A Direct Linear Reconstruction Method for Spectrally Resolved 3D Bioluminescence Tomography

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Abstract: Spectrally-resolved BioLuminescence optical Tomography (sBLT) is used to recover images of Luciferase activity within a 3D mouse model using multi-wavelength emission data from multiple internal bioluminescence sources. The images show that the location of sources can be reconstructed accurately using a fast, direct and linear reconstruction algorithm.

OCIS codes: (170.6960) Tomography; (100.3010) Image reconstruction techniques; (100.3190) Inverse problems

1. Introduction

The development and widespread implementation of bioluminescence imaging has resulted in cancer research undergoing a significant advance in the ability of investigators to determine cell growth in vivo [1]. In vivo bioluminescence imaging involves cells which are tagged with a reporter gene that encodes the sequence for a light-generating enzyme, luciferase. Luciferase is produced by the cell, and is a protein that can generate visible light through the oxidation of an enzyme-specific substrate. This process requires the presence of oxygen and ATP as a source of energy. Part of the chemical energy is released as visible light so that this signal becomes an optical indicator of cellular activity in vivo. Luciferase has, therefore, become a frequently used reporter in many biochemical and cell culture assays.

Tumor metastases studies have been completed with luciferase imaging, showing tumor cell invasion, which otherwise could not be observed [2]. Imaging of wound healing has also exploited this approach to visualize the process over several days [3]. Hardy et al, [4] have also demonstrated that luciferase bioluminescence from lymphocytes could be used to follow trafficking in vivo. As these imaging and measuring tools progress, it is important to have improved imaging and image reconstruction methods to accurately visualize and quantify the bioluminescence signals in vivo in order to gain fundamental insight into tumor growth, regression, immune response and cellular response to therapy. Specifically it is essential to develop 3D imaging methods to combine information from multiple imaging technologies, and further, to use the information from different sources to generate improved and increased understanding of the processes under investigation.

Recent interest in modeling and reconstruction algorithms for BLT has increased [5, 6] and led to the general consensus that non-spectrally resolved intensity-based BLT results in a non-unique problem[7]. However, the light emitted from firefly Luciferase is widely distributed over the band of wavelengths from 500 nm to 650 nm and above. When not attenuated, it produces a peak emission near 560 nm, but when detected from within an animal appears to have a peak closer to 600 nm with measurable emissions as much as 50 nm above and below this peak [1]. Using a spectrometer it is possible to measure the emission at the surface of the tissue in discrete steps of 10 nm ranging from 550 nm to 650 nm, although for deeper sources strong optical absorption of tissue at the lower wavelengths might preclude accurate measurements with adequate signal to noise. This paper demonstrates the development of a 3D algorithm used for multi-wavelength 3D sBLT image reconstruction in a mouse model. It is demonstrated through reconstructed images from simulated noise-added data that recovery with multi-wavelength sBLT produces images which are spatially accurate and superior to the conventional surface imaging.

2. Methods

The mathematical basis for sBLT is the Boltzmann transport equation, a physical model for light propagation in tissue. Under the assumption that scattering dominates absorption for red light in tissue, the Boltzmann transport equation can be simplified to the diffusion approximation, which for a continuous light source is given by:

$$-\nabla \cdot D(\boldsymbol{r})\nabla\Phi(\boldsymbol{r}) + \mu_a\Phi(\boldsymbol{r}) = B(\boldsymbol{r}) \qquad (1)$$

where $B(r)$ is an internal bioluminescence source, $\Phi(r)$ is the photon fluence rate at position r, $D=1/3(\mu_a+\mu_s')$ is the diffusion coefficient, μ_a is the optical absorption coefficient and μ_s' is the reduced scatter coefficient. A Robin (Type

