Quantitative fluorescence tomography using a trimodality system: *in vivo* validation

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Abstract. A fully integrated trimodality fluorescence, diffuse optical, and x-ray computed tomography (FT/DOT/XCT) system for small animal imaging is reported in this work. The main purpose of this system is to obtain quantitatively accurate fluorescence concentration images using a multimodality approach. XCT offers anatomical information, while DOT provides the necessary background optical property map to improve FT image accuracy. The quantitative accuracy of this trimodality system is demonstrated *in vivo*. In particular, we show that a 2-mm-diam fluorescence inclusion located 8 mm deep in a nude mouse can only be localized when functional *a priori* information from DOT is available. However, the error in the recovered fluorophore concentration is nearly 87%. On the other hand, the fluorophore concentration can be accurately recovered within 2% error when both DOT functional and XCT structural *a priori* information are utilized together to guide and constrain the FT reconstruction algorithm. [DOI: 10.1117/1.3467495]

Keywords: multimodality; fluorescence tomography; x-ray computed tomography; *a priori* information; *in vivo*; quantitative imaging; optical imaging; molecular imaging.

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1 Introduction

Fluorescence tomography (FT) has become a promising molecular imaging modality for small animals in recent years.[1–3] Unfortunately, the quantitative accuracy of currently available FT systems remains low due to the highly diffusive nature of light propagation in biological tissues.[4] In particular, two distinct requirements should be met to achieve quantitatively accurate FT. First, the tissue optical heterogeneity needs to be taken into account. In optical imaging, photon propagation is determined by the optical property distribution within the whole medium. Hence, the background optical property distribution needs to be available and used as functional *a priori* information prior to the reconstruction of FT parameters. Several studies have also confirmed that diffuse optical tomography (DOT) guided fluorescence tomography reveals more accurate location and concentration information of the fluorophore.[5–7] Accordingly, the background optical property map is obtained by DOT and used as the functional *a priori* information to correct the effect of optical background heterogeneity on the photon propagation in this study.

The second requirement for quantitative FT is that structural *a priori* information needs to be obtained from another high spatial resolution anatomical imaging modality. The recovered fluorescence concentration highly depends on depth as well as size of fluorescence inclusions due to the ill-posedness of its inverse problem. Previously, the structural *a priori* information from another high spatial resolution imaging modality has been demonstrated to improve the FT reconstruction accuracy significantly.[8–12]

To be able to utilize both functional and structural *a priori* information for quantitative fluorescence tomography, we have built a fully integrated gantry-based trimodality FT/DOT/x-ray computed tomography (XCT) system for small animal imaging. Two integral parts of this trimodality system are the XCT, which offers anatomical information, and the DOT, which provides background optical distribution. When combined, both modalities contribute to improving FT. The performance of the system has been demonstrated with phantom studies previously.[13,14] In this work, the trimodality FT/DOT/XCT system is validated *in vivo*. Transparent tubes with known amounts of ICG are implanted in nude mice subcutaneously or deep in tissue. The use of implanted ICG tubes provides a gold standard to evaluate the system in a controlled manner. We have demonstrated that the ICG concentration in the tubes can be recovered accurately only by using functional *a priori* information from DOT for background optical heterogeneity correction, together with structural *a priori* information from XCT.

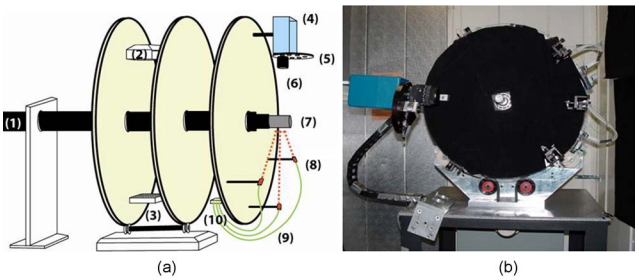

Fig. 1 (a) Schematic diagram of the FT/DOT/XCT system. The components seen in the schematic diagram are: 1. the sample holder, 2. x-ray source, 3. x-ray detector, 4. CCD camera, 5. filter wheel, 6. lens, 7. phantom, 8. fiber optic collimator, 9. optical fibers, and 10. fiber optic switch. (b) The picture of the system from the front view showing the FT/DOT components.

