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Fluorescence Tomographic Imaging using a Hand-held Probe based Optical Imager: Extensive Phantom Studies

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Abstract

Hand-held probe based optical imagers are popular towards breast imaging due to their potential portability and maximum patient comfort. Recently, a novel hand-held probe based optical imager has been developed and its feasibility for three-dimensional fluorescence tomographic imaging demonstrated. Herein, extensive tomography studies were performed on large slab phantoms (650 ml) in order to assess the performance limits of the hand-held imager. Experiments were performed using different target volumes (0.1–0.45 cc), target depths (1–3 cm), and fluorescence (Indocyanine Green) absorption contrast ratios in a non-fluorescing (1:0) and constant fluorescing backgrounds (1000:1-5:1). The estimated sensitivity and specificity of the hand-held imager is 43% and 95%, respectively.

1. Introduction

Fluorescence-enhanced optical imaging is an emerging technology towards early-stage diagnostic imaging of the breast tissue. In recent years, hand-held optical imaging devices are an alternate attraction since they can be developed as a portable device with the ability to imaging any tissue volume/curvature with maximum patient comfort [1–6]. Hand-held optical imagers developed to date have been focused on spectroscopic applications, and not tomographic imaging (unless when used with a second imaging modality, such as ultrasound [5]). Recently, a hand-held probe based optical imager was developed at the Optical Imaging Laboratory towards three-dimensional (3-D) fluorescence tomographic imaging applications. The imager uses a 5×10 sq. cm hand-held probe that has the ability to simultaneously illuminate and collect light signals for rapid data acquisitions over larger areas and with improved signal strength [7–8]. The 3-D fluorescence tomography feasibility of this hand-held based optical imager has been demonstrated on tissue phantoms of large volumes (650 ml) [8].

In the current work, extensive tomography studies were carried out using the hand-held optical imager on large tissue phantoms under various experimental conditions. The focus of this work is to assess the performance limits of the hand-held imager, with a goal to translate the technology to the clinic. Fluorescence-enhanced optical tomography studies were carried out under various experimental conditions of target depth (up to 3.0 cm), target volume (as small as 0.1 cc), and target to background (T:B) absorption contrast ratio (as small as 5:1), using frequency-domain measurements. A preliminary evaluation of the diagnostic sensitivity and specificity of the system is reported.

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2. Materials and Methods

2.1 Instrumentation

The hand-held based optical imager primarily consists of a hand-held probe that is connected to the laser diode (80mw, Sanyo DL7140-201S, Thorlabs Inc., Newton, NJ) and image intensified (FS9910, ITT Night Vision, VA) charge coupled device (CCD) camera (PI-SCX 7495-0002, Roper Scientific, Trenton, NJ) along with appropriate power supplies and electronic components to operate in both the continuous-wave (CW) and frequency-domain modes of imaging [8]. Initially, modulated light at 100MHz is launched onto the tissue phantom at multiple (6) point locations simultaneously via the hand-held probe. The generated modulated signal (attenuated input signal and/or fluorescence signal) is collected simultaneously from 165 point locations spaced over a $5 \times 10 \text{ cm}^2$ hand-held probe area. This collected signal is in turn simultaneously detected using the custom-built gain-modulated intensified CCD (ICCD) camera. Modulation at the source and detector end is generated using individual oscillators, which are phase-locked. By changing the phase delay (from 0 to 2π) between these oscillators, phase sensitive steady-state images is acquired and processed (via Fast Fourier Transforms) in order to obtain frequency-domain measurements (in terms of amplitude and phase shift). More details of instrumentation and data acquisition procedure can be found elsewhere [8]. Appropriate optical filters are used to separate the attenuated input near-infrared (NIR) light from the fluorescence signal (at a higher wavelength).

2.2 Phantom Studies

Extensive phantom studies were performed using large slab geometries in order to assess the current performance limits of the hand-held imager. The performance was assessed in terms of the limits in target size, target depth, and target: background (T:B) optical contrast ratio recovery from the tomographic images. In Study-I, experiments were performed wherein a target was present in a homogeneous (i.e. uniform absorption/scattering medium) tissue phantom, with a T:B > 1:1. In Study-II, experiments were performed wherein a target was either absent or present with a T:B=1:1 in a homogeneous phantom.

