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Combined Fluorescence and X-Ray Tomography for Quantitative *In Vivo* Detection of Fluorophore

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Abstract

Initial results from a novel dual modality preclinical imager which combines non-contact fluorescence tomography (FT) and x-ray computed tomography (CT) for preclinical functional and anatomical *in vivo* imaging are presented. The anatomical data from CT provides *a priori* information to the FT reconstruction to create overlaid functional and anatomical images with accurate localization and quantification of fluorophore distribution. Phantoms with inclusions containing Indocyanine-Green (ICG), and with heterogeneous backgrounds including iodine in compartments at different concentrations for CT contrast, have been imaged with the dual modality FT/CT system. Anatomical information from attenuation maps and optical morphological information from absorption and scattering maps are used as *a priori* information in the FT reconstruction. Although ICG inclusions can be located without the *a priori* information, the recovered ICG concentration shows 75% error. When the *a priori* information is utilized, the ICG concentration can be recovered with only 15% error. Developing the ability to accurately quantify fluorophore concentration in anatomical regions of interest may provide a powerful tool for *in vivo* small animal imaging.

Keywords

Fluorescence Tomography; Diffuse Optical Tomography; X-Ray Computed Tomography

Introduction

In vivo fluorescence imaging is becoming a powerful tool for scanning small animals (1-5). Commercial availability has enabled this technique to be widely used in biological laboratories for imaging small animals longitudinally (6-8). This technique utilizes near-infrared fluorophores as exogenous contrast agents, which is similar to immuno-fluorescence labeling. This labeling mechanism has allowed fluorescence imaging to be extensively applied for cancer imaging as well as stem cell tracking (9-15). However, most commercial fluorescence imaging systems utilize planar imaging technique, which generates a 2D projection fluorescence image of a plane parallel to the detector. The depth and fluorophore concentration information cannot be determined using such a simple planar imaging approach. On the other hand, fluorescence tomography is able to recover the cross-sectional image of the fluorophore distribution from multiple projection images (16-18). It is still difficult, however, to recover quantitatively accurate fluorophore concentrations due to the ill-posedness of the FT inverse problem (19-20).

The ability to accurately quantify fluorophore concentration *in vivo* creates a powerful tool for cancer research. Preclinical cancer models with malignant cells accumulating fluorescent biomarkers can be used to track tumors over time with longitudinal *in vivo* studies testing the efficacy of various chemical and radiological therapies (15,21). Quantitative fluorescence tomography also creates a new molecular imaging tool for researchers to perform cell-based therapies, such as stem cell therapy (13-15). *In vivo* detection of cells has a broad range of potential applications in various diseases covering the fields of neurology, oncology, and cardiology. The ability to non-invasively monitor cell trafficking *in vivo* longitudinally is a pressing need for emerging cellular therapeutic strategies. Therefore, there is a great need for quantitative FT.

A multi-modality approach is a promising solution for achieving quantitative FT. It has been applied previously to combine functional/molecular imaging modalities with high spatial resolution anatomical imaging modalities (22-25). Functional imaging modalities include positron emission tomography (PET), single photon emission computed tomography (SPECT), and fluorescence tomography (FT). In all three cases there is little or no anatomical information contained in the reconstructed images, making accurate localization difficult. On the other hand, anatomical imaging modalities, such as x-ray computed tomography (CT) and magnetic resonance imaging (MRI), can provide accurate images of anatomy and structure. A combined system such as PET/CT, SPECT/CT, PET/MRI or SPECT/MRI allows the functional activities to be perfectly co-registered on anatomical images. More importantly, the anatomical information can be further used to guide and constrain the reconstruction of the functional imaging modalities thus improving PET and SPECT images (23-24). Currently, dual modality PET/CT and SPECT/CT systems are used both clinically and pre-clinically and the overlaid images are used to accurately anatomically locate function *in vivo* (26-28).

Likewise, anatomical information can also be used to improve FT images, which is also a low spatial resolution functional/ molecular imaging modality (19,29-31). In this work, CT is used as the anatomical *a priori* information to improve the quantitative accuracy of FT. One can also combine FT with other imaging modalities that could provide anatomical *a priori* information such as MRI (19,32-33). Unfortunately, in the case of MRI one must use a fiber optics-based system that results in many practical limitations such as the need to make good contact with the skin. This may not be a trivial issue with small animals with heavy fur. The necessity of using fibers in contact with skin instead of a non-contact CCD camera is due to the fact that CCDs do not work in high magnetic fields. The system presented here takes advantage of the high-resolution capability of CT to provide the anatomical information that could be used as *a priori* data in the FT reconstruction.

