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Nonlinear Bayesian inversion methods reveal detail in fluorescence optical diffusion tomography images.

Hidden Pictures

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Place a flashlight under your hand, and you'll see a dim, red glow around the edges of your fingers. Contrary to intuition, human tissue is not opaque to light, but the light does not travel straight through. In the near-IR (NIR) spectral region (650 to 1000 nm), however, tissue's absorption of the radiation is relatively low, which has opened the door for optical diffusion tomography (ODT).

ODT uses NIR light measured at the tissue surface to reconstruct internal absorption and scattering properties.[1,2] Using appropriate mathematical inversion strategies, the method can image features that are obscured under several centimeters of tissue. While ODT does not have the spatial resolution of MRI or x-ray tomography, it offers a number of important advantages. It is safe, portable, relatively economical, and offers high temporal resolution. Used with spectroscopy, ODT can distinguish among different biochemical components, such as oxygenated and deoxygenated blood. As a result, ODT and NIR spectroscopy have been applied in clinical studies of brain function and hemodynamics, because blood flow and relative oxygenation can provide observable contrast in areas of brain activity.

Optical contrast also shows up in tumors. Blood concentration may be higher near tumors due to the increased growth of blood vessels. Also, relative blood oxygenation provides an indicator of the metabolic rate, which is correlated with tumor malignancy. Knowledge of these differences has led to extensive work in the area of optical breast imaging.

One way to improve the specificity and contrast of ODT in tumor imaging applications is through the use of fluorescent diagnostic agents. Just as the growth of new blood vessels may cause higher blood concentration, the growth of new blood vessels also may cause

injected fluorophores to accumulate in diseased tissue. Recent advances in specific, tumor-targeted contrast agents have enabled molecular imaging, in which the contrast is the result of events on a molecular scale, such as the expression of a receptor for the dye, instead of structural features.[3]

Fluorescence optical diffusion tomography (FODT) is an extension of ODT that reconstructs the fluorescent yield and lifetime, as well as the absorption and scattering. It works by sending light at the fluorophore's excitation wavelength into the tissue. The light can be pulsed, sinusoidally modulated, or unmodulated, depending on the requirements for speed, cost, and resolution. The sources, which are typically lasers coupled into an array of optical fibers, are illuminated one at a time. The fluorophore absorbs the incident light and then decays to its ground state with some characteristic time constant, emitting some of the light at a longer wavelength. An array of detectors—usually fibers or bundles coupled into photomultiplier tubes or avalanche photodiodes—collects the emitted light.

A Complex Task

The multiple scattering of light in tissue makes ODT and FODT quite complicated. The simple backprojection algorithms used in some forms of tomography are not sufficient to the task of reconstructing the light's path. To model the distribution of light throughout the random medium, researchers use a partial differential equation known as the diffusion equation. The unknown absorption and scattering coefficients, which are reconstructed in ODT, appear as coefficients in the diffusion equation, which creates a nonlinear inverse problem.

The inverse problem is typically computationally intensive and the computation tends to suffer from stability problems. Photons scatter in three dimensions, requiring the full 3-D numerical solution of the diffusion equation. Because of these difficulties, the inverse problem must be solved iteratively with robust modeling and optimization techniques. Calculations must incorporate prior information about the solution, typically by inserting a stabilizing or smoothing term in the inversion.

Recently, our group has introduced nonlinear Bayesian inversion methods for reconstructing the absorption, scattering, and fluorescence parameters in ODT and FODT.[4,5] We have validated the methods by reconstructing the fluorescent yield, fluorescence lifetime, and absorption coefficient in a series of experimental studies.[6,7]

The transport of light amplitude-modulated at a frequency ω through a highly scattering medium can be modeled using the photon diffusion equation:

$$\nabla \cdot [D(r)\nabla\phi(r,\omega)] - [\mu_a(r) + j\omega/c]\phi(r,\omega) = -S(r,\omega)$$

where r is position, $\phi(r,\omega)$ in watts per square centimeter is the complex modulation envelope of the photon flux, and $S(r,\omega)$ is the source term. The absorption coefficient is $\mu_a(r)$ in inverse centimeters and the diffusion coefficient $D(r)$, measured in centimeters, is inversely related to scattering. The modulation frequency ω is typically on the order of 100 MHz. This equation may be solved numerically using finite-element or finite-difference methods.