2.2.1 Study I: Experiments with T:B > 1:1—Experimental studies were performed on a $10 \times 10 \times 10 \text{ cm}^3$ cubical phantom that was filled with 1% Liposyn (Liposyn II, 20%, Henry Schein, Melville, NJ) up to 6.5 cm height (i.e. a phantom volume of 650 ml). A hollow clear plastic sphere filled with $1 \mu\text{M}$ Indocyanine Green (ICG) in 1% Liposyn solution was used to mimic a single target (or tumor). Sodium polyaspartate (MW 3000–8000) was used as a stabilizing agent for ICG [9]. The optical properties of the background and fluorescent target are given in Table 1 (similar to previous studies [8]).

In Study I-A, all experimental cases have T:B = 1:0, wherein ICG was present only in the target and not the background (i.e. perfect uptake scenario). Experiments were carried out using different target volumes (0.45 cc, 0.23 cc and 0.1 cc) and depths (1 cm to 3 cm) as described in Table 2 (Cases 1 to 6). Cases with large target volumes placed closer to the surface were not included in the current study (presented elsewhere [8]).

In Study I-B, all experimental cases have T:B = x :1 (here, $x = 1000$ to 5), wherein ICG was present in both the target and background at different concentrations (i.e. imperfect uptake scenario). Experiments were carried out using a 0.45 cc target located up to 2.5 cm deep and at various fluorescence absorption contrast ratios (x :1 = 1000:1 to 5:1), as described in Table 2 (cases 7 to 28). In these studies, the ICG concentration in the target was maintained at $1 \mu\text{M}$ and the ICG concentration in the background was altered in order to obtain the T:B contrast ratio of interest.

2.2.2 Study II: Experiments with T:B=1:1 or with no target—Experimental studies in Study II were also performed using a 650 ml tissue phantom filled with 1% Liposyn solution (similar to Study I). The optical properties of the fluorescing background and target (if any) were similar to background properties of the imperfect uptake cases as described in Table 1. In Study II-A, all experimental cases had no target embedded, and instead the ICG concentration was varied from 0.001 μM to 0.2 μM in background as described in Table 3 (cases 29 to 36). In study II-B, all experimental cases have T:B=1:1, wherein ICG was present in equal concentrations (0.01 μM) in target and background. Experiments were carried out using a single 0.45 cc target located at various depths (1–2 cm) as described in Table 3 (cases 37 to 49). Experimental cases 37–41 all have the same target depth ($z=1$ cm) but different target locations along the x-y axis. Similarly, experimental cases 42–46, and 47–49 have 1.5 and 2 cm deep targets, respectively, under varying target x-y locations.

2.3 Image Reconstructions

Three-dimensional fluorescence-enhanced optical tomography was carried out using a computational efficient version of the approximate extended Kalman filter (AEKF) based algorithm [8,10]. Initially, a finite-element based Galerkin approach was implemented using the adjoint formulation [11], towards the forward model simulations. A $10 \times 6.5 \times 10$ cm³ phantom was generated and discretized into tetrahedral elements using Gambit 2.1.6 software (Fluent Inc., Lebanon, NH). The simulated phantom consisted of 10835 nodes and 53467 volume elements. The actual source strengths from six sources were measured using an optical power meter (PM 100, Thorlabs Inc., Newton, NJ) and accounted for in the forward model.

Three-dimensional image reconstructions were performed using a computationally efficient version of the Approximate Extended Kalman Filter (AEKF) algorithm, in order to obtain the 3-D optical property map of the fluorescence absorption coefficient at excitation wavelength (μ_{axf}). The Kalman filter is a linear state Bayesian estimator used in process control for estimating a state recursively, based on its previous observations. The advantages of this Bayesian estimator include: (i) the use of measurement error (error between measurement repetitions) and model error (due to the mathematical simplifications of the diffusion equation) to weight the updates in each iteration and regularize the matrix inversion; and (ii) the estimation of parameter error values (or the confidence in the parameter value), apart from the estimation of the parameter value itself, and using these parameter error values to damp and regularize the inversions. The AEKF algorithm recursively minimizes the variance of parameter error (i.e. error in μ_{axf} for this study), given the estimates of measurement, system, and parameter error covariances [8]. In all the experimental cases, the parameter error covariance is defined as the error in the spatially distributed (unknown) parameter values (μ_{axf}), which is used to damp into the inversion for better convergence. The initial parameter error covariance was set to an empirical value of 0.001 for all cases. The measurement error was estimated from the variances of the repeated measurements obtained at each detector point; the system error was estimated as $\frac{1}{4}$ of the measurement error. The reconstructions were assumed to have converged when the root mean square output error (RMSE) was less than 1%, or the total number of iterations exceeded 50.

