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Tomographic phosphorescence lifetime multiplexing

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Abstract

We present a tomographic reconstruction algorithm for recovering distributions of multiple phosphorescent dyes within turbid media from time-resolved measurements, using either point or spatially patterned sources and detectors. The algorithm employs a multi-exponential analysis of time-resolved data, followed by tomographic inversion of the decay amplitudes to recover independent yield distributions for each lifetime present in the medium. Using Monte Carlo simulations, we computationally demonstrate that this two-step inversion approach provides several-fold improvement in quantitative and localization accuracy compared to a direct inversion of the time domain phosphorescence. We also demonstrate the tomographic reconstruction of up to three phosphorescent lifetimes embedded in thick tissue. The proposed algorithm can allow quantitative multiplexed tomography of luminescent and phosphorescent dyes for a wide range of *in vivo* applications.

Keywords

(170.3010) Image reconstruction techniques; (170.3880) Medical and biological imaging; (170.6920) Time-resolved imaging; (170.3650) Lifetime-based sensing; (170.6960) Tomography

Lifetime-sensitive luminescent and phosphorescent probes have been developed for a variety of biomedical applications, such as for imaging oxygen in tissue [1–4] and for multichannel bioimaging [5]. While existing applications of phosphorescence lifetime have primarily focused on *ex vivo* or microscopic imaging [3,4], the ability to recover distributions of phosphorescent probes within scattering media can extend these applications to non-invasive imaging in the whole body [6–8]. The recent development of oxygen sensitive probes with absorption closer to the near infrared region is an important step in this direction, since it can allow deep tissue imaging of phosphorescent probes [1,6].

The emission of phosphorescence occurs on microsecond to millisecond timescales, which is much longer than the nanosecond timescales for photon scattering and absorption within tissue. The long phosphorescence lifetimes implies that the spatial and temporal evolutions of phosphorescence in a turbid medium are independent of each other. In this Letter, we show that this spatio-temporal independence allows the time-resolved phosphorescence in diffuse media to be mathematically expressed as a matrix product of spatial and temporal factors. Furthermore, we show that this factorization can be exploited in a two-step, multi-

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exponential analysis-based time domain approach to obtain accurate tomographic reconstructions of multiple phosphorescent dyes within a turbid medium. This procedure is analogous to the asymptotic time domain (ATD) approach for fluorescence lifetime multiplexing [9], which exploits the late arriving (or asymptotic) photons in the time domain fluorescence signal. In the case of phosphorescence, the entire TD signal is in the asymptotic limit, so that the ATD formalism provides an exact and complete description of phosphorescence within turbid media. We present a rigorous theoretical formalism for tomographic inversion of phosphorescence using the ATD approach that allows the use of either point or spatially patterned sources and detectors. Using Monte Carlo simulations with spatially modulated sources and detectors, we demonstrate that the ATD approach provides a dramatic improvement in relative quantitation and localization accuracy over a direct inversion of TD phosphorescence for recovering up to three phosphorescent dyes with distinct lifetimes within a turbid medium.

Consider a bounded medium of support Ω obeying the radiative transfer equation (RTE) [10], embedded with N phosphorescent dyes with lifetimes $\tau_n = 1/\Gamma_n$ and associated phosphorescence yield distributions $\eta_n(\mathbf{r})$. Neglecting reemission of phosphorescence (first-order approximation), the TD phosphorescence signal for impulsive excitation at source position $\mathbf{r}_s$ and detection at $\mathbf{r}_d$ on the surface takes the form

$$U_F(\mathbf{r}_s, \mathbf{r}_d, t) = \int d^3r \sum_{n=1}^N [W(\mathbf{r}_s, \mathbf{r}_d, \mathbf{r}) e^{-\Gamma_n t}] \eta_n(\mathbf{r}), \quad (1)$$

where $W = G^x(\mathbf{r}_s, \mathbf{r}) G^m(\mathbf{r}, \mathbf{r}_d)$ is a time-independent, continuous-wave (CW) weight function, also called the sensitivity matrix, expressed as a product of the Green's functions, G^x and G^m , for light transport in the medium at the excitation (λ_x) and emission (λ_m) wavelengths. Only steady state or CW functions for light transport are relevant since the lifetimes ($\sim 10^{-6}$ s – 10^{-3} s) are much longer than timescales for light scattering in biological tissue ($\sim 10^{-9}$ s). The above equation can also be written as

$$U_F(\mathbf{r}_s, \mathbf{r}_d, t) = \sum_{n=1}^N a_n(\mathbf{r}_s, \mathbf{r}_d) e^{-\Gamma_n t}, \quad (2)$$

where the time-independent decay amplitudes are related to corresponding yield distributions:

$$a_n(\mathbf{r}_s, \mathbf{r}_d) = \int d^3r W(\mathbf{r}_s, \mathbf{r}_d, \mathbf{r}) \eta_n(\mathbf{r}). \quad (3)$$

