Published in final edited form as:

Opt Lett. 2008 April 15; 33(8): 782–784.

Influence of optical heterogeneities on reconstruction of spatial phosphorescence lifetime distributions

Sofia V. Apreleva and Sergei A. Vinogradov*

Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Abstract

We have previously demonstrated that phosphorescence lifetime imaging (PLI) allows for unbiased determination of absolute oxygen levels in homogeneously absorbing/scattering media. We computationally show that tomographic PLI can perform equally well in heterogeneous environments relying only on surface measurements of phosphorescence.

The theoretical basis of tomographic phosphorescence lifetime imaging [PLI] [1] is closely related to that of fluorescence lifetime imaging (FLI) [2,3]. However, large differences between lifetimes of singlet (10^{-9} - 10^{-8} s) and triplet (10^{-5} - 10^{-4} s) states bring about significant differences in the data acquisition and image reconstruction approaches used in these two methods. Optical parameters μ_a, k [μ_a -absorption coefficient (mm^{-1}), k -diffusion coefficient (mm)], and fluorescence lifetimes τ are strongly coupled at the level of the FLI inverse problem, reflecting the fact that photon diffusion and emission of fluorescence occur on the same time scale. Consequently, small errors in recovery of distributions $\{\mu_a\}$ and $\{k\}$ [4] lead to large errors in the recovery of life-time distributions $\{\tau\}$. To battle against the ill-posedness of the FLI inverse problem, independent measurements of the excitation field are required [5].

The emission of phosphorescence occurs 10^4 - 10^5 times slower than the photon diffusion, and consequently, the kernel of the PLI inverse problem does not involve phosphorescence lifetimes [1]. As a result, lifetime distributions $\{\tau\}$ should be less sensitive to uncertainties in the recovery of distributions $\{\mu_a\}$ and $\{k\}$. Herein, we study this property of PLI and computationally show that time-gated measurements of phosphorescence provide sufficient information for the reconstruction of lifetime images (and, subsequently, $p\text{O}_2$ images) even when images of absorption and scattering cannot be reconstructed with adequate accuracy.

Experimentally measured boundary quantity $\Psi(m_s, m_d, \text{ and } t_d)$, i.e., emission collected after delay time t_d for source s and detector d , is given by the integral [1]

$$\begin{aligned} \Psi(m_s, m_d, t_d) &= \int_{t_d}^{\infty} \Gamma(m_s, m_d, t) dt \\ &= \frac{A}{2} \int_v G_{\text{em}}(m_d, \mathbf{r}) G_{\text{ex}}(m_s, \mathbf{r}) I_0(\mathbf{r}) \exp\left(-\frac{t_d}{\tau(\mathbf{r})}\right) d^3 \mathbf{r}, \\ I_0(\mathbf{r}) &= \varphi_0 \mu_a^p(\mathbf{r}, \lambda_{\text{ex}}), \end{aligned} \quad (1)$$

where m_s and m_d are the source and detector coordinates, $G_{\text{em}}(m_d, \mathbf{r})$ and $G_{\text{ex}}(m_s, \mathbf{r})$ are the solutions of the steady-state diffusion equations for emission and excitation photons, $\mu_a^p(\mathbf{r}, \lambda_{\text{ex}})$ is the local absorption by the phosphorescent probe (the product of the probe's concentration and the molar extinction coefficient), ϕ_0 is the probe's phosphorescence quantum

*Corresponding author: vinograd@mail.med.upenn.edu

OCIS codes: 170.3880, 170.6920.

yield in the absence of quencher ($pO_2=0$), and τ is the phosphorescence lifetime. Constant A depends on the refraction parameter R , $A=(1+R)/(1-R)$. In our simulations, the excitation photon density distribution $G_{ex}(m_s, \mathbf{r})$ was calculated based on the total absorption μ_a^l ,

$\mu_a^l(\mathbf{r}, \lambda_{ex}) = \mu_a^m(\mathbf{r}, \lambda_{ex}) + \mu_a^p(\mathbf{r}, \lambda_{ex})$, where $\mu_a^m(\mathbf{r}, \lambda_{ex})$ is the background absorption by the medium (tissue without the probe). Distribution $G_{em}(m_s, \mathbf{r})$ was calculated assuming zero absorption by the probe at the emission wavelength $[\mu_a^p(\mathbf{r}, \lambda_{em})=0]$. Diffusion equations were solved by the finite element method (FEM) using the Robin boundary conditions.