For perfect uptake experimental cases (Study I-A), the initial guess of the reconstruction parameter (μ_{axf}) was arbitrarily set to a small value of 0.003 cm⁻¹. Additionally, subtracted measurements [8] were used towards image reconstructions for Study I-A. Subtracted measurements were obtained by accounting for the non-fluorescing background's excitation leakage during data post-processing (prior to image reconstructions). For imperfect uptake experimental cases (Study I-B) and other cases with ICG in background (Study II-A and II-B), μ_{axf} was set to its true value of ICG concentration presented in the phantom background. Additionally, the raw fluorescence measurements were used during reconstructions without

subtracting the background signal, since the strong fluorescing background dominates the non-fluorescing excitation leakage from the same background. Additionally, use of subtracted measurements for imperfect uptake cases is unrealistic in a clinical environment.

Contour slice plots of the reconstructed parameter (μ_{axf}) at various depths in the tissue phantom are presented in 3-D using TecPlot 360 (Tecplot Inc., Bellevue, WA). These plots provide a qualitative estimation of the reconstructed target's size and location. For quantitative estimation of the reconstructed target's details, a cut-off value in μ_{axf} was selected based on the first break point of histogram plot of μ_{axf} . The reconstructed target was compared to that of the true target, in terms of: (i) target transverse (x-y axis) recovery and depth recovery based on the estimated centroid location of the reconstructed target; (ii) target volume; and (iii) T:B contrast ratio. Herein, the recovered T:B contrast ratio is determined as the ratio between volume weighted and averaged μ_{axf} value of all the elements whose μ_{axf} is above the cut-off value and that of the rest of the elements.

The recovered T:B contrast ratio was also used towards differentiating positive and negative cases in optical tomography. If this T:B contrast ratio was greater than a predetermined threshold (see Equation 1), the reconstruction was considered a positive test (i.e. target(s) were recovered or differentiable from the background) and vice-versa for a negative test. The choice of this threshold value is described in more detail in Section 3.1 (Results and Discussions) and is given by

$$\frac{Average(\mu_{axf} nodes_{target})}{Average(\mu_{axf} nodes_{background})} > Threshold \quad (1)$$

The image reconstruction results from all the experimental studies (Study I and II) were categorized as positive or negative, and the performance of the imaging system was evaluated using sensitivity and specificity analyses.

2.4 Sensitivity and Specificity Studies

The diagnostic sensitivity of an imaging modality is defined as the probability of obtaining a positive test result among patients with the disease (here a target present with T:B > 1:1), and the diagnostic specificity is the probability of obtaining a negative test result among patients without the disease (here a target present with T:B = 1:1 or no target). The sensitivity and specificity can be calculated based on Equations 2 and 3, respectively.

$$Sensitivity = \frac{TP}{TP+FN} \quad (2)$$

$$Specificity = \frac{TN}{TN+FP} \quad (3)$$

where definitions of TP, FP, FN and TN are true positive, false positive, false negative, and true negative, respectively (see Figure 2).