Equations (1)–(3) are valid for arbitrary complex shaped, heterogeneous tissue that obey the RTE or diffusion equations and offer two distinct approaches for tomographic inversion of phosphorescence. In Eq. (1), the full TD weight matrix (quantity in square brackets) is inverted to obtain the yield distributions, η_n , from the raw TD data, U_F . Equation (3), on the

other hand, separates the forward problem into independent forward problems for each lifetime, so that the yield, η_n , for each lifetime can be obtained from the corresponding decay amplitudes, a_n , by inverting the CW weight function W . We will show below that the latter approach offers dramatic improvement in accuracy of relative quantitation and localization. Equations (1)–(3) can be expressed in the spatial frequency domain (SFD) by performing a Fourier transformation with respect to the source and detector coordinates $\mathbf{r}_s$ and $\mathbf{r}_d$. In particular, Eqs. (2) and (3) take the following form in the SFD:

$$\tilde{U}_F(\mathbf{k}_s, \mathbf{k}_d, t) = \sum_{n=1}^N \tilde{a}_n(\mathbf{k}_s, \mathbf{k}_d) e^{-\Gamma_n t}, \quad (4)$$

$$\tilde{a}_n(\mathbf{k}_s, \mathbf{k}_d) = \int d^3r \tilde{W}(\mathbf{k}_s, \mathbf{k}_d, \mathbf{r}) \eta_n(\mathbf{r}), \quad (5)$$

where $\mathbf{k}_s$ and $\mathbf{k}_d$ are the source and detector spatial frequencies and $\tilde{W}$ is the SFD-CW sensitivity function for phosphorescent light transport, and is obtained as a 2-D spatial Fourier transform of the weight function W in Eq. (1).

To more clearly see the advantage of Eq. (3) for tomographic multiplexing, it is useful to write the forward problem in a matrix form. Discretizing the problem into V medium voxels, L time points and M pairs of sources and detectors (either point or patterned) at the medium boundaries, the forward problem in Eq. (1) can be expressed as a matrix equation:

$$y = \mathcal{W} \eta. \quad (6)$$

Here, y is a $(ML \times 1)$ data vector, $\mathcal{W}$ is the full $(ML \times NV)$ TD weight matrix [quantity within square brackets in Eq. (1)], $\eta = [\eta_1, \eta_2, \dots, \eta_N]^T$ is a $(NV \times 1)$ vector of unknown yields for both lifetimes. As mentioned above, the spatial and temporal dependence of $\mathcal{W}$ are independent due to the long phosphorescence lifetimes. Thus, the matrix $\mathcal{W}$ can be written as a product of two factors, expressed in the matrix form as

$$\mathcal{W} = A \bar{W}, \quad (7)$$

where $A = [\exp(-t/\tau_1) \otimes I, \exp(-t/\tau_2) \otimes I, \dots, \exp(-t/\tau_n) \otimes I]$ is a $(ML \times MN)$, overdetermined “basis” matrix containing Kronecker products of exponential decay terms and the $(M \times M)$ identity matrix, I , and $\bar{W} = \text{diag}(W, W, \dots, W)$ is a $(NM \times NV)$ block diagonal matrix containing the CW weight matrices W along the diagonal. Using the above factorization, Eq. (6) can now be written as

$$y = A \bar{W} \eta. \quad (8)$$

The inversion of TD phosphorescence can now proceed in two ways. In the first way, which we call ATD method, we use Eq. (8) and first invert the well-conditioned matrix A using its Moore–Penrose pseudoinverse $A^\dagger$, followed by a Tikhonov inversion of the matrix $\bar{W}$ [9]. The inverse problem takes the form

$$\eta_{\text{ATD}} = \bar{W}^T (\bar{W}\bar{W}^T + \lambda I)^{-1} a, \quad (9)$$

where $a = A^\dagger y = [a_1, \dots, a_N]^T$ is a $(MN \times 1)$ vector of decay amplitudes for the N lifetimes and M pairs of source-detector spatial frequencies. The conventional approach for time-resolved phosphorescence tomography [6,8], which we call the direct TD (DTD) approach, does not exploit the two-step nature of the inverse problem, but rather performs a direct inversion of the full TD weight matrix $\mathcal{W}$ in Eq. (6). The Tikhonov form of the DTD inversion is

$$\eta_{\text{DTD}} = \mathcal{W}^{-1} y = \mathcal{W}^T (\mathcal{W}\mathcal{W}^T + \lambda I)^{-1} y. \quad (10)$$

