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Ultrasound-modulated bioluminescence tomography

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We propose a method to reconstruct the density of a luminescent source in a highly scattering medium from ultrasound-modulated optical measurements. Our approach is based on the solution to a hybrid inverse source problem for the diffusion equation.

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The development of tools for molecular imaging has had a transformative effect on biomedical research [1]. There are multiple applications including mapping gene expression and following the course of infection in a single animal, among others. Optical methods hold great promise for molecular imaging, due to their spectroscopic sensitivity to chemical composition, nondestructive nature, and relatively low cost [2]. One particularly popular modality, known as bioluminescence imaging, makes use of a bioluminescent marker, most often the luciferin-luciferase system, as a reporter of molecular activity [3,4]. In a typical experiment, genetically modified light-emitting cells are introduced into a model organism and a CCD camera is used to record the intensity of emitted light. The resulting images convey information about the spatial distribution of the labeled cells. However, the images are not tomographic nor are they quantitatively related to the number density of the cells. One approach to this problem is to reconstruct the number density (optical source) from measurements of multiply scattered light, a method known as bioluminescence tomography (BLT) [5–13]. The corresponding inverse problem is a classical inverse source problem (ISP) and it is well known that such problems do not have unique solutions [14]. That is, more than one source can give rise to the same measurements. Uniqueness can be restored under strong mathematical assumptions, requiring *a priori* knowledge of the source geometry.

To overcome the problem of nonuniqueness in BLT requires a fundamentally new approach. In this Rapid Communication, we propose an imaging modality termed ultrasound modulated bioluminescence tomography (UMBLT), which is in the spirt of several recently developed hybrid imaging methods. In hybrid imaging (also called multiwave imaging), an external field is used to control the material properties of a medium of interest, which is then probed by a second field [15–33]. In the physical setting we consider, the source density is spatially modulated by an acoustic wave, while measurements of the emitted light are recorded. We find that it is possible to *uniquely* reconstruct the source density by an algebraic formula. Moreover, the reconstruction is stable in the sense that an error in the measurements is linearly related to the error in recovering the source. We note that the inverse problem of UMBLT has a quite different mathematical structure than those that arise in other hybrid imaging modalities such as acousto-optic tomography (AOT) [18]. In particular, the inverse problem of AOT is an inverse scattering problem which consists of solving a *nonlinear* partial differential equation. In contrast, the inverse problem of UMBLT is an inverse source problem which is formulated as a *linear* partial differential equation. Indeed, we believe that this is the first inverse source problem that has been considered in the context of hybrid imaging.

We note that our results are particularly timely in view of recent exciting work by Huynh *et al.* [34]. These authors report experiments in which a focused ultrasound beam is used to enhance the resolution of bioluminescence images. Such experiments provide the necessary data to reconstruct the source density in UMBLT.

We begin by recalling the mathematical formulation of BLT. We consider a highly scattering medium in which light propagates as a diffuse wave [35]. The energy density u of the wave is assumed to obey the time-independent diffusion equation

$$-\nabla\cdot\left[Dn^2\nabla\left(\frac{u}{n^2}\right)\right]+\alpha u = S \quad \text{in} \quad \Omega, \tag{1}$$

$$u+\ell\frac{\partial u}{\partial n}=0 \quad \text{on} \quad \partial\Omega. \tag{2}$$

Here Ω is a three-dimensional bounded domain, n is the index of refraction, α and D are the absorption and diffusion coefficients of the medium, S is the source density, and ℓ is the extrapolation length. We note that in bioluminescence imaging the source is *incoherent* and emits light over a broad range of frequencies. Thus, for the remainder of this Rapid Communication, we assume that the intensity is measured over a relatively narrow band of frequencies so that the frequency dependence of the absorption and diffusion coefficients can be neglected.

The inverse problem of BLT is to determine the source density S everywhere in the volume Ω from measurements of the intensity on $\partial\Omega$. As previously mentioned, this problem does not have a unique solution, due to the existence of nonradiating sources; such sources generate fields that vanish everywhere in their exterior. This difficulty may be overcome, to some extent, if it is known that S is constant on a fixed

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number of regions of known shape. It is also possible to determine geometrical properties of the source, such as its spatial extent.