3. Results and Discussion

3.1 Study I: Experiments with T:B>1:1

The phantom studies performed in Study I employed both perfect uptake (Study I-A) and imperfect uptake (Study I-B) experimental cases. Three-dimensional image reconstructions were performed for 26 out of 28 experimental cases. Two cases were excluded as described in the next paragraph. The recovered T:B contrast ratios from the 26 reconstruction cases are plotted (on a logarithmic scale) against the distance-off between the true and reconstructed target centroid location (see Figure 3). The 26 experimental cases were distinctly differentiated into two groups in terms of the recovered T:B contrast ratio and distance-off. Fourteen experimental cases recovered a very low T:B contrast ratio ranging between 1.1 to 1.7, and the remaining 12 experimental cases recovered a T:B contrast ratio > 20 . Hence, the choice of the threshold value (see Equation 1) was distinct from these plots and could be chosen anywhere between 2 and 20, in an attempt to differentiate the positive and negative tests from the reconstruction results. In this study, a threshold value of 2 was chosen, to avoid misinterpreting too many targets as artifacts by choosing a higher threshold value of 20. The tomography results from the 14 experimental cases (case 5, 7, 8, 11, 12, 15, 16, 19, 20, 22, 24, 26, 27 and 28) with recovered T:B contrast ratio < 2 were considered as *Negative*, and the remaining 12 experimental cases were considered as *Positive*. Additionally, it was observed that when the recovered T:B contrast ratio was less than 2, the distance-off between the true and reconstructed target's centroid was larger (> 0.5 cm) in comparison to that observed at higher T:B contrast ratios. This further indicates that all negative tests possibly recovered artifacts and not the actual targets. The recovered target volume was also calculated and compared to true target volume for all cases in Study I. However, no obvious correlation was observed between recovered volume error and recovered contrast ratio, which in turn determines whether the reconstruction result is positive or negative. Hence the parameter of recovered target volume is not included here, for the consideration of limiting discussion to parameters that is crucial to sensitivity and specificity study.

For all perfect uptake experimental cases (1 to 6, Study I-A), a subtraction-based post-processing technique was employed for eliminating excitation leakage. By subtracting the background noise (i.e. measurements obtained from homogeneous non-fluorescing phantoms) from fluorescence optical measurements obtained from a given experimental case, the excitation leakage was minimized to the maximum extent possible. However, the amplitude measurements post to subtraction in cases 1 (0.45 cc target volume, 3-cm depth, T:B=1:0) and 3 (0.23 cc target volume, 2-cm depth, T:B=1:0) were less than 0. This indicated that the acquired raw measurements for these experimental cases were at noise level and no valuable signal was detected from the target. Hence image reconstructions were not performed for these experimental cases (1 and 3), and they were automatically considered as *Negative* (after reconstructions). From perfect uptake based experimental cases (Study I-A), the depth of the reconstructed target using our imaging system is 1 cm for a 0.1 cc target, 1.5 cm for a 0.23 cc target, and 2.5 cm for a 0.45 cc target. These results imply that the tomographic reconstructions are limited from recovering positive targets, when the target size becomes smaller and/or deeper. This can be attributed to the exponentially attenuating fluorescence signal from a smaller/deeper target, which may approach the noise floor of the imaging system and hence cannot be differentiated with respect to the background.

For all imperfect uptake experimental cases (7–28, Study I-B), the subtraction technique was not employed since the approach may not be directly applicable to *in-vivo* studies (in future). From the 3-D reconstruction results, it was observed that the maximum recoverable target depth is 1.5 cm for T:B contrast ratio $\geq 200:1$, 1 cm for T:B contrast ratio as small as 25:1. The target was not recovered (i.e. *Negative* test result) when T:B contrast ratio was $\leq 10:1$. The 2-D contour slice plots at different phantom depths (along z-axis) are shown in Figure 4 for example

cases 17 (0.45 cc target volume, 1.5-cm depth, T:B=200:1) and 25 (0.45 cc target volume, 1-cm depth, T:B=25:1) in order to qualitatively illustrate the recovered target location and T:B optical contrast.

A single target was recovered without any artifacts in both the example cases, although the reconstructed target depth appeared closer than the true depth. This is due to the limitation of acquiring and employing only reflectance measurements during image reconstructions, which inherently tends to reconstruct targets closer to the surface, as also observed by other researchers in the past [12–13]. Additionally, unlike the perfect uptake cases, the fluorescence amplitude detected on the imaging surface for imperfect uptake cases is contaminated by strong fluorescing background signal. The smaller the T:B contrast ratio, the easier it was for the fluorescence signal from the target to be masked by the background fluorescence signal. Hence a target can be recovered at 1.5 cm deep when T:B contrast is equal to 200:1, but can only be recovered at 1 cm deep when T:B contrast ratio drops to 25:1.

