

Dual-modality Optical Tomography: Fluorescent and Bioluminescent Imaging Tomography of Embedded Sources *In vivo*

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Abstract

The development of instrumentation which captures both *in vivo* bioluminescent and fluorescent signals on a cooled, sensitive charge-coupled device has offered flexibility in reporter detection for functional molecular imaging. The addition of transillumination capabilities for fluorescence excitation provides improved detection sensitivity to deep fluorescent sources whose signal at the surface may be obscured by autofluorescence excited with reflectance-mode imaging. Images of fluorescence for varying transillumination source positions offer constraining information for quantification and localization of fluorescent sources deep within tissue.

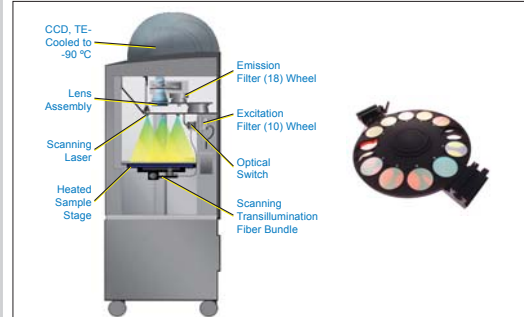
In this work, a new fluorescence imaging tomography algorithm (FLIT) based on diffusion theory in a homogeneous medium is described. Boundary conditions for photon propagation between tissue and air are appropriately modeled with surface topography extraction from structured light images of the animal. Separate Green's functions model the propagation of photons in tissue after injection of the excitation light into the body at the surface opposite the camera lens, and the subsequent fluorescence emission to the imaged portion of the surface. The excitation and emission Green's functions are combined to form a functional to solve for the fluorophore distribution and quantification. Treatment of tissue autofluorescence with spectral unmixing will be discussed.

We will present results of our fluorescence tomographic algorithm with confirmation from co-registered CT scans. We show tomographic solutions of a homogeneous tissue phantom embedded with fluorescent dye for validation of our algorithm. To demonstrate the accuracy of our fluorescence imaging tomography and diffuse bioluminescence imaging tomography (DLIT) algorithms *in vivo*, animals were implanted with both calibrated luminescent beads and silastic pillows filled with fluorescent dyes and imaged in an dual modality optical imaging system and a micro-CT system. Co-registration of the tomographic solutions with segmented CT shows excellent superposition with submillimeter accuracy in the phantom case.

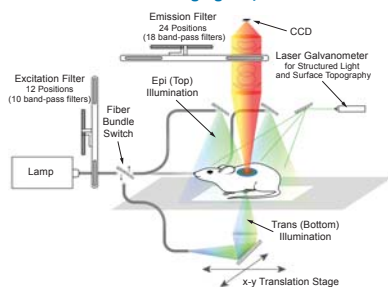
With these validations, dual optical tomographic analysis was performed on *in vivo* studies in which animals were orthotopically injected with PC3M-luc cells and administered a fluorescent tumor probe intravenously. There is good agreement between the bioluminescent and fluorescent solutions.

Materials and Methods

Dual Modality *In vivo* Optical Imaging Instrument

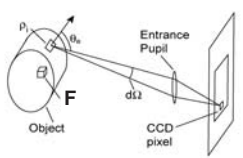


In vivo Bioluminescence Imaging Acquired without excitation light



In vivo Fluorescence Imaging Transmission-Mode Excitation

Boundary Conditions for Fluorescence Imaging Tomography



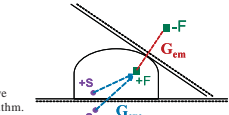
The instrument is absolutely calibrated so that count units measured at the CCD are converted to radiance units at the surface. Appropriate boundary conditions convert the radiance data to photon density data ρ .

The Green's functions are modeled with a combination of the partial current boundary condition and extrapolated boundary condition.

$$\rho = s_{exc} G_{exc} G_{em} [\delta x^3 Q.E. \sigma n]$$

δx^3 = Voxel volume [mm³]
Q.E. = Quantum Efficiency of fluorescence conversion
 σ = Excitation photon cross section [mm²]
 n = Fluorophore concentrations [number/mm³]

Estimate this quantity F [number/mm²] using a Non-Negative Least Squares algorithm.