III or mixed) boundary condition is used to account for reflection and refraction at the tissue surface. The fluence rate data can be represented by an operator, which is linear in terms of the bioluminescence source. It is assumed that absorption and scatter are known from separate measurements and reconstructions [8]. Thus, the BLT image reconstruction problem is posed as a solution to the minimization:

$$\hat{\chi} = \arg\min_{\mu_a,\kappa} \| (y - F(B(r))) \| \quad (2)$$

where y are the measured data, $F(B(r))$ are the data calculated using equation (1) given an estimate of the internal bioluminescence distribution model, and ||.|| is the weighted L2-norm, representing the square root of the sum of the squared elements. If the location of the bioluminescence source $B(r)$ is known a priori, the solution of the problem can be constrained to calculate the strength of $B(r)$ to minimize $\hat{\chi}$ in equation (2). However if no prior information about source location is available, the problem is inherently more difficult, since both the source strength and its spatial distribution may vary. However, the photon fluence rate, which is the measured observable, is linear in $B(r)$; therefore, a practical approach to solving this problem constructs the image of the bioluminescence source as a sum of basis distributions whose weights are estimated from the measured response. Taking advantage of the linearity of the model it is possible to create a set of independent basis solutions for the source, $B = \sum_{i=1}^{N} a_i b_i$, where the coefficients a_i are the weight functions for multiple unit sources b_i at each node i in the 3D model containing a total number of nodes N, which in matrix form is given as, $B = ab$. The size of B is reduced if a coarser basis is used for the source relative to the discretization of Φ in equation (1). Substituting this matrix expression into equation 2 and solving for a, in a least square manner, results in a *single step linear expression*, $a=W^T(WW^T+\lambda I)^{-1}y$, where W is a matrix containing the solution of equation (1) for all possible source positions N and y is the measured surface flux. Here, λ is a regularization parameter and I is the identity matrix. Although the Hessian matrix WW^T is invertible, the use of λ becomes necessary in the presence of noise in the data. In this work, λ = 0.001% of the maximum of the diagonal of the Hessian is used. Instead of using data from a single wavelength, multi-wavelength sBLT combines data-sets that are measured from the same domain containing the same bioluminescence distribution, over a range of usable wavelengths such that $a=\hat{W}^T(\hat{W}\hat{W}^T+\lambda I)^{-1}\hat{y}$, where $\hat{W}=[W_{\lambda 1}; W_{\lambda 2}; W_{\lambda 3}; \ldots; W_{\lambda n}]$ are the weight matrices of all n of cascaded wavelengths and $\hat{y} =[y_{\lambda 1}; y_{\lambda 2}; y_{\lambda 3}; \ldots; y_{\lambda n}]$ is the corresponding measured surface data for each wavelength. The solution a is a vector corresponding to the number of unknowns that define the bioluminescence source distribution.

3. Results

A 3D model of the MOBY mouse [9], Fig 1, was used to generate Bioluminescence data for two sources placed deep within the model. The 3D model contained 13031 nodes corresponding to 65958 linear tetrahedral elements and a total of 400 detector positions were uniformly distributed in a grid in the area indicated in Fig 1. The mouse was assumed to be homogeneous soft tissue containing 20 μMolar of total blood with 75% oxygen saturation and 60% water. Both the scatter amplitude and power of the tissue were assumed to be unity. Over the 600 – 650 nm range of wavelengths the absorption coefficient varied from 0.0281 to 0.0058 mm^{-1} and the reduced scattering from 1.6667 to 1.5385 mm^{-1} across the wavelengths used. These background optical properties can be obtained using spectral reconstruction at NIR wavelengths as discussed elsewhere[8]. Two bioluminescence sources were modeled deep within the tissue as shown in Fig 3 and the resulting surface fluence was calculated for a range of wavelengths as shown in Fig 2. Images of bioluminescence source distribution were reconstructed, Fig 3, using the methods described above, using a total of 6 wavelengths, ranging from 600-650 nm in steps of 10 nm. The time for the calculation of the weight matrix for the model was approximately 4 minutes per wavelength (using a dual Xeon 3.4GHz, 4GB RAM), giving a total reconstruction time of approximately 21 minutes including matrix inversion.

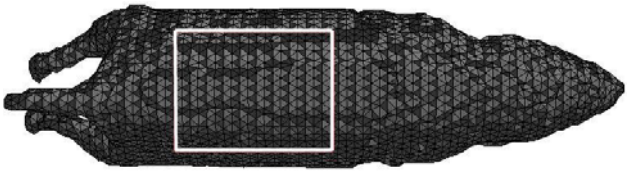

Figure 1. Three dimensional model of the mouse used. The white rectangular area indicated the surface area from which boundary data where measured.

