

Sensitivity Characterization of a Time-Domain Fluorescence Imager: eXplore Optix

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Abstract

Empirical relations of fluorescence signal to background ratio is established from experimental data over a wide range of parameters, and the sensitivity of eXplore Optix using Cy5.5 for known tissue optical properties is predicted.

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Introduction

The interest in fluorescence imaging has increased steadily in the last decade [1][15]. Using fluorescence techniques, it is feasible to visualize and quantify the function of genes and the expression of enzymes and proteins deep into tissues. When applied to small animal research, optical imaging based on fluorescent marker probes can provide valuable information on the specificity and efficacy of drugs at reduced cost and with greater efficiency than current nuclear imaging techniques. Meanwhile, fluorescence techniques represent an important class of optical methods being applied to in vitro and in vivo biomedical diagnostics, towards noninvasive clinical applications.

ART developed a time domain in vivo small animal fluorescence imager, eXplore Optix [16]. Using the measured time-resolved fluorescence signal, fluorophore location and concentration can be quickly estimated [17]. Fluorescence imaging often involves the injection of an extrinsic fluorophore, typically chemically bound with drug molecules or activatable after interaction with specific enzymes. Then, an external light source is applied to excite the fluorophore and the fluorescent signal is recorded accordingly. A key issue in practical application of fluorescence imaging is the background signal, that in general comes from four sources: autofluorescence from the tissue sample, residual signal due to imperfect clearance of the targeted probe, leakage of the excitation laser light due to imperfect fluorescent filters, and fluorescence from theoretical components in the data acquisition channel. Regardless of the exact technology employed, background signal can not be completely eliminated, although it does not affect the data quality when the targeted fluorescence signal is strong. However, when the fluorescence signal is weak, background signal could dominate the measurement. As a result, it limits the detecting sensitivity of an imaging system. In this paper, we present some results of the sensitivity characterization of eXplore Optix mainly limited by background.

Due to light diffusion in tissue, the fluorescent as well as the background signal are a function of tissue optical properties, e.g. absorption and scattering coefficients, and fluorophore concentration and location, etc. We use Cy5.5 fluorophore submerged at different positions in tissue-like phantoms with various optical properties to explore the capability of eXplore Optix. By defining a threshold of signal to background ratio (SBR), empirical relations of fluorescence signal with the variables aforementioned are established that allow us to predict the detectable depth of a given fluorophore concentration or the minimum detectable concentration at a given location for known tissue optical properties.

Device, material and method

Detailed description of eXplore Optix can be found elsewhere [16], [17]. Basically, a 1450 nm diode laser (FDU from PROCOUNT, Berlin, Germany) is used as a light source. A photomultiplier tube (PMT) (Hamamatsu, Japan) coupled with a time correlated single photon counting (TCSPC) system (Beckor & Hiki, Berlin, Germany) is used as a fluorescence signal detector. A combination of filters (Omega Optical, Brattleboro, Vermont and Barr Associates, Westford, Massachusetts) was installed in the system for fluorescence measurements. A translation stage and galvanometric mirrors enable raster scanning along x and y directions for imaging. For Cy5.5 experiments, a 670 nm laser is used. The average laser power was kept at about 1 mW. Actual laser power delivered to the target is adjusted by a computer-controlled variable neutral density filter wheel.

The liquid phantom is achieved by mixing 10% Lipoven II (Abbott Laboratories, Montreal, Canada), demineralized water and India ink. The quantity of each composition is selected by a predetermined recipe [18] to ensure the optical properties are similar to tissue ($\mu_a = 0.05 - 1.5 \text{ mm}^{-1}$ and $\mu_s = 0.005 - 0.05 \text{ mm}^{-1}$). Solid fluorescent inclusions are made from polyurethane resin, titanium dioxide (TiO_2), as well as the Cy5.5 fluorophore.

Three groups of experiments were done to explore the sensitivity of eXplore Optix. In each group, two of the three parameters, fluorophore concentration C , μ_a , and μ_s , are fixed, and the third varies. A fluorescent inclusion is imaged at different depths inside the liquid phantom, and both fluorescent and background signal are recorded.