To address the above mentioned difficulties, we introduce an acoustic wave field that spatially modulates the source. This *internal control* of the medium provides information that is not available in conventional ISPs. To proceed, we consider the medium to be a collection of particles (cells) suspended in a fluid in which the acoustic wave propagates. Some of the particles absorb and scatter light, while others act as sources and emit light. If a small amplitude acoustic wave is incident on the medium, then each particle will experience an acoustic radiation force and oscillate about its local equilibrium position. We assume that the acoustic pressure is a standing plane wave of the form $p = A\,\cos(\omega t)\cos(\mathbf{k}\cdot\mathbf{x}+\varphi)$, where ω is the frequency, A is the amplitude, $\mathbf{k}$ is the wave vector and φ is the phase of the wave. For simplicity, we have assumed that the speed of sound c_s is constant with $k = \omega/c_s$. If the particles have positions $\mathbf{x}_i$, then their number density is $\rho(\mathbf{x}) = \sum_i \delta(\mathbf{x}-\mathbf{x}_i)$. It can be seen that the number density is spatially modulated according to

$$\rho_\epsilon(\mathbf{x}) = \rho_0(\mathbf{x})[1+\epsilon\,\cos(\mathbf{k}\cdot\mathbf{x}+\varphi)], \tag{3}$$

where ρ_0 is the number density in the absence of the acoustic wave and $\epsilon = A/(\rho c_s^2) \ll 1$ is a small parameter [18]. Now, the source density is proportional to the density of light-emitting cells and is thus given by

$$S_\epsilon(\mathbf{x}) = S_0(\mathbf{x})[1+\epsilon\,\cos(\mathbf{k}\cdot\mathbf{x}+\varphi)], \tag{4}$$

where S_0 is the source density in the absence of the acoustic wave. The optical properties of the medium are also acoustically modulated. In particular, the index of refraction of the fluid in which the particles are suspended is modulated due to Brillouin scattering and is given by

$$n(\mathbf{x}) = n_0[1+\epsilon\gamma\,\cos(\mathbf{k}\cdot\mathbf{x}+\varphi)], \tag{5}$$

where n_0 is the unmodulated index of refraction and γ is the elasto-optical constant. We note that $\gamma \approx 0.3$ in water. In [18] it was shown that the absorption and diffusion coefficients are modulated according to

$$\alpha_\epsilon(\mathbf{x}) = \alpha_0(\mathbf{x})[1+\epsilon(2\gamma+1)\cos(\mathbf{k}\cdot\mathbf{x}+\varphi)], \tag{6}$$

$$D_\epsilon(\mathbf{x}) = D_0(\mathbf{x})[1+\epsilon(2\gamma-1)\cos(\mathbf{k}\cdot\mathbf{x}+\varphi)]. \tag{7}$$

Making use of the above results, we see that (1) and (2) become

$$-\nabla\cdot D_\epsilon\nabla u_\epsilon + \alpha_\epsilon u_\epsilon = S_\epsilon \quad \text{in} \quad \Omega, \tag{8}$$

$$u_\epsilon + \ell\frac{\partial u_\epsilon}{\partial n} = 0 \quad \text{on} \quad \partial\Omega, \tag{9}$$

where $u_\epsilon = u/n^2$.

The inverse problem is to recover S_0 from knowledge of u_ϵ on $\partial\Omega$. Here we assume that α_0 and D_0 are known everywhere in Ω as determined, for instance, by an optical tomography experiment. It will prove useful to consider the auxiliary problem

$$-\nabla\cdot D_0\nabla v_j + \alpha_0 v_j = 0 \quad \text{in} \quad \Omega, \tag{10}$$

$$v_j + \ell\frac{\partial v_j}{\partial n} = f_j \quad \text{on} \quad \partial\Omega, \quad j = 1,\ldots,N, \tag{11}$$

where f_j are boundary sources. If we multiply (10) by u_ϵ and (8) by v_j, take the difference of the resulting equations and integrate over Ω, we obtain the identity

$$\Sigma_\epsilon^{(j)} = \int_\Omega d^3x[(D_\epsilon - D_0)\nabla u_\epsilon\cdot\nabla v_j + (\alpha_\epsilon-\alpha_0)u_\epsilon v_j - v_j S_\epsilon], \tag{12}$$

where we have integrated by parts and applied the boundary conditions (9) and (11). The surface term $\Sigma_\epsilon^{(j)}$ is defined by

$$\Sigma_\epsilon^{(j)} = \int_{\partial\Omega} d^2x\left[u_\epsilon D_0\frac{\partial v_j}{\partial n} - v_j D_\epsilon\frac{\partial u_\epsilon}{\partial n}\right]. \tag{13}$$