The DTD inverse problem in Eq. (10) can be expressed in an alternate form by exploiting the factorized form in Eq. (7). Substituting for $\mathcal{W}$ from Eq. (7) into Eq. (10), we arrive at the following expression for the DTD inversion:

$$\eta_{\text{DTD}} = \bar{W}^T (\bar{W}\bar{W}^T + \lambda C_a / C_y)^{-1} a, \quad (11)$$

where $C_a = (A^T A / \sigma_y^2)^{-1}$, $C_y = \sigma_y^2 I$ is the data covariance matrix and $\lambda = (\sigma_y / \sigma_\eta)^2$, with σ_y and σ_η as the variances in the measurement and yield. Comparison of Eqs. (9) and (11) reveals the key distinction between DTD and ATD, which is that the inverse operator in Eq. (9) (quantity within parenthesis) is block diagonal, with each submatrix on the diagonal representing a lifetime component in the problem. This implies zero crosstalk between the two lifetimes. On the other hand, the inverse operator in Eq. (11) is not block diagonal (reflecting that the forward problems are not independent) due to the term C_a . We will see that this difference results in significant performance difference between the two methods. We also note that the size of the matrix to be inverted in Eq. (11), $\bar{W}\bar{W}^T$ is $NM \times NM$, which is much smaller than the $LM \times LM$ matrix for full TD inversion in Eq. (10), $\mathcal{W}\mathcal{W}^T$, since typically $N \ll L$. Thus Eq. (11) offers a dramatic reduction in the computation of DTD which can otherwise be prohibitive for more than a few time gates. Since the basis of deriving Eq. (11) from Eq. (10) is Eq. (7), which is an exact equation for phosphorescence, Eq. (11) is an exact expression for a computationally efficient implementation of DTD inversion of phosphorescence.

We next computationally validate the advantages of ATD [Eq. (9)] over DTD [Eq. (11)] for phosphorescence tomography using Monte Carlo (MC) simulations, performed using “MCX,” a GPU-based accelerated MC computing platform [11]. We consider a slab medium

(6 cm × 4 cm × 1.8 cm, 1 mm³ voxels) with absorption $\mu_a^x = \mu_a^m = 0.1 \text{ cm}^{-1}$, scattering $\mu_s^x = \mu_s^m = 10 \text{ cm}^{-1}$, anisotropy of 0.01, and refractive index of 1.37. Two 1 mm³ fluorescent inclusions of equal concentration are placed at a height of $z = 10 \text{ mm}$, with varying lateral separations of 10, 2, and 0 mm. We first consider a case where the inclusions have lifetimes of 100 and 150 μs . The simulations were performed in the SFD with sources and detectors consisting of 2-D sinusoidal patterns of the form $S_n = 0.5[1 + \cos(2\pi(k_x x + k_y y) + \phi_n)]$ (shown in Fig. 1), where the three phases $\phi_n = 2n\pi/3$ ($n = 0, 1, 2$) are used to eliminate the D.C. background [12,13]. k_x and k_y ranged from 0 to 0.5/cm in six steps, resulting in 36 sources at $z = 0$. The detectors consisted of the same 36 patterns at $z = 1.8 \text{ cm}$. Shot noise (2.6%, SNR = 32 dB) was added to the MC TD data. The source and detector TD Green's functions $G^{x,m}$ and, subsequently, the CW weight function $\bar{W}$ were calculated for the 36 source and detector patterns and for 64 time gates between 0 and 1.2 ms (resulting in 1296×64 measurements). Next, the decay amplitudes, $\tilde{a}_1$ and $\tilde{a}_2$, corresponding to the lifetimes, τ_1 and τ_2 , were recovered from the decay portion of the TD signal for all M pairs of source and detector spatial frequencies, using a linear biexponential fit with the known lifetimes. The linear fit employed all 64 gates in the signal. Recall that the ATD and DTD inverse problems in Eqs. (9) and (11) allow inclusion of a large number of gates without much computational cost, while the use of more than a few gates would be computationally intractable using the standard DTD expression in Eq. (10). The amplitudes a_n 's were next used to recover the yield distributions for the two lifetime components using the ATD [Eq. (9)] and DTD [Eq. (11)] inversion methods. The regularization, λ , was chosen to be equal for both the ATD and DTD reconstructions.