References

- [1] J. R. Lakowicz, *Topics in Fluorescence Spectroscopy*, Plenum Press, New York, 1994.
- [2] R. Cubeddu, A. Pifferi, P. Taroni, and G. Valentini, "Tumor detection in vivo by measurement of fluorescence decay time constant," *Opt. Lett.* **20**, 2553-2555 (1995).
- [3] M. A. O'Leary, D. A. Boas, J. D. Li, B. Chorno, and A. G. Yeh, "Fluorescence lifetime imaging in turbid media," *Opt. Lett.* **21**, 158-160 (1996).
- [4] B. B. Dine, F. Liu, and R. R. Alfano, "Time-resolved fluorescence and photon migration studies in biomedical and model random media," *Rep. Prog. Phys.* **64**, 227-292 (1997).
- [5] J. Chang, H. L. Graber, and R. L. Barbour, "Imaging of fluorescence in highly scattering media," *IEEE Trans. Biomed. Eng.* **44**, 810-822 (1997).
- [6] H. B. Jiang, "Frequency-domain fluorescent diffusion tomography: a finite-element-based algorithm and simulations," *Appl. Opt.* **37**, 5337-5343 (1998).
- [7] M. J. Epstein, D. E. Dougherty, T. L. Troy, and E. M. Sevick-Muraca, "Biomedical optical tomography using dynamic parameterization and Bayesian conditioning on photon migration measurements," *Appl. Opt.* **38**, 2138-2150 (1999).
- [8] D. Hanley, V. Chernomordik, Gennat, M. Low, and A. Ganjukhite, "Fluorescence measurement of localized, deeply embedded physiological processes," in *Medical Imaging*, C. Chen, A. V. Chapatov, Eds., Proc. SPIE **3978**, 277-282 (2000).
- [9] V. Ntziachristos and R. Weisleder, "Experimental three-dimensional fluorescence reconstruction of diffuse media using a normalized Born approximation," *Opt. Lett.* **26**, 893-895 (2001).
- [10] K. Vishwanath, B. W. Teague, and M. A. Mycek, "Quantitative fluorescence lifetime spectroscopy in turbid media: comparison of theoretical, experimental and computational methods," *Phys. Med. Biol.* **47**, 3357-3405 (2002).
- [11] A. B. Millan, S. Oh, K. J. Webb, C. A. Bouman, Q. Zhang, D. A. Boas, and R. P. Millan, "Fluorescence optical tomography," *Appl. Opt.* **42**, 3081-3094 (2003).
- [12] A. D. Völkel and A. H. Welch, "Fluorescence tomography with simulated data based on the equation of radiative transfer," *Opt. Express* **11**, 1019-1021 (2003).
- [13] R. B. Schatz, J. Rippl, and V. Ntziachristos, "Experimental fluorescence tomography of tissues with noninvasive measurement," *IEEE Trans. Medical Imaging* **23**, 450-500 (2004).
- [14] Melissa Gao, George Lewis, Gordon M. Turner, Antoinette Soubrier, and Vasilis Ntziachristos, "Effects of background fluorescence in fluorescence molecular tomography," *Appl. Opt.* **44**, pp. S468-S474, (2005).
- [15] Kevin R. Gademski, Paul F. Menez, Joseph E. Hayward, and Michael S. Patterson, "Quantification of fluorophore concentration in vivo using two simple fluorescence-based measurement techniques," *J. Biomed. Opt.* **10**, 034007 (2005).
- [16] P. Gallant, A. Bekkerov, G. Ma, F. Lesage, Y. Wang, D. Hill, L. McIntosh, "A quantitative time-domain optical imager for small animals in vivo fluorescence studies," OSA 2004 Biomed. Opt. Topical Meeting (Miami Beach, Florida), April 14-17, 2004.
- [17] David Hill, Guobin Ma, Frédéric Lesage, and Yong Wang, "Simple time-domain optical method for estimating the depth and concentration of a fluorescent inclusion in a turbid medium," *Opt. Lett.* **28**, 2258-2260 (2003).
- [18] M. L. Vernon, J. Fiedette, Y. Peincheval, S. Caron, P. Beaulieu, "Fabrication and Characterization of a Solid Polyurethane Phantom for Optical Imaging Through Scattering Media," *Appl. Opt.* **38**, 4317-4251 (1999).

Results and discussion

Shown in Fig. 1 are typical fluorescence signal to background ratios (SBR defined as $(\text{rawFluorescenceSignal}/\text{Background})$) versus inclusion depth. Clearly it decreases exponentially as expected. By defining SBR=0.05 as detectable and SBR=1 as quantifiable (illustrated in Fig. 2), one can get the detectable and quantifiable depths of a inclusion versus μ_a , μ_s and C (as shown in Fig. 3). Combining the three fittings and extrapolating them, one is able to get a function of SBR over μ_a , μ_s and C . Then one can predict the detectable and quantifiable depths for given concentration, or the minimum detectable and quantifiable concentrations at given depth (see Fig. 4 and Fig. 5, for example).

Figure 1: Typical fluorescent signal to background ratio of a 100 nM Cy5.5 inclusion versus its depth inside a liquid phantom with $\mu_a = 0.05 \text{ mm}^{-1}$ and varied μ_s . The five curves from top to bottom corresponds to $\mu_s = 0.6, 0.8, 1.0, 1.2$, and 1.6 mm^{-1} .

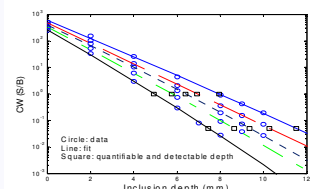


Figure 2: Definition of quantifiable and detectable SBR. Left panel, for SBR=1, information extracted from the signal is consistent, so is quantifiable. Right upper panel, SBR=0.08, signal from inclusion can be distinguished from surrounding background, so is detectable. Right lower panel, SBR=0.03, signal from inclusion is buried in the surrounding background, so it is nondetectable.

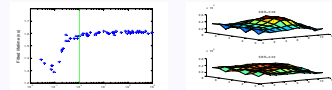


Figure 3: Detectable and quantifiable depth of a inclusion versus μ_a , μ_s , and fluorophore concentration, when the other two parameters are fixed.

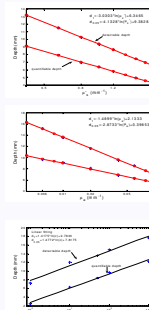


Figure 4: Detectable (left) and quantifiable (right) depth of a 100 nM Cy5.5 fluorescent inclusion over μ_a and μ_s space.

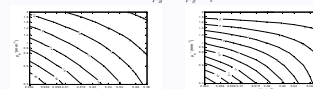


Figure 5: Minimum detectable (left) and quantifiable (right) concentration in nM of Cy5.5 fluorescent inclusion at 4 mm depth inside the medium over μ_a and μ_s space.

Summary

Cy5.5 fluorescence signal and background were measured in various conditions within tissue-like phantoms. Empirical relations of fluorescence signal to background ratio are established from experimental data over a wide range of parameters that allow us to predict the sensitivity of eXplore Optix for known tissue optical properties.