References

1. Kuo et al., "Three-dimensional reconstruction of *in vivo* bioluminescent sources based on multi-spectral imaging," JBO, 12, 024007, 2007.

Acknowledgements

Philip Salmon, Ph.D. - SkyScan
Daniel Stearns, Ph.D.

Results

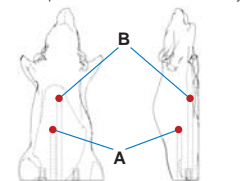
Fluorescence Imaging Tomography

Algorithm

- Acquire photographic, and fluorescent images at a number of trans-illumination source positions. Include a structured light image.
- Use structured light image to reconstruct surface vertices of mouse.
- From surface radiance images, determine the photon density just inside the surface on every element of the surface vertices.
- Parameterize the light sources in the object volume as voxels.
- $\rho = s_{exc} G_{exc} G_{em} [\delta x^3 Q.E. \sigma n]$
- Solve the linear system of equations to determine the fluorescent source strengths.
 - Non-negative least squares
 - Regularization can be used to treat inexact theory.
 - Voxel gridding
 - Initially coarse for well-posed linear problem.
 - Adaptively refined in size to converge on location and strength.

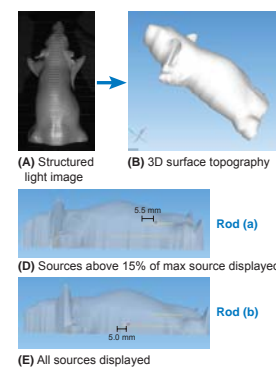
Tissue Phantom

- Resin with scatterer and absorber
- Two rod positions
- Rod tip embedded with AlexaFluor 750 dye

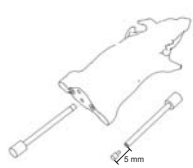


Fluorescent imaging of AF750 dye in Rod Tip A (4×10^{13} dye molecules) trans-Exc: 745 nm, Em: 800 nm

Fluorescent images acquired with trans-illuminated excitation sources. Excitation source locations are indicated as crosshairs in each image.



The surface (B) of the subject is determined from structured light images (A) for appropriate modeling of boundary conditions at the air-tissue interface.



(C) Fluorescent dyes are placed in hollow ends of rods made of the same material as the tissue phantom. Caps at the rod ends are 5 mm in length.

(D) Reconstruction of dye in rod inserted into Bore A, shown as red voxels. The long, narrow yellow surfaces indicate the location of the bores. Note that the voxel location is approximately 5 mm from the end of the bore, the dye's location in the rod due to the length of the rod end cap.

(E) Reconstruction of dye in rod inserted into Bore B.

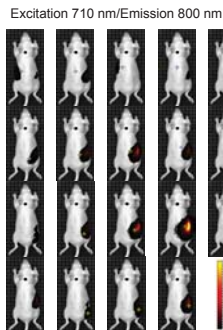
Dual Modality Optical Imaging *In vivo*

Implantation study

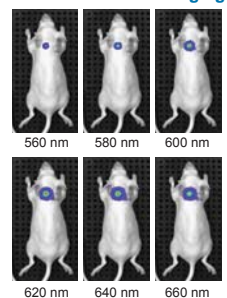
- 10^{15} molecules AF750 fluorescent dye in a silastic tubing pillow implanted in abdominal cavity
- Calibrated luminescent bead implanted in animal scruff

Trans-illumination Fluorescence Imaging

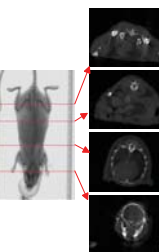
Excitation 710 nm/Emission 800 nm



Bioluminescence Imaging



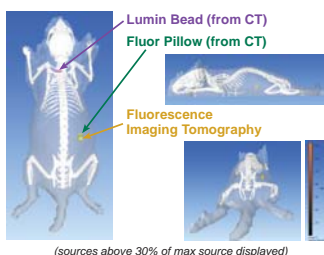
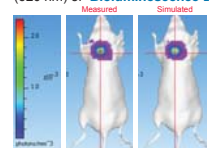
Micro-CT Data



Photon Density Fit for single trans-illumination source position of Fluorescence Data