Figures 2(a)–2(c) show 2-D slices of the DTD phosphorescence yields, η_1 (100 μs) and η_2 (150 μs), as the red and green components of a single RGB image, normalized to the maximum of both yields. Figures 2(d)–2(f) show the corresponding line profiles along the X-direction, through the true location of the inclusions for each yield distribution. Similarly, Figs. 2(g)–2(i) and Figs. 2(j)–2(l) respectively, show 2-D slices of the ATD reconstructions and the corresponding line profiles. It is clear that DTD results in significantly higher crosstalk (~40%), which can be seen for the 10 mm case [Fig. 2(d)] as a shoulder in the line profiles at the incorrect location. The crosstalk is less apparent for the 2 and 0 mm separations, but is manifest as a significant error in localization >1 mm for the 2 mm separation. The ATD approach results in <2% error in recovering the relative quantitation and provides nearly perfect localization for all three separations. The significantly better performance of ATD can be attributed to the block diagonal form of the ATD inverse problem in Eq. (9), which implies zero crosstalk between η_1 and η_2 .

We next compared the crosstalk performance of ATD and DTD for a wide range of lifetime separations typically encountered in phosphorescence applications [1,4]. TD phosphorescence was simulated using MCX for a single inclusion at 10 mm, with a lifetime ranging from 100–200 μs in steps of 10 μs , with the other measurement parameters identical to the simulations in Fig. 2(a). For each lifetime, the inversion was performed assuming two lifetimes were present in the same inclusion, one fixed at $\tau_1 = 100 \mu\text{s}$ and the other lifetime varied from $\tau_2 = 110$ –200 μs in steps of 10 μs . The crosstalk was then calculated as the

averaged fraction of the recovered yield for τ_1 (τ_2) when only the τ_2 (τ_1) is present in the inclusion (i.e., crosstalk = $[\eta_1/\eta_2|\tau_2 \text{ only} + \eta_2/\eta_1|\tau_1 \text{ only}]/2$). This simulation setup is ideal for calculating crosstalk, since any reconstructed yield in the second lifetime component is spurious and represents the crosstalk as defined by the off-diagonal blocks of the model resolution matrix (or point spread function) [9]. Figure 3(a) shows that DTD results in nearly 75% crosstalk for short lifetime separations and improves to ~32% as the lifetime separation is nearly doubled. The ATD approach, on the other hand, provides nearly zero crosstalk for the entire range of lifetime separations considered.

While the simulations in Fig. 2 assumed equal phosphorescence yields ($\eta_1 = \eta_2$), the yields of individual lifetimes can be arbitrary in a realistic scenario. The influence of crosstalk is more strongly realized when the yields are unequal. Figure 3(b) shows the error in relative quantitation as a function of lifetime separation between two overlapping phosphorescent inclusions in the same simulation geometry as Fig. 3(a). The error is shown for three different ratios of true yield, namely, $\eta_1/\eta_2 = 1:1$, $1.5:1$ and $2:1$, where η_1 is the yield for the 100 μs lifetime and η_2 is yield for the varying lifetime. From Fig. 3(b), it is seen that while the DTD inversion results in significant quantitation error >30% for unequal yields, ATD provides less than ~8% error for lifetime separations above 10% and less than ~2% error for lifetime separations above 50%. These simulations indicate the robustness of the ATD approach for providing accurate relative quantitation across a range of lifetime separations and phosphorescence yield strengths.

Finally, we consider the tomographic recovery of three phosphorescent inclusions with distinct lifetimes. Three 1 mm³ phosphorescent inclusions with equal yield and lifetimes of $\tau_1 = 100 \mu\text{s}$, $\tau_2 = 150 \mu\text{s}$, and $\tau_3 = 200 \mu\text{s}$ are located at depths of $z = 13 \text{ mm}$, $z = 10 \text{ mm}$, and $z = 7 \text{ mm}$. The use of varying depths was to illustrate the robustness of recovering relative quantitation across regions with varying sensitivity. Figure 4 shows 2-D slices of the reconstructed yield and the corresponding line profiles for all three lifetimes as the red, green, and blue components of a single RGB image. It is clear that the DTD results in significant crosstalk for all three lifetimes, with more than 50% crosstalk in the 150 μs inclusion from both the 100 and 200 μs inclusions. The resulting relative quantitation ratio for DTD is $\eta_1:\eta_2:\eta_3 = 0.82:0.60:1$. The ATD once again results in negligible crosstalk for all three lifetimes and results in a relative quantitation ratio of $\eta_1:\eta_2:\eta_3 = 1:0.81:0.96$ for the three yield distributions.