In addition to anatomical *a priori* information, diffuse optical tomography (DOT) is used for optical background heterogeneity correction to further improve the FT accuracy (29,34-35). The propagation of the excitation light from sources to the fluorophore and that of the emitted light from the fluorophore to the detectors depends on the optical parameters of the background at the excitation and emission wavelengths. Hence, a rigorous reconstruction of the fluorescence property maps should include the reconstruction of optical properties of the tissue at both excitation and emission wavelengths. Conventional FT systems acquire only fluorescence measurements based on the assumption of known background optical properties (2,17-18,36). In highly heterogeneous structures, the effect of the heterogeneous optical properties on the fluorescence reconstruction will be substantial (34,37). Therefore, DOT measurements are acquired prior to the fluorescence data to be able to obtain absorption and scattering maps of the animal tissue at the excitation and emission wavelengths. These individualized maps are used as optical morphological *a priori* information to improve the FT reconstruction. Moreover, the information obtained from the CT system is used as anatomical or structural *a priori* information and the improvement obtained is two-fold in that CT *a*

priori information will not only improve the FT reconstruction but also the DOT reconstructions.

In this work, we have built a combined FT/CT system that uses CT for anatomical *a priori* information as well as diffuse optical tomography (DOT) for additional optical, functional *a priori* information. We demonstrated improved quantitative accuracy of recovered fluorophores with a prototype combined FT/CT system on a single gantry with a common phantom holder. Optical components have been integrated with a preclinical cone beam CT gantry (38-40). The resulting combined FT/CT system has been tested for quantitative accuracy in determining fluorophore concentration in ROIs within phantoms with both homogeneous and heterogeneous backgrounds.

Methods

The anatomical *a priori* information is obtained with a preclinical cone beam CT system. The optical *a priori* information is obtained by a DOT method. The FT data is acquired by measuring the emitted light from fluorophores using proper filters to eliminate the contribution of the excitation light. The fluorophore concentration map is then reconstructed using these measurements. Figure 1 shows pictures of the combined FT/CT system. The optical components and the CT components are fixed on a single rotating gantry side by side such that the FT and CT imaging circles (for the trans axial tomographic images rendered from cone beam data) lie on parallel planes and are centered on the symmetry axis of the rotating gantry. The distance between the FT and CT reconstruction circles is about 20 cm and a common table translates the object between the modalities.

Figure 2 shows a schematic of the combined FT/CT system. The components of the optical system are an array of lasers and a CCD camera fitted with various filters through a motorized filter wheel. The configuration of these optical components allows for the acquisition of all the data required for both DOT and FT. The DOT acquisition employs two different lasers that output light at the emission and excitation wavelengths of the fluorophore, 785 nm (75 mW, Thorlabs, Newton, NJ) and 830 nm (150 mW, Intelite, Genoa, NV). The selection of the laser wavelength is based on the fluorophore used in the experiment, ICG, which has the excitation and emission wavelength at 785nm and 830nm (40-41). The output of the lasers was combined using a 50/50 optical splitter. The three laser positions were sequentially activated by a 1×3 optical switch (Dicon Fiberoptics). A cooled CCD camera (Perkin Elmer, Cold Blue) was used to capture images from multiple views. The CCD camera was fixed on the gantry, 30 cm away from the rotation center. The three laser positions are 45 degrees apart from each other. First, the excitation laser is turned on and DOT data collected. Then, the second laser is turned on to acquire the DOT data at emission wavelength. Finally, the excitation laser is activated again but a band-pass filter is used in front of the CCD to eliminate the excitation light and acquire only the emission light from the fluorophore.

The CT system is comprised of a cone beam x-ray generator and a flat panel x-ray detector array. Cone beam x-ray projection images are acquired at up to 512 equally spaced view angles. The distance from the center of the reconstruction circle to both the source and detector are independently adjustable allowing for a wide range of magnifications. For the images presented here, the x-ray tube was operated at 50 kVp and 0.5 mA. The flat panel sensor had an active area of 12 cm × 12 cm and pixel size of 50 μm (c7942GP, Hamamatsu Photonics). Planar images were acquired from 256 projections over 360° degree rotation in a step and shoot mode. Trans-axial images were reconstructed using a Feldcamp cone beam filtered back projection algorithm. A standard box car filter was used.