Next, we perform an asymptotic expansion of u_ϵ and $\Sigma_\epsilon^{(j)}$ in the small parameter ϵ:

$$u_\epsilon = u_0 + \epsilon u_1 + \epsilon^2 u_2 + \cdots, \tag{14}$$

$$\Sigma_\epsilon^{(j)} = \Sigma_0^{(j)} + \epsilon\Sigma_1^{(j)} + \epsilon^2\Sigma_2^{(j)} + \cdots. \tag{15}$$

We find that to order $O(1)$,

$$\Sigma_0^{(j)} = \int_\Omega d^3x\, v_j S_0. \tag{16}$$

At $O(\epsilon)$ we have

$$\Sigma_1^{(j)}(\mathbf{k}) = \int_\Omega d^3x[(2\gamma-1)D_0\nabla u_0\cdot\nabla v_j + (2\gamma+1)\alpha_0 u_0 v_j - v_j S_0]\cos(\mathbf{k}\cdot\mathbf{x}+\varphi). \tag{17}$$

The intensity measured by a point detector on $\partial\Omega$, which collects light in the outward normal direction, is given by $I_\epsilon = c/(4\pi)(1+\ell^*/\ell)u_\epsilon$ [36]. Here ℓ^* is the transport length, which is related to the diffusion coefficient by $D = 1/3c\ell^*$. Making use of the boundary conditions (2) and (11) we see that (13) becomes

$$\Sigma_\epsilon^{(j)} = \frac{4\pi}{3}\frac{\ell^*}{\ell+\ell^*}\int_{\partial\Omega} d^2x\, f_j I_\epsilon\cos(\mathbf{k}\cdot\mathbf{x}+\varphi). \tag{18}$$

Evidently $\Sigma_1^{(j)}$ can be determined from experiment. Thus, by varying the wave vector $\mathbf{k}$ and the phase φ and inverting a Fourier transform, we can recover the so-called internal functional

$$H_j = (2\gamma-1)D_0\nabla u_0\cdot\nabla v_j + (2\gamma+1)\alpha_0 u_0 v_j - v_j S_0 \tag{19}$$

from measurements. That is,

$$H_j(\mathbf{x}) = \int\frac{d^3k}{(2\pi)^3}e^{-i\mathbf{k}\cdot\mathbf{x}}\left[\Sigma_1^{(j)}(\mathbf{k};0) + i\,\Sigma_1^{(j)}(\mathbf{k};3\pi/2)\right], \tag{20}$$

where the dependence of $\Sigma_1^{(j)}$ on φ has been made explicit.

The inverse problem now consists of recovering the source S_0 from the internal functional H_j. We emphasize that this

is an unusual inverse problem, since the data H_j is known everywhere in Ω. This situation can be compared with that of the ISP, where the data is known only on $\partial\Omega$. The ISP is thus underdetermined, which leads to the previously mentioned problem of nonuniqueness. In contrast, we will see that the availability of internal data in UMBLT allows for the unique recovery of S_0. We first consider the case of a single boundary source. Equation (19) then becomes

$$\frac{H}{v} = (2\gamma - 1)D_0\nabla \ln v \cdot \nabla u_0 + (2\gamma + 1)\alpha_0 u_0 - S_0, \quad (21)$$

which is well defined since v does not vanish in Ω. Using the fact that

$$S_0 = -\nabla \cdot D_0\nabla u_0 + \alpha_0 u_0, \quad (22)$$

we can eliminate S_0 from (21). We then find that u_0 obeys the equation

$$-(L - 2\gamma\alpha_0)u_0 = \frac{H}{v} \quad \text{in} \quad \Omega, \quad (23)$$

$$u_0 + \ell\frac{\partial u_0}{\partial n} = 0 \quad \text{on} \quad \partial\Omega, \quad (24)$$