We have presented an algorithm for tomographic lifetime multiplexing of phosphorescent dyes embedded in a turbid medium such as biological tissue. The algorithm is based on a two-step analysis, consisting of multi-exponential fits followed by separate reconstructions of individual 3-D yield distributions of all the lifetime components present in the medium. Using Monte Carlo simulations, we computationally demonstrated that this approach provides high accuracy for recovering the relative quantitation and localization of up to three phosphorescent inclusions in a turbid medium obeying the RTE equation. The smallest spatial separation considered in this Letter (2 mm) is more than 10-fold smaller than that of previous works. The ability to accurately localize and quantify such closely spaced as well as overlapping, phosphors illustrates the powerful advantages of the ATD approach for quantitative tomographic phosphorescence lifetime multiplexing. A novel aspect of the

methodology presented herein is the incorporation of spatially patterned sources and detectors for phosphorescence tomography. This allows for the well-known advantages of structured light imaging, such as faster imaging speed, improved spatial resolution, contrast, and dynamic range performance [14–16] to be extended to whole-body phosphorescence imaging. Therefore, the approach presented here can enable quantitative tomographic lifetime multiplexing using phosphorescent and luminescent probes for a variety of biological applications.

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Fig. 1.

Examples of source and detector spatial patterns used as input to the Monte Carlo simulations. The patterns were of the form $S_n = 0.5[1 + \cos(2\pi(k_x x + k_y y) + \phi_n)]$. The lowest (0) and highest (0.5 cm^{-1}) frequencies along the x and y dimensions are shown.

Fig. 2.

Comparison of (a)–(f) DTD with (g)–(l) ATD reconstructions of two phosphorescent inclusions (indicated by “x”) with lifetimes of 100 and 150 μ s and equal emission yields, embedded at a height of 10 mm in a 1.8 cm thick turbid slab, with separations of 10 (left), 2 (center), and 0 mm (right). The 2-D slices of the (a)–(c) DTD and (g)–(i) ATD yield reconstructions η_1 and η_2 for 100 and 150 μ s lifetimes are shown as red and green components of a single RGB image, normalized to the maximum of both yields (thus, the yellow color indicates the equal yield for the 0 mm separation case). (d)–(f) Normalized line profiles along the X-direction through the location of the inclusions of DTD and (j)–(l) ATD reconstructed η_1 (red) and η_2 (green), shown with true inclusion locations (vertical gray lines).

Fig. 3.

(a) Comparison of crosstalk using DTD (blue) and ATD (red) reconstructions as a function of lifetime separation. Time-resolved phosphorescence was simulated for a single inclusion of lifetime 100 μs at a depth of $z = 10$ mm for the same simulation conditions as in Fig. 2. The ATD and DTD reconstructions were performed assuming two lifetimes, one fixed at $\tau_1 = 100$ μs and the other varied between $\tau_2 = 110$ –200 μs . (Crosstalk is defined in the text.) (b) Error in the reconstructed relative quantitation (η_1/η_2) as a function of the lifetime separation between two overlapping phosphors located at a height of 10 mm in the same simulation setup as in (a). Quantitation error is shown for DTD (blue) and ATD approach (red) for three different ratios of true yields: $\eta_1/\eta_2 = 1:1$ (dashed–dotted line), 1.5:1 (dotted line), and 2:1 (solid line).

Fig. 4.

Comparison of the DTD and ATD reconstructions for three phosphorescent inclusions of equal yield and with lifetimes of $\tau_1 = 100 \mu\text{s}$, $\tau_2 = 150 \mu\text{s}$, and $\tau_3 = 200 \mu\text{s}$. The inclusions are separated by 10 mm along the lateral (“X”) direction and were located at depths of $z = 13 \text{ mm}$, 10 , and 7 mm in a 1.8 cm thick slab medium with the same optical properties as in Fig. 1. The top row shows the DTD and ATD reconstructions for the three lifetimes as the red (η_1), green (η_2), and blue (η_3) components of a single RGB image. The bottom row shows the corresponding line profiles along the X-direction through the location of the inclusions.