For the FODT problem, light at wavelength λ_x excites a fluorophore, which emits light at a longer wavelength λ_m. In general, the absorption coefficients and diffusion coefficients are not the same at the two wavelengths. The problem thus requires two coupled diffusion equations: one to describe the excitation light, and the second to describe propagation of the emission light. In this model, the fluorescence parameters are the lifetime $\tau(r)$, in seconds, which represents the exponential decay constant, and the fluorescent yield $\eta(r)$, in inverse centimeters, which incorporates the fluorophore's quantum efficiency and absorption. Suppose we know the diffusion coefficient, for example; then the absorption coefficients at both λ_x and λ_m, and the fluorescent parameters of yield and lifetime are unknown, and we must reconstruct them. The tomography measurements for each of these unknowns must be made with sources and detectors at the appropriate wavelengths (see figure 1).

A statistical framework that incorporates models of instrument noise and the behavior of typical images can solve the inverse problem. Let x denote one of the unknown images, and let y denote its corresponding data set. We compute the maximum *a posteriori* (MAP) estimate using Bayes' rule:

$$x_{MAP} = \arg\max_x \{p_{Y|X}(y|x) \times p_X(x)\}$$

where $p_{Y|X}(y|x)$ is the data likelihood and $p_X(x)$ is the prior density for the image. The data likelihood describes the fit between the data and the image. It incorporates instrument noise but no additional assumptions about smoothness or other image properties. The prior

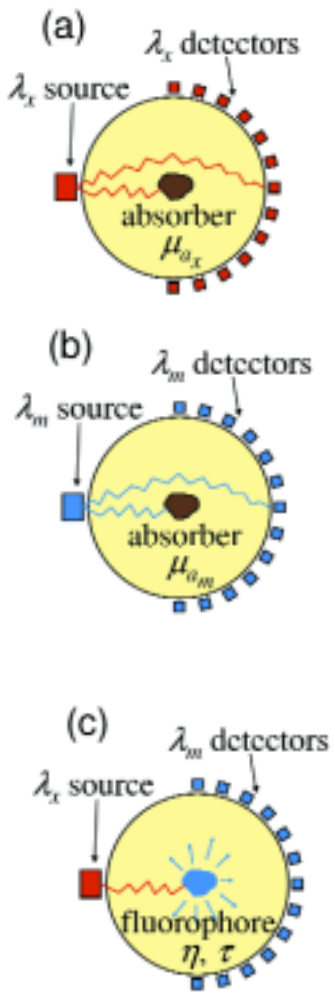

Figure 1 Measurement approach for reconstructing all unknowns involves using appropriate source and detector wavelengths for reconstructing absorption at λ_x (a), absorption at λ_m (b), and fluorescence yield and lifetime (c).

model incorporates statistical properties of typical images, such as smoothness, and must be present to ensure stability of the inversion. Above, we presented an independent Gaussian model for the data likelihood and a generalized Gaussian Markov random field model for the prior distribution. We solve this second equation numerically by use of an iterative optimization method.

Testing the Theory

To validate our framework for solving the ODT and FODT problems, we performed an imaging experiment to reconstruct the absorption and fluorescence of the clinical contrast agent indocyanine green (ICG) embedded within a tissue-simulating phantom. Such tissue phantoms are easily reproducible and have known optical properties that we can use to assess the reconstruction accuracy.

The tissue phantom comprised a 2-cm glass sphere containing ICG and 1% Intralipid, a fat emulsion, embedded within a 1% Intralipid suspension. The total thickness of the lipid suspension was 5.7 cm, with the sphere in the center. A measurement box with fiberoptic connectors contained the phantom. We selected source fiber positions on the bottom of the box. We coupled 20 mW of output from a tunable, mode-locked titanium-doped sapphire pulsed laser (Spectra-Physics; Mountain View, CA) into the source fibers, using a galvanometer-based optical scanner as a switch. The top of the phantom box was open, and a time-gated, intensified CCD camera captured images of the phantom's top surface.