The recovered T:B contrast ratio for imperfect uptake cases (Study I-B) have been plotted with respect to true target depth in Figure 5. For a given true T:B contrast ratio, the recovered T:B contrast inversely varied with the true target depth. For all *Positive* cases, the target depth was always recovered at ~0.7 cm regardless of true target depth. The possible reason is that the increased true target depth and the decreased T:B contrast ratio has a similar effect on the detected fluorescing signals from the phantom surface. The reconstruction studies were also performed using different initial μ_{axf} background value for selected cases, and it was observed that an arbitrary choice of the initial μ_{axf} background value did not significantly impact the image reconstruction results. Improvements in reconstruction algorithms are currently carried out in order to improve the recovered T:B contrast ratio, independent of the true target depth.

Currently, alternate approaches using the hand-held optical imager (in terms of implementing noise filtering tools during data post-processing, and probe head design to acquire trans-illumination measurements as well) are attempted in our Optical Imaging Laboratory, in order to improve the target depth recovery at smaller T:B contrast ratios using smaller targets (i.e. extend the performance capabilities of the system). In addition, research is carried out by various other research groups to develop more tumor-specific contrast agents in order to increase the T:B contrast ratio towards improved early-stage or deep target detection.

3.2 Study II: Experiments with T:B=1:1 or with no target

In Study II-A, experiments with no target present (cases 29 to 36) were performed in order to study the effect of fluorescence background signal on target recovery. The fluorescence background signal acquired from the phantom surface is unevenly distributed (due to non-uniform simultaneous source strengths) and tends to have much stronger signal closer to the source points. Hence, image reconstruction studies were performed in order to study the ability of the imaging system to differentiate background fluorescence (noise) signal with no target as a *Negative* test result. Study II-B experiments with T:B contrast ratio of 1:1 (case 37 to 49) was performed in order to study the effect of normal tissue structure on target recovery (by using a structurally different target in a homogeneous background with similar ICG concentrations throughout). The image reconstructions were performed in order to study the ability of the imaging system to differentiate fluorescing heterogeneity from structural heterogeneity.

From image reconstructions, it was observed that 20 out of 21 experimental cases in Study II (A&B) recovered T:B contrast ratio less than 2 (threshold value). This demonstrates the robustness of the imaging system in avoiding false positive cases by not recovering structural heterogeneity or fluorescence background noise in most of the cases.

3.3 Sensitivity and Specificity Analyses

The recovered T:B contrast ratios for all experimental cases from Study I (28 cases) and II (21 cases) are plotted in Figure 6. The results are categorized into true/false and positive/negative cases using the chosen threshold value and the true target condition. The sensitivity and specificity of this optical imaging system were estimated at 43% and 95%, respectively based on phantom studies. The sensitivity and specificity of different fluorescence optical systems have only been studied on small animals and human subjects (*ex-vivo*) as a spectroscopic tool and not tomographic to date. These sensitivity and specificity values reported to date for the different fluorescence based optical systems ranged from 70% to 100%, and 75% to 100%, respectively [14–18]. Although reporting the sensitivity and specificity is more appropriate for clinical studies, an understanding of a system's performance at the laboratory level is crucial towards clinical translational efforts. The obtained sensitivity and specificity values indicate that the current imaging system requires extensive improvement in detecting deeper and smaller targets with smaller T:B contrast ratios. Currently, modifications in the hand-held probe head and alternate reconstruction algorithms are attempted towards continued improvement of the hand-held optical imaging system.

4. Conclusion

Fluorescence-enhanced optical imaging studies have been performed using a hand-held based optical imaging system, operating in the frequency-domain. Extensive phantom studies have been carried out under various experimental conditions of T:B contrast ratios (1:0, 1000:1 to 5:1), target volumes (0.45 – 0.10 cc), and target depths (1–3 cm) in order to assess the performance of the imager towards tomographic imaging. Under perfect uptake conditions (i.e. T:B = 1:0), a 0.45 cc target was tomographically reconstructed when located up to 2.5 cm deep. As the volume of the target was decreased to 0.1 cc, it was detectable only up to 1 cm deep from tomography studies. Under imperfect uptake conditions (i.e. T:B > 1:1), the detection of the same 0.45 cc target dropped to a 1.5 cm depth for a T:B = 200:1 and 1 cm depth for a T:B = 25:1. The background fluorescence in imperfect uptake cases appear as noise in the measurements, limiting the recovery of deeper targets, especially with a drop in the T:B contrast ratio. Experiments performed using targets with T:B = 1:1 or no targets at all, have successfully demonstrated the ability of the system in not recovering false targets in 20 out of 21 cases. Based on all 49 experimental cases, the estimated sensitivity and specificity of this novel hand-held probe based optical imager is around 43% and 95%, respectively.