For a solution to the inverse problem we used the maximum entropy method (MEM), in which the entropy S is maximized subject to constraints imposed by the preselected value of chi-squared χ^2 :

$$S = - \sum_j p_j \log \frac{p_j}{\lambda_j},$$

$$\chi^2 = \sum_n \sum_i^K \left\{ \frac{(\Psi_{ni} - y_{ni})^2}{\sigma_{ni}^2} \right\}, \quad (2)$$

where p_j are the components of vector $\mathbf{p}$, consisting of all the parameters $\{\mu_a\}$, $\{k\}$, and $\{\tau\}$; λ_j are the initial values for p_j ; y_{ni} are the boundary measurements; σ_{ni}^2 is the variance; N is the number of the source-detectors pairs; K is the number of delays t_d ; and L is the total number of parameters in the search. Previously, we used a recursive version of the MEM to reconstruct phosphorescence intensity distributions for each delay t_d [1] and then exponentially fitted these distributions to obtain the lifetime image $\{\tau\}$. Here, the data for all delays t_d s were simultaneously inverted varying all the parameters $\{\mu_a\}$, $\{k\}$, and $\{\tau\}$. A large-scale MEM algorithm [6] was employed, which conducts searches in subspaces, spanned over only three basis vectors $\mathbf{e}_\mu$ ($\mu=1-3$):

$$\begin{aligned} \mathbf{e}_1 &= \mathbf{p} (\nabla S) |\nabla S|^{-1}, \\ \mathbf{e}_2 &= \mathbf{p} (\nabla \chi^2) |\nabla \chi^2|^{-1}, \\ \mathbf{e}_3 &= \mathbf{p} (\nabla \nabla \chi^2 \mathbf{e}_1) - \mathbf{p} (\nabla \nabla \chi^2 \mathbf{e}_2), \end{aligned} \quad (3)$$

where ∇S and $\nabla \chi^2$ are the gradients of S and χ^2 , $|\nabla S|$ and $|\nabla \chi^2|$ are their norms, and $\nabla \nabla S$ and $\nabla \nabla \chi^2$ are the corresponding Hessians. The notation $\mathbf{a}(\mathbf{b})$ designates point-wise multiplication, i.e., the vector with components $a_j b_j$. A significant advantage of this method is that it avoids inversion of large matrices, considerably gaining speed. For example, in the methods based on full Hessian inversion, the speed is roughly proportional to 2^L , whereas for this algorithm, the speed linearly increases with L .

Finding the gradient and Hessian of χ^2 requires partial derivatives $\partial \Psi / \partial \mu_a$ and $\partial \Psi / \partial k$, which were calculated using the FEM [7]. For simplicity, we assumed that the absorption heterogeneities were due only to the variations in the absorption of tissue μ_a^m , while the absorption by the probe was held constant throughout the volume, $\mu_a^p(\mathbf{r}, \lambda_{ex}) = 0.006 \text{ mm}^{-1}$ [1]. Accordingly, everywhere in the text herein μ_a refers to μ_a^m , unless otherwise stated. For example, it is the images of distributions $\{\mu_a^m\}$ that are shown in Fig. 1. Under this assumption, distributions $\{\mu_a\}$ and $\{k\}$ at the wavelengths of excitation and emission can be related to each other by simple proportionality relationships, $\mu_a(\mathbf{r}, \lambda_{em}) = a \times \mu_a(\mathbf{r}, \lambda_{ex})$, $k(\mathbf{r}, \lambda_{em}) = b \times k(\mathbf{r}, \lambda_{ex})$, and the partial derivatives can be expressed as

$$\begin{aligned} \frac{\partial \Psi_m}{\partial \mu_{aex}} &= \frac{A}{2} \int_V \left\{ \tau(\mathbf{r}) I(\mathbf{r}) \exp\left(-\frac{t_d}{\tau(\mathbf{r})}\right) \times \left[a G_{em}(m_d, \mathbf{r}) \frac{\partial G_{ex}(m_s, \mathbf{r})}{\partial \mu_{aex}} + G_{ex}(m_s, \mathbf{r}) \frac{\partial G_{em}(m_d, \mathbf{r})}{\partial \mu_{aex}} \right] \right\} d\mathbf{r}, \\ \frac{\partial \Psi_m}{\partial k_{ex}} &= \frac{A}{2} \int_V \left\{ \tau(\mathbf{r}) I(\mathbf{r}) \exp\left(-\frac{t_d}{\tau(\mathbf{r})}\right) \times \left[b G_{em}(m_d, \mathbf{r}) \frac{\partial G_{ex}(m_s, \mathbf{r})}{\partial k_{ex}} + G_{ex}(m_s, \mathbf{r}) \frac{\partial G_{em}(m_d, \mathbf{r})}{\partial k_{ex}} \right] \right\} d\mathbf{r}, \\ \frac{\partial \Psi_m}{\partial \tau} &= \frac{A}{2} \int_V \left\{ \tau(\mathbf{r}) I(\mathbf{r}) \exp\left(-\frac{t_d}{\tau(\mathbf{r})}\right) G_{em}(m_d, \mathbf{r}) G_{ex}(m_s, \mathbf{r}) \times \left(\frac{1}{\tau(\mathbf{r})} + \frac{t_d}{\tau^2(\mathbf{r})} \right) \right\} d\mathbf{r}, \end{aligned} \quad (4)$$