We performed baseline measurements without the fluorophore present for calibration. After collection of the baseline data, we added 0.125 µM ICG into the sphere. We tuned the laser to the excitation and emission peaks for ICG and added appropriate filters to allow us to reconstruct the unknown absorption and fluorescence parameters. We sampled point spread functions from the camera image, put them through a Fourier transform, and selected three frequency components. Applying the inversion method generated the reconstructions, which were qualitatively and quantitatively reasonable (see figure 2).

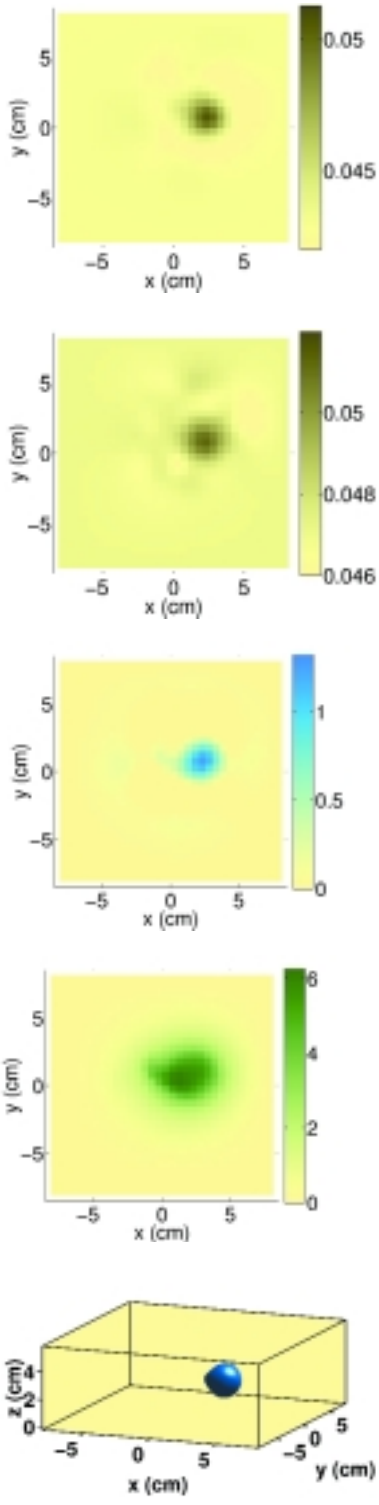

Figure 2 Reconstruction slices show (from top) absorption at λ_x in cm^{-1}, absorption at λ_m in cm^{-1}, fluorescence yield in cm^{-1}, and fluorescence lifetime in 10^{-10} s. The data yields an isosurface.

Researchers have made significant progress in developing ODT and FODT. However, much work remains to improve the capability for real-time clinical applications. We have applied inversion algorithms based on multigrid techniques and other recent methods, including shape-based algorithms, to dramatically reduce the computational requirements, but we still need faster, more flexible inversion algorithms. Application of these methods, along with ongoing improvement in modeling approaches, instrumentation, and fluorescent contrast agents, should pave the way toward widespread clinical use. **oe**

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Acknowledgments

This work was funded by the National Science Foundation under contract CCR-0073357. Jonathan Stott and David Boas acknowledge support from Advanced Research Technologies Inc.

References

1. S. Arridge, Inverse Problems 15, p. R41 (1999).

2. D. Boas, D. Brooks, et al., IEEE Signal Processing Magazine 18, p. 57 (2001).

3. V. Ntziachristos, C. Bremer, et al., Eur. Radiol. 13, p. 195 (2003).

4. A. Milstein, J. Stott, et al., "Fluorescence optical diffusion tomography using multiple frequency data," J. Opt. Soc. Am. A, to appear.

5. S. Oh, C. Bouman, et al., "A general framework for nonlinear multigrid inversion," IEEE Trans. Image Processing, to appear.

6. A. Milstein, S. Oh, et al., Appl. Opt. 42, p. 3081 (2003).

7. S. Oh, A. Milstein, et al., J. Opt. Soc. Am. A 19, p. 1983 (2002).