Photon Density Fit for single wavelength (620 nm) of Bioluminescence Data



Fluorescence Imaging Tomography reconstruction of AF750 fluorescent dye pillow registered with segmented CT data

Results (continued)

Dual Modality Optical Imaging *in vivo*

Implantation study



Bioluminescence Imaging Tomography reconstruction of luminescent bead registered with segmented CT data

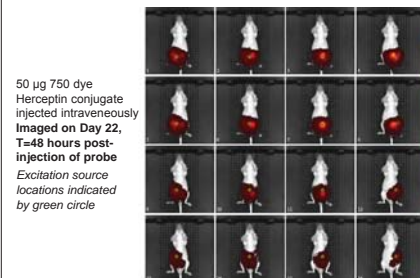
Dual Modality Optical Tomography

In vivo study

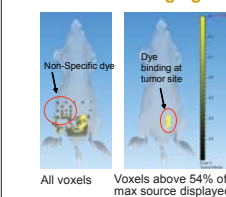
- 5×10^5 PC3M-luc cells injected orthotopically to the prostate
- 50 μ g AF750 dye Herceptin conjugate injected IV on Day 20

Fluorescence Data

Ex: 745nm Em: 800 nm



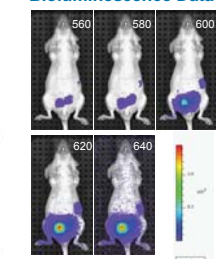
Fluorescence Imaging Tomography



All voxels

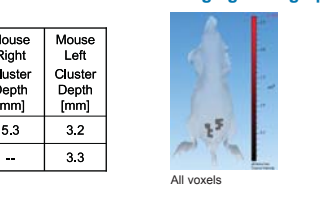
Voxels above 54% of max source displayed

Bioluminescence Data



5×10^5 PC3M-luc cells injected orthotopically to the prostate imaged on Day 22

Bioluminescence Imaging Tomography



All voxels

	Mouse Right Cluster Depth [mm]	Mouse Left Cluster Depth [mm]
DLIT	5.3	3.2
FLIT	--	3.3

The tomographic reconstruction from fluorescent images matches the animal left cluster of voxels from the bioluminescent tomography estimation. Fluorescent images from both transmission and reflectance excitation indicate that the probe concentrated in the abdominal area on animal left.

Spectral Unmixing Prior to Fluorescence Imaging Tomography

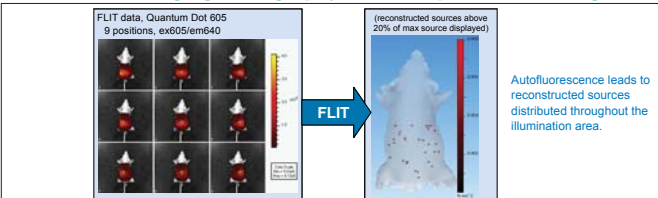
Purpose

- Improve the 3D localization and clarification when target fluorophore signal is at a level comparable to tissue autofluorescence or another fluorophore.

Method

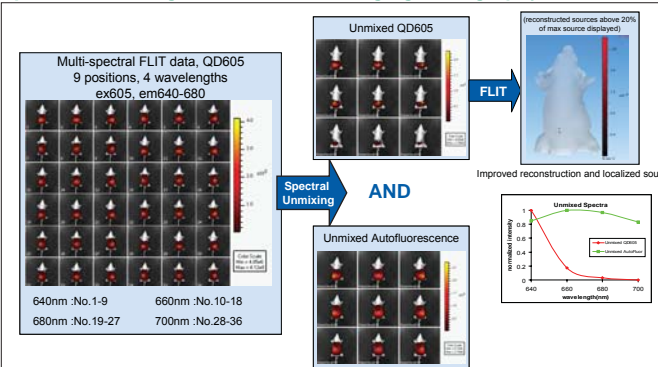
- Multi-spectral transillumination imaging at multiple positions
- Perform spectral unmixing at all the positions and separate target fluorophore from background
- Execute FLIT reconstruction using unmixed fluorophore images

Fluorescence Imaging Tomography without Spectral Unmixing



Autofluorescence leads to reconstructed sources distributed throughout the illumination area.

Spectral Unmixing + Fluorescence Imaging Tomography



Improved reconstruction and localized source