where $\mu_{\text{aex}} = \mu_a(\mathbf{r}, \lambda_{\text{ex}})$ and $k_{\text{ex}} = k_{\text{ex}}(\mathbf{r}, \lambda_{\text{ex}})$. At wave-lengths $\lambda_{\text{ex}} = 635$ and $\lambda_{\text{em}} = 780$ nm [1], constants a and b have values of 0.85 and 0.9, respectively [8].

Notably, the assumption about the uniformity of the probe's distribution is not required by the method. It was used to emphasize the fact that the contrast in the phosphorescence images is determined only by the degree of oxygen quenching and not by the probe accumulation in the regions of hypoxia [9]. Distributions $\{\mu_a^m\}$ and $\{\mu_a^p\}$ can be independently reconstructed without affecting the reconstruction of lifetime distributions.

Our simulations were designed to demonstrate the effects of optical heterogeneities on the ability of the method to reconstruct images of phosphorescence lifetimes solely from the surface distributions of phosphorescence. A 2D-cylindrical model ($\varnothing = 8$ cm) with 18 evenly spaced peripheral source/detector sites (306 pairs) was chosen (Fig. 1). The probe's constants were $k_q = 150 \text{ mm Hg}^{-1} \text{ s}^{-1}$ and $\tau_0 = 260 \mu\text{s}$. (Lifetime τ is related to oxygen pressure $p\text{O}_2$ by the Stern-Volmer relationship, $1/\tau = 1/\tau_0 + k_q \times p\text{O}_2$.) Optical parameters and oxygen levels (phosphorescence lifetimes) were chosen to approximate those found in some biological tissues [8,10]. Two hypoxic regions were positioned as shown in Fig. 1 (left-most column). Region A was characterized by a lower $p\text{O}_2$ and longer phosphorescence lifetime

($p\text{O}_2^A = 5 \text{ mm Hg}$, $\tau^A = 230 \mu\text{s}$), while region B had a higher $p\text{O}_2$ and shorter lifetime

($p\text{O}_2^B = 17 \text{ mm Hg}$, $\tau^B = 160 \mu\text{s}$). The background was characterized by

$p\text{O}_2^{bg} = 64 \text{ mm Hg}$ ($\tau^{bg} = 75 \mu\text{s}$). The heterogeneities in μ_a and k were placed in the object either in the same location as the hypoxic region (region B) or in separate independent locations (regions C and D).

The reconstructions (Fig. 1, columns a-c) were performed using a two-step approach: step 1—constant values of μ_a (0.004 mm^{-1}) and k (0.29 mm), typical for biological tissues [11], were assumed throughout the entire volume, and the lifetime distribution $\{\tau\}$ was recovered using the data integrated only after large delays t_d ($t_d > 150 \mu\text{s}$) to emphasize contributions of the hypoxic region; step 2—thus obtained distribution $\{\tau\}$ was used as the initial guess in the full-scale optimization, using data collected at all delays t_d with the parameters $\{\mu_a\}$, $\{k\}$, and $\{\tau\}$ unrestricted and simultaneously varied.

In the first experiment (Fig. 1, column a), the medium was optically homogeneous, with absorption and scattering parameters constant throughout the volume, $\mu_a = 0.003 \text{ mm}^{-1}$, and $k = 0.302 \text{ mm}$. In the following three experiments (Fig. 1, columns b and c), gradually increasing heterogeneities in absorption and scattering were introduced at positions B, C, and D (see the left-most column). The corresponding values of μ_a and k are shown at the bottom of Fig. 1. Normally distributed noise was added to the data to produce signal-to-noise ratios close to those observed in the previously described experiments (for definitions and examples see [1]).

Images of phosphorescence lifetimes, recovered using fixed distributions $\{\mu_a\}$ and $\{k\}$ exactly matching those in the simulations, are shown in the first (upper) row of Fig. 1. In this reconstruction, step 1 was omitted and only the lifetimes $\{\tau\}$ were unrestricted in step 2. Thus obtained $\{\tau\}$ images are not influenced by errors in $\{\mu_a\}$ and $\{k\}$ and, therefore, can be considered a reference for all the other reconstructions. Remarkably, all $\{\tau\}$ images obtained in blind reconstructions (second row) practically matched those in the reference row, even though distributions $\{\mu_a\}$ and $\{k\}$ (third and fourth rows) were very poorly recovered. Recovery of images $\{\tau\}$ showed good accuracy both spatially and in terms of absolute values. For example, in case Fig. 1b (see second row), the average lifetimes (τ_{av}) for regions A and B were found to be 223 and 146 μs , respectively, (obtained by averaging over the original regions shown on the left) and 79 μs for the background; the original values were 230, 160, and 75

μs , respectively. A slight decrease ($<10\%$) in τ_{av} for regions A and B and the respective increase for the background is due to the lifetime-mixing effect, as previously observed and rationalized [1].