In all the experimental cases with T:B > 1:1, the tomographic reconstructions recovered the target closer to the imaging surface than the true target depth, due to the limitation of acquiring only reflectance measurements from the hand-held probe. Hence, the hand-held optical imager represents both a 2-D detection probe with greater accuracy and an imaging system with lesser accuracy on the depth reconstructions. The addition of trans-illumination measurements in the future can improve the accuracy in reconstructing the target depth, apart from its 2-D location. Currently, a second generation hand-held optical probe based imager is developed in the Optical Imaging Laboratory, with unique abilities to acquire both reflectance as well as trans-illumination measurements (as in x-ray mammography), such that the device is adaptable for detection as well as imaging.

In addition to the development of the second generation probe, modifications in the data acquisition techniques are implemented in order to acquire both excitation and fluorescence (emission) signals during experimental studies. These measurements when used together via appropriate referencing techniques (e.g. Emission/Excitation signals at each detection point) may improve the tomographic capabilities of the imager. Additionally, use of homogeneous phantoms (with uniform fluorescence concentration) as calibration units can help account for the background fluorescence signal from imperfect uptake studies as well. However,

development of a single standard calibration phantom is challenging and may be challenging (yet possible) towards future *in-vivo* studies.

The first *in-vivo* fluorescence tomography (feasibility) study on human subjects was reported by Corlu et al [19] using a soft-compression, parallel-plane transmission geometry and a sequential illumination/ area collection based frequency-domain technique. Besides this *in-vivo* study, fluorescence tomographic studies are limited to phantoms and small animals. The current extensive work on phantoms is a precursor to our future *in-vivo* efforts towards translating fluorescence optical tomography, using a portable device, to the clinic. In addition, the ongoing research efforts are: (i) performance of *in-vitro* tomographic imaging using chicken breast based phantom models in an attempt to mimic heterogeneity in the background; and (ii) development of appropriate co-registration tools in an attempt to acquire positional and optical information in real-time towards a co-registered image for *in-vivo* tomographic analysis using the hand-held device [20].

In summary, extensive tissue phantom studies were performed to assess the robustness of this hand-held probe based ICCD imager towards future clinical translation. The preliminary reported sensitivity and specificity results obtained from phantom studies are currently used as guidance for improving the system in terms of both instrumentation and data analysis. As an initial assessment study of this hand-held optical imager, feasibility of 3-D target localization towards early-diagnosis is the immediate future direction. In the future, the accuracy of the recovered target volume and optical properties will be addressed upon further development of system instrumentation, data acquisition and reconstruction algorithm. The ultimate goal of this research is to translate the hand-held optical imager towards 3-D diffuse and fluorescence tomographic imaging in a clinical environment, towards breast imaging, at various stages of the disease.

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Figure 1.

(a) The laboratory set-up of the hand-held probe based optical imager, with illustration of the frequency-domain based imaging, (b) The hand-held probe, with the illumination-collection fiber layout.

True Target Condition		
Tomography Results	$T:B > 1:1$	$T:B = 1:1$ or no target
	$T:B > s$ TP True Positive	FP False Positive
	$T:B < s$ FN False Negative	TN True Negative

Figure 2.

The diagnostic sensitivity and specificity in the context of the current optical tomography studies. Here “ $T:B$ ” represents target to background contrast ratio, “ s ” represents “Threshold value” (shown in Equation 1) for differentiating positive and negative tomographic results.

Figure 3.

The recovered T-B contrast and recovered distance-off for all experimental cases (1 to 28) in phantom study I with tumor mimicking target. Experimental cases 1 and 3 were not included.

Figure 4.