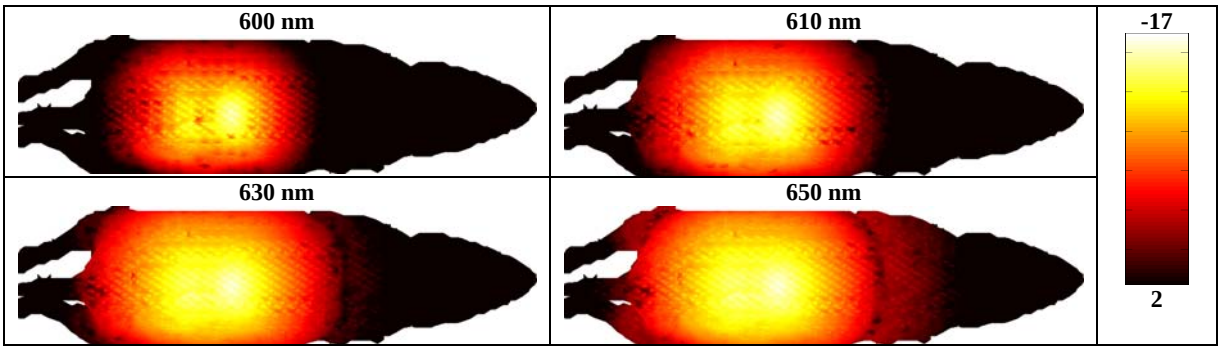

Figure 2. The log of surface fluence due to two separate internal sources of 5 mm radius placed at 3.5 and 7.5 mm beneath the external top surface. The sources are 10 mm apart in the horizontal axis.

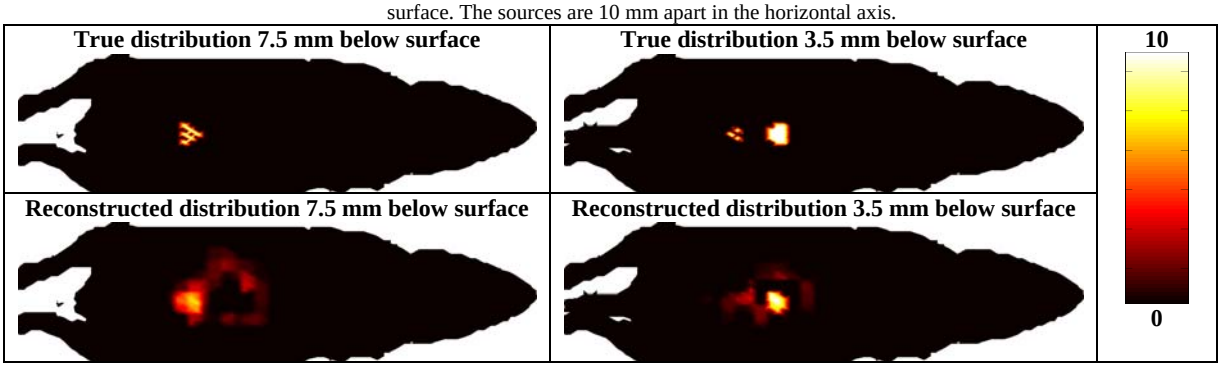

Figure 3. The true (top row) and reconstructed (bottom row) distribution of the Bioluminescence distribution.

4. Discussion

In this paper a modeling and linear single step image reconstruction algorithm for sBLT is presented which demonstrates BLT image recovery from 3D multi-wavelength data. Multi-wavelength emission provides a means of estimating the depth of an object, due to the wavelength dependent attenuation of tissue. Images generated from noisy simulated data have been presented which show that the position of the unknown multiple bioluminescence sources can be reconstructed accurately using a fast linear reconstruction methods. As a comparison to the conventional surface imaging, Fig 2, the reconstructed images demonstrate that although two sources are visible using conventional imaging, their depth and relative strengths can be accurately resolved using sBLT tomographic reconstructions.

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6. References

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