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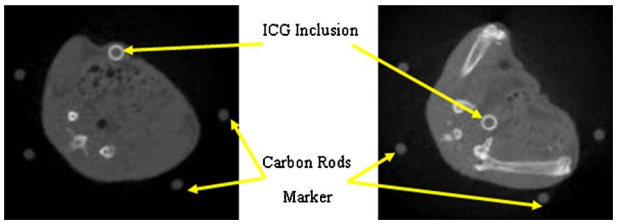

Fig. 2 XCT images for (a) the first and (b) second cases. The positions of the ICG inclusions and the carbon rods are indicated by yellow arrows. (Color online only.)

2 Method

The schematic diagram and picture of the FT/DOT/XCT system are both shown in Fig 1. The CT images were obtained with a preclinical cone-beam CT system. For optical data acquisition, a cooled charge-coupled device (CCD) camera (Perkin Elmer, Cold Blue, Massachusetts) was coupled with a macrolens focused at the object. The fluorophore ICG (IC-Green, Akorn, Illinois), was excited by a 785-nm laser diode (80 mW, Thorlabs, Newton, New Jersey). The optical property of mice was assumed to be similar at excitation and emission wavelength, and hence, DOT measurements were also acquired at 785 nm prior to FT measurements. For optical data analysis, a diffusion equation for light propagation modeling was used. A Levenberg-Marquardt nonlinear optimization algorithm was used for reconstruction. When structural *a priori* information from XCT was available, Laplacian-type soft *a priori* was utilized to guide and constrain the FT reconstruction algorithm.

All animal procedures were approved by the Institutional Animal Care and Use Committee at University of California, Irvine. 2-mm-diameter transparent thin wall glass tubes were implanted in 5 to 6 weeks old nude mice. 1% Intralipid and 500-nM ICG were added as the scatterer and fluorophore, respectively. The performance of the system was evaluated with two different cases. For the first case, the tube was implanted subcutaneously through an approximately 5-mm incision between the skin and the underlying muscle. The depth of the tube, approximately 1.5 mm, was determined from the XCT cross sectional images [Fig. 2(a)]. On the contrary, the tube was placed deep inside of the abdomen cavity for the second case. The XCT image showed that the tube was 8 mm under the skin this time [Fig. 2(b)]. This was more difficult due to deeper location of the inclusion in heterogeneous tissue.

A custom holder with four carbon rods was used not only to support the animal but also to serve as fiducial markers for accurate coregistration of XCT and optical images. The exterior boundary of the animal was obtained from XCT images and used to generate finite element mesh for optical analysis. The coregistration procedure is programmed in a manner to minimize user dependency. The reconstructions were performed using three different combinations of functional and structural *a priori* information for each case. The details of these combinations are summarized as follows.

No a priori information. The ICG concentration was reconstructed assuming homogeneous background, which was the mean absorption coefficient of animal tissue, μ_a=0.01 mm^{-1}. XCT structural *a priori* information was not used either.

Diffuse optical tomography functional a priori information alone. The ICG concentration was reconstructed using a background optical property map obtained from the DOT measurements. XCT structural *a priori* information was again not used.

Both diffuse optical tomography functional and x-ray computed tomography structural a priori information. The ICG concentration was reconstructed using a background optical property map obtained from DOT measurements. Meanwhile, the structural *a priori* information containing the fluorophore location obtained from the XCT image was also used to guide and constrain the FT reconstruction.

The reconstruction results obtained using these three methods were compared to demonstrate the benefit of using DOT functional and/or XCT structural *a priori* information in improving FT reconstruction results.

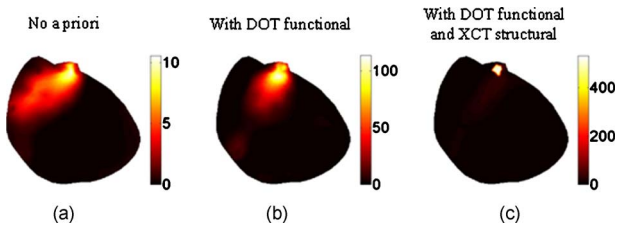

Fig. 3 The reconstructed ICG concentration maps for the subcutaneously implanted inclusion (first case). (a) The inclusion can be identified from the reconstructed ICG map, even without any *a priori* information. (b) On the other hand, the recovered ICG concentration is more accurate when DOT functional *a priori* information is utilized. (c) Finally, when both XCT structural and DOT functional *a priori* information is used to guide the FT reconstruction together, the ICG concentration is recovered within 2% error, 510 nM.