where $Lu_0 := -\nabla \cdot D_0\nabla u_0 - (2\gamma - 1)D_0\nabla \ln v \cdot \nabla u_0$. If 0 is not an eigenvalue of $L - 2\gamma\alpha_0$ with the above prescribed boundary conditions (which holds with suitable smallness conditions on α_0 or Ω) [37], we can uniquely solve (23) for u_0 with

$$u_0 = -(L - 2\gamma\alpha_0)^{-1}\frac{H}{v}. \quad (25)$$

Once u_0 is known, we can obtain the source S_0 from (22). It follows immediately that S_0 can be reconstructed with Lipschitz stability. That is, errors in H propagate linearly to errors in S_0. More precisely, suppose that H and H' are the internal data corresponding to the sources S_0 and S_0', respectively. We then have the stability estimate

$$\|S_0 - S_0'\|_{L^2(\Omega)} \leqslant C\|H - H'\|_{L^2(\Omega)}, \quad (26)$$

where C is a fixed constant [37]. See [37] for the case when 0 is an eigenvalue of $L - 2\gamma\alpha_0$.

Next we consider the inverse problem with multiple boundary sources. Note that since the coefficients α_0 and D_0 are assumed to be known, the solutions v_j can be computed numerically and thus additional experiments do not need to be performed. To proceed, we assume that $(\nabla v_j, v_j)$ form a basis for every point in Ω. It can be seen that this condition holds if the boundary sources f_j are appropriately chosen [37]. Assuming this is the case, (19) forms a system of linear equations for the vector field $\mathbf{A} = (2\gamma - 1)D_0\nabla u_0$ and the function $f = (2\gamma + 1)\alpha_0 u_0 - S_0$ of the form $Mg = H$. Here $g = (f, \mathbf{A})$, $H = (H_1, \ldots, H_4)$, and

$$M = \begin{pmatrix} v_1 & & (\nabla v_1)^t \\ & \vdots & \\ v_4 & & (\nabla v_4)^t \end{pmatrix}. \quad (27)$$

Solving the above equations for f and $\mathbf{A}$ we obtain

$$f = \sum_j (M^{-1})_{1j} H_j, \quad (28)$$

$$A_i = \sum_j (M^{-1})_{i+1,j} H_j. \quad (29)$$

Since $\mathbf{A}/D_0$ is a gradient field, it follows that

$$u_0(\mathbf{x}) - u_0(\mathbf{x}_0) = \frac{1}{2\gamma - 1}\int_\Gamma \frac{1}{D_0}\mathbf{A} \cdot d\mathbf{x}, \quad (30)$$

where Γ is an arbitrary path beginning at a point $\mathbf{x}_0 \in \Omega$ and ending at $\mathbf{x}$. Using the above results, we find that the source S_0 may be obtained from the formula

$$S_0(\mathbf{x}) = S_0(\mathbf{x}_0) + (2\gamma + 1)[\alpha_0(\mathbf{x})u_0(\mathbf{x}) - \alpha_0(\mathbf{x}_0)u_0(\mathbf{x}_0)] - f(\mathbf{x}) + f(\mathbf{x}_0), \quad (31)$$

which is the main result of this Rapid Communication. As before, it is readily seen that S_0 can be reconstructed with Lipschitz stability. The corresponding stability estimate is of the form

$$\|S_0 - S_0'\|_{L^2(\Omega)} \leqslant C\sum_j \|H_j - H_j'\|_{L^2(\Omega)}, \quad (32)$$

where we have assumed that $S_0(\mathbf{x}_0) = S_0'(\mathbf{x}_0)$.

We now illustrate the above reconstruction procedure with numerical simulations. For simplicity, we consider the case of an infinite homogeneous medium. The absorption and diffusion coefficients are given by $\alpha_0 = 1.0\ \text{ns}^{-1}$ and $D_0 = 1.0\ \text{cm}^2\ \text{ns}^{-1}$, which is typical for biological tissue at optical wavelengths. The f_j are taken to be unit-amplitude point sources which occupy the vertices of a square of dimensions $L \times L$. Since the inverse problem is linear, it suffices to restrict our attention to a point source, which we place at the center of the square. In this setting, it is possible to compute the data H_j in closed form. In Fig. 1 we present a reconstruction of S_0 in