Reconstruction results of phantom study using 2-D contour slice plot at different phantom depths (z-axis) from 0.4 cm to 1.2 cm. The target located at 1.5 cm deep with contrast ratio 200:1 (case 17) and 1 cm deep with contrast ratio 25:1 (case 25).

Figure 5.

The recovered T:B contrast ratio with respect to true target depth in Study I-B of phantom studies under imperfect uptake ratios, using a 0.45 cc target in all cases.

Figure 6.

The recovered T:B target contrast ratio (in logarithmic scale) for all experimental cases in both Study I and Study II related to fluorescence tomography studies on tissue phantoms. Among them, cases 1 and 3 were considered false negative but their recovered contrast ratios were not shown in the figure as no reconstructions were performed (due to lack of non-noisy fluorescence measurements).

Table 1

Optical properties of fluorescent target and background for all phantom studies.

Optical Properties	μ_{ax} (cm ⁻¹)	μ_{am} (cm ⁻¹)		μ_{sx} (cm ⁻¹)	Φ	τ (nsec)		
	μ_{axf}	μ_{axi}	μ_{amf}	μ_{ami}				
Target	0.3	0.04	0.506	0.046	10.87	9.82	0.01088	0.56
Background (T:B=1:0)	0.00	0.04	0.00	0.046	9.42	6.95		
Background (T:B≥1:1)	0.3/(T:B)	0.04	0.506/(T:B)	0.046	9.42	6.95		

μ_{axf} , μ_{amf} : Absorption coefficient due to the fluorophores at excitation and emission wavelengths, respectively
 μ_{axi} , μ_{ami} : Absorption coefficient due to the chromophores at excitation and emission wavelengths, respectively
 μ_{sx} , μ_{sm} : Reduced scattering coefficient due to the chromophores at excitation and emission wavelengths, respectively
 Φ and τ : quantum efficiency and life time for ICG, respectively

Table 2

The experimental details in terms of target volume, depth and T-B contrast ratio in phantom study I with T:B>1.

Experimental cases		Target volume (cm ³)	Target depth (cm)	Contrast ratio
Study I-A (Perfect Uptake)	Case 1	0.45	3	1:0
	Case 2	0.45	2.5	1:0
	Case 3	0.23	2	1:0
	Case 4	0.23	1.5	1:0
	Case 5	0.10	1.5	1:0
	Case 6	0.10	1	1:0
Study I-B (Imperfect Uptake)	Case 7	0.45	2.5	1000:1
	Case 8	0.45	2	1000:1
	Case 9	0.45	1.5	1000:1
	Case 10	0.45	1	1000:1
	Case 11	0.45	2.5	500:1
	Case 12	0.45	2	500:1
	Case 13	0.45	1.5	500:1
	Case 14	0.45	1	500:1
	Case 15	0.45	2.5	200:1
	Case 16	0.45	2	200:1
	Case 17	0.45	1.5	200:1
	Case 18	0.45	1	200:1
	Case 19	0.45	2	100:1
	Case 20	0.45	1.5	100:1
	Case 21	0.45	1	100:1
	Case 22	0.45	1.5	50:1
	Case 23	0.45	1	50:1
	Case 24	0.45	1.5	25:1
	Case 25	0.45	1	25:1
	Case 26	0.45	1.5	10:1
	Case 27	0.45	1	10:1
	Case 28	0.45	1	5:1

The phantom and target detail in phantom Study II with T:B >1 or with no target (labeled “N/A.”) represents no target is embedded.

Table 3

	Exp cases	Background ICG Concentration (μM)	Target Depth (cm)	Target Volume (cm ³)	Contrast Ratio
Study II-A	Case 29	0.001	N/A	N/A	N/A
	Case 30	0.002	N/A	N/A	N/A
	Case 31	0.005	N/A	N/A	N/A
	Case 32	0.01	N/A	N/A	N/A
	Case 33	0.02	N/A	N/A	N/A
	Case 34	0.04	N/A	N/A	N/A
	Case 35	0.1	N/A	N/A	N/A
Study II-B	Case 36	0.2	N/A	N/A	N/A
	Case 37–41	0.01	1	0.45	1:1
	Case 42–46	0.01	1.5	0.45	1:1
	Case 47–49	0.01	2	0.45	1:1