3 Results and Discussion

The XCT images for both cases are shown in Fig. 2, and the results of the reconstructed ICG concentration maps are shown in Figs. 3 and 4. For subcutaneously implanted ICG inclusion (depth 1.5 mm), the fluorophore can be resolved in

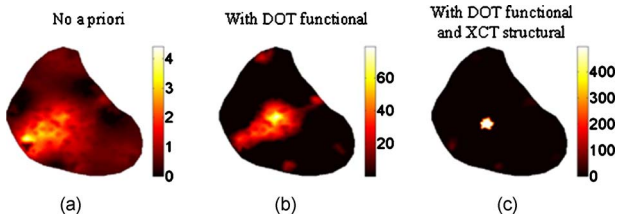

Fig. 4 The reconstructed ICG concentration maps for the inclusion deeply embedded inside of the mouse (second case). (a) The inclusion cannot be located at all without any *a priori* information. (b) ICG inclusion, on the other hand, can be localized when DOT functional *a priori* information is provided. The recovered ICG concentration, however, is still underestimated by 80%, 62 nM. (c) When both DOT functional and XCT structural *a priori* information is used, the ICG concentration is recovered within 3% error, 487 nM.

the reconstructed ICG map, even without optical background heterogeneity correction [Fig. 3(a)]. However, the recovered mean ICG concentration of the inclusion was very low, 8.2 nM, compared to the real value, 500 nM. On the other hand, artifacts are significantly reduced and the recovered ICG concentration is more accurate, but are still one fifth of the original value, 110 nM, when only the optical background correction is provided by DOT [Fig. 3(b)]. Finally, when the location of the fluorescence inclusion is obtained from XCT and used to guide the FT reconstruction together with the DOT functional *a priori* information, the ICG concentration is recovered within 2% error, 510 nM, as shown in Fig. 3(c).

When the ICG inclusion is deeply embedded inside the animal (depth of 8 mm), it is more difficult to recover the fluorescence concentration. The inclusion cannot be located at all without any *a priori* information [Fig. 4(a)]. On the other hand, it can be localized when the optical background property is obtained from DOT [Fig. 4(b)]. The recovered ICG concentration in the inclusion, however, is still underestimated by 80%, 62 nM. Finally, when both DOT functional and XCT structural *a priori* information is used, the ICG concentration is recovered within 3%, 487 nM [Fig. 4(c)].

In conclusion, a trimodality FT/DOT/XCT system for small animal imaging is presented in this work. We demonstrate that a small fluorescence inclusion can be accurately recovered *in vivo* using this system. Without the XCT structural *a priori* information, the accuracy of the recovered fluorophore concentration highly depends on the depth of the inclusion as well as background optical parameters. On the other hand, fluorophore concentration of the inclusion can be accurately recovered independent of its depth using both DOT functional *a priori* information and XCT structural *a priori* information.

Previously, XCT has been used as anatomical priors to improve FT reconstruction. In most cases, the XCT and FT images are acquired in separate settings. Then XCT anatomical information is coregistered and used to aid visualization of fluorophores or to improve the quantitative accuracy of FT.[9,11,15] On the other hand, an alternative approach is to build integrated systems to acquire both FT and anatomical images in the same setting.[12,16] Accurate coregistration of optical and anatomical images is the key advantage of this approach.

In our study, the addition of the XCT component improves FT in three steps. First, the XCT provides a straightforward and accurate way to extract an exterior boundary of the object. Second, XCT provides anatomical images of the animal, on which the fluorescence activity can be colocalized. Even without the utilization of structural *a priori* information, the fluorophore activity can be accurately coregistered on the high-resolution structural XCT image. Last but not the least, the anatomical information obtained from XCT is used to guide the FT reconstruction. As we demonstrate in this *in vivo* study, the quantitative FT can only be achieved when XCT structural *a priori* information of the fluorophore is used.

However, we also demonstrate in our previous work that structural *a priori* by itself is not enough to obtain accurate FT maps.[6,8] In essence, the strength of the trimodality system described here comes from its ability to offer both XCT structural and DOT functional *a priori* information that can be employed to reconstruct quantitatively accurate fluorophore concentration images. In summary, we validate the quantitative accuracy of a trimodality FT/DOT/XCT system with *in vivo* studies.

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