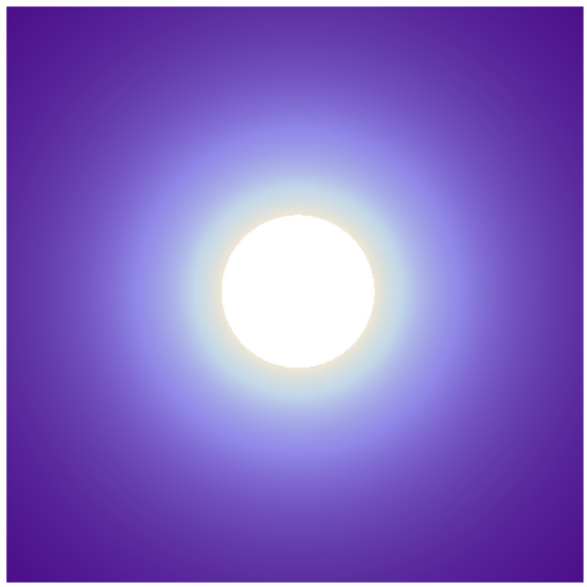

FIG. 1. (Color online) Reconstructed image of a point source. The field of view is $L/10 \times L/10$.

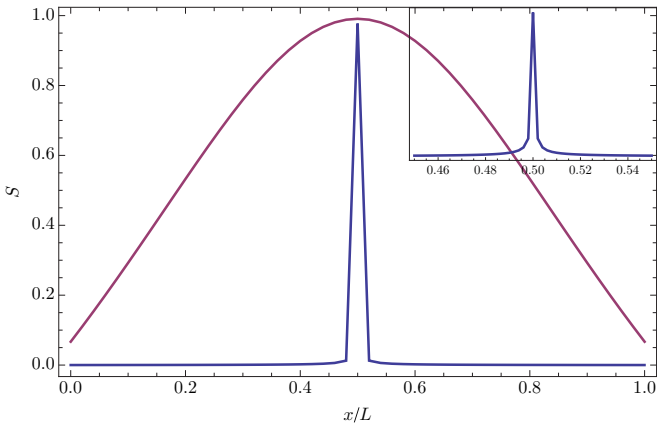

FIG. 2. (Color online) One-dimensional profile of the reconstructed source in arbitrary units. The inset shows the peak in greater detail. The curve shown in red (higher) is the transmitted intensity due to the source.

the plane containing the source. Here we take $L = 1$ cm and the integration in (30) is performed with a step size of $L/100$. Figure 2 shows a one-dimensional profile of the reconstructed source along a line passing through the center of the source. It can be seen that the resolution, as measured by the full width at half maximum (FWHM) is approximately $L/50$. We note that this must be considered to be a best-case estimate since the effects of noise have not been considered. However, the stability estimate (32) indicates that there will be relatively little degradation of the resolution in the presence of noise. It is instructive to contrast the above results with those that can be obtained by conventional bioluminescence imaging. To this end, also shown in Fig. 2 is the transmitted intensity due to the source measured on a line coinciding with an edge of the square region in which the measurements are performed. The FWHM of the intensity is approximately $L/5$. Thus the resolution of the reconstructed image is a factor of 10 higher than in conventional bioluminescence imaging.

We close with several remarks. (i) In general, the absorption coefficient α_0 and diffusion coefficient D_0 will not be known with high spatial resolution, as would be the case if they were determined from optical tomography experiments [35]. Thus, it would be of interest to determine the effect of errors in α_0 and D_0 on reconstruction of the source S_0. It may be anticipated from the stability estimate (32), that such errors would propagate linearly. However, a more refined analysis is necessary to separate the effects of low- and high-frequency errors in α_0 and D_0. (ii) The diffusion equation (1) is valid when the energy density varies slowly on the scale of the transport mean free path. This condition breaks down when the acoustic wavelength is sufficiently small. It would thus be useful to extend the theory we have developed to the regime in which light propagation is described by the radiative transport equation [35]. (iii) In many biomedical applications, the speed of sound in tissue is not constant. Our results generalize straightforwardly to this case. In particular, we note that for known, sufficiently localized fluctuations in the sound speed, recovery of the internal functional is possible by a suitably modified Fourier transform [38].

In conclusion, we have developed a hybrid imaging method for reconstructing the source density in bioluminescence tomography. Our approach is based on the solution to an inverse problem for the diffusion equation with interior control of boundary measurements.

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