepsilon M}))_{\mathfrak{Q}_\ell} \geq 0 \quad \forall \mathbf{q} \in \mathfrak{Q}_{ad}. \end{aligned}$$

When  $Q_{ad,j} \subset Q_j$  are subspaces, the inequality is reduced to a variational equation

$$(U(\mathbf{p}_{\varepsilon M}) - \mathbf{f}, U(\mathbf{q}))_{\mathbf{G}} + \varepsilon (\mathbf{p}_{\varepsilon M}, \mathbf{q})_{\mathbf{\Omega}} + M (\ell(\mathbf{p}_{\varepsilon M}), \ell(\mathbf{q}))_{\mathbf{\Omega}_l} = 0 \quad \forall \mathbf{q} \in \mathbf{\Omega}_{ad}.$$

- The solution  $\mathbf{p}_{\varepsilon M}$  of Problem 3.2 depends continuously on the data.
- Suppose  $0 \in Q_{ad,j}$ . Then as  $M \rightarrow \infty$ ,  $\mathbf{p}_{\varepsilon M} \rightarrow \mathbf{p}_{\varepsilon} = (\omega_{ij} p_{\varepsilon,j})$  in  $\mathbf{\Omega}$ , where  $\mathbf{p}_{\varepsilon} = (p_{\varepsilon,j})$  with  $p_{\varepsilon,j} = S(\mathbf{p}_{\varepsilon,*j})$ , and  $\mathbf{p}_{\varepsilon} \in \mathbf{Q}_{ad}$  is the unique solution of the problem

$$J_{\varepsilon}(\mathbf{p}_{\varepsilon}) = \inf \{J_{\varepsilon}(\mathbf{q}) : \mathbf{q} \in \mathbf{Q}_{ad}\}, \quad J_{\varepsilon}(\mathbf{q}) = \frac{1}{2} [\|\mathbf{W}(\mathbf{q}) - \mathbf{f}\|_{\mathbf{G}}^2 + \varepsilon \|\mathbf{q}\|_{\mathbf{Q}}^2],$$

where  $\mathbf{Q} = Q_1 \times \cdots \times Q_{j_0}$  and  $\mathbf{Q}_{ad} = Q_{1,ad} \times \cdots \times Q_{j_0,ad}$ .

- Assume  $\mathbf{S}_{0M}$ , the solution set of Problem 4.2 with  $\varepsilon = 0$ , is nonempty. Then  $\mathbf{S}_{0M}$  is closed and convex. Moreover,

$$\mathbf{p}_{\varepsilon M} \rightarrow \mathbf{p}_{0M} \text{ in } \mathbf{\Omega}, \text{ as } \varepsilon \rightarrow 0,$$

where  $\mathbf{p}_{0M} \in \mathbf{S}_{0M}$  satisfies

$$\|\mathbf{p}_{0M}\|_{\mathbf{\Omega}} = \inf \{\|\mathbf{q}\|_{\mathbf{\Omega}} : \mathbf{q} \in \mathbf{S}_{0M}\}.$$

In particular, if the solution set  $\mathbf{S}_{0M} = \{\mathbf{p}_M\}$  is a singleton. Then

$$\mathbf{p}_{\varepsilon M} \rightarrow \mathbf{p}_M \text{ in } \mathbf{\Omega}, \text{ as } \varepsilon \rightarrow 0.$$

One particular multispectral BLT problem is discussed in [15], where some numerical results are reported.

Finally, we remark that the RTE based BLT problem is being under investigation.

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