Unlike lifetime distributions, absorption and diffusion distributions did not reproduce well, except for the homogeneous case (Fig. 1a). The diffusion model in PLI is considered quasi-static [1] owing to the very long time scale of the phosphorescence emission. It is well known that static measurements are inadequate for accurate reconstructions of absorption/scattering coefficients. Recovered images typically suffer from significant cross talk between the parameters, which can be clearly seen in Fig. 1, reconstructions b and c (third and fourth rows). Nonetheless, inability to accurately recover distributions $\{\mu_a\}$ and $\{k\}$ did not significantly affect reconstructions of the phosphorescence lifetime images $\{\tau\}$, which behaved almost as an invariant with respect to $\{\mu_a\}$ and $\{k\}$. This suggests that a well-defined minimum exists in the τ subspace, whereas the minimum in the (μ_a, k) subspace is rather poorly defined. Topologically, the surface of the optimization functional near the minimum is likely to resemble a “canyon” with steep walls (cross section in the τ dimension) and a flat bottom [cross section in the (μ_a, k) dimension]. Such stability of the $\{\tau\}$ distribution is a consequence of the fact that the convolution kernel in the PLI inverse problem in Eq. (1) depends only on parameters μ_a and k and is independent of phosphorescence lifetimes τ .

In conclusion, we have computationally demonstrated that PLI can be performed in optically heterogeneous media solely based on the surface measurements of phosphorescence. This result has a direct implication for the construction of future PLI instruments, which will not require the capability of measurements of excitation fields but record only surface measurements of the phosphorescence.

References and Notes

1. Apreleva SV, Wilson DF, Vinogradov SA. Appl. Opt 2006;45:8547. [PubMed: 17086268]
2. Patterson MS, Pogue BW. Appl. Opt 1994;33:1963.
3. O’Leary MA, Boas DA, Li XD, Chance B, Yodh AG. Opt. Lett 1996;21:158.
4. Here and throughout the text, we use the symbols $\{ \}$ to designate a distribution, an image, or a set of parameters
5. Soubret A, Ripoll J, Ntziachristos V. IEEE Trans. Med. Imaging 2005;24:1377. [PubMed: 16229423]
6. Skilling J, Bryan RK. Mon. Not. R. Astron. Soc 1984;211:111.
7. Paulsen KD, Jiang HB. Med. Phys 1995;22:691. [PubMed: 7565358]
8. Taroni P, Pifferi A, Torricelli A, Comelli D, Cubeddu R. Photochem. Photobiol. Sci 2003;2:124. [PubMed: 12664972]
9. Increase in the local probe concentration can be observed in tumors, which are typically characterized by higher blood content
10. Fantini S, Walker SA, Franceschini MA, Kaschke M, Schlag PM, Moesta KT. Appl. Opt 1998;37:1982. [PubMed: 18273118]
11. Dehghani H, Pogue BW, Poplack SP, Paulsen KD. Appl. Opt 2003;42:135. [PubMed: 12518832]

Fig. 1.

(Color online) Left-most column, spatial distributions of lifetimes $\{\tau\}$ (top), absorption coefficients $\{\mu_a\}$ (middle), and diffusion coefficients $\{k\}$ (bottom) used in simulations. The lifetimes in regions A, B, and in the back-ground were $\tau^A=230$, $\tau^B=160$, and $\tau^{bg}=75$ μs , respectively. The background values for μ_a and k were 0.003 mm^{-1} and 0.302 mm in all of the simulations. In the regions of heterogeneity (B, C, and D), μ_a and k changed as shown in the table (bottom), reflecting an increase in the optical heterogeneity (from a to c). First row, lifetime distributions $\{\tau\}$ reconstructed using fixed *a priori* known distributions $\{\mu_a\}$ and $\{k\}$, matching those in the original simulations. Second row, blind reconstructions $\{\tau\}$ performed without *a priori* knowledge of distributions $\{\mu_a\}$ and $\{k\}$. Third and fourth rows, distributions $\{\mu_a\}$ and $\{k\}$ simultaneously obtained with distributions $\{\tau\}$ (second row) in blind reconstructions.