To demonstrate the performance of the FT/CT system, we have constructed multi-modality phantoms with multiple compartments to mimic background optical heterogeneity. The structure of the phantom was obtained by the CT system and was used as anatomical *a priori* information. In all cases, the absorption maps (optical *a priori* information) are obtained from DOT at 785 nm and 830 nm and Indocyanine-Green (ICG) was used as the fluorophore. Furthermore, iodine was added to the different compartments at different concentrations for CT contrast. The performance of the system was evaluated with two phantom studies. The first one has a symmetrical circular geometry with a heterogeneous background.

For the first study, a cylindrical phantom with off center cylindrical highly absorptive inclusions was prepared where a 3.6 mm inclusion was filled with ICG and located 10 mm away from the center of the phantom. The ICG concentration in the inclusion was 0.67 μM . The ICG was confined in a glass tube. A heterogeneous optical background was obtained by adding an absorptive inclusion with three times higher absorption compared to the background. This absorptive inclusion was 14 mm in diameter and located 5 mm offset from the center of the phantom. Optical property in the background was 0.006 mm^{-1} and 0.0055 mm^{-1} for the absorption coefficients. The scattering coefficient was 1.0 mm^{-1} at both 785 nm and 830 nm wavelengths. Iodine solution was also added to the object to make the structure visible in the CT image. Figure 3 shows the structural *a priori* information obtained from the FT/CT system. The glass tube that holds the ICG is seen clearly in the reconstructed CT image (left image in Figure 3). The reconstructed absorption map of the phantom at 785 nm using DOT measurements clearly shows the absorptive inclusion (right image in Figure 3). Both the CT and DOT measurements were acquired sequentially on the combined FT/CT system. Then FT data was acquired and the ICG concentration was reconstructed both with and without the *a priori* information.

For the second study, an arbitrary shaped phantom with an off center cylindrical inclusion was prepared using agarose powder. This FT system is intended for small animal fluorescence imaging in free space and an irregular shaped phantom was constructed to evaluate the system performance in a more realistic setting for *in vivo* preclinical imaging. ICG (IC-Green, Akorn, Inc) was also used as the fluorophore in this study. The phantom had a 4.2 mm ICG inclusion located 5.5 mm under the surface. The true ICG concentration is 334 nM and the ICG concentration maps were reconstructed with and without the CT anatomical *a priori* information. In this case, homogeneous background was used.

Results

The results for the first study are shown in Figure 4. If no *a priori* information was used (Figure 4.a), the 3.6 mm ICG inclusion could be located in the reconstructed fluorophore concentration image. However, the image had serious artifacts and the mean recovered ICG concentration in the ROI is only 0.12 μM (86% error). Furthermore, the full width at half maximum (FWHM) of the recovered object in the horizontal direction is 11.4 mm. On the other hand, when structural *a priori* information from XCT is used, the 3.6 mm ICG inclusion can be more accurately located without serious artifacts, Figure 4.b. The recovered ICG concentration is 0.64 μM , within 5% error from the true value. We have demonstrated in this case that the fluorophore concentration can only be accurately located by using both functional *a priori* information from DOT and structural *a priori* information from the XCT image during the reconstruction of the FT image.

The results for the second study are shown in Figure 5. The structure of the asymmetrical phantom can be seen from the CT image (Figure 5.a) and the recovered ICG concentration with and without the *a priori* information are shown in Figure 5.b and Figure 5.c, respectively. Without the *a priori* information, ICG inclusions can be located, however, the mean recovered

ICG concentration in the ROI is only 86 nM (75% error). On the other hand, when the *a priori* information from XCT image is utilized during the FT reconstruction, the ICG concentration can be recovered with only 15% error (Figure 4.c). We demonstrate that the FT system alone is able to localize the position of the fluorophore, but the accurate concentration information can only be achieved when the structural information from XCT is also used to guide the reconstruction.

Discussion

We have developed a novel multi-modality pre-clinical imager by combining non-contact FT and x-ray CT. The anatomical data from CT and optical data from DOT provide *a priori* information to the FT reconstruction to create functional/molecular images with more accurate localization and accurate quantification of fluorophore concentration. The demonstrated combined FT/CT system has the potential for *in vivo* quantitative molecular imaging. Recently, molecular imaging using fluorescence techniques has shown great promise in cell detection. The true fluorophore concentration cannot be recovered unless proper *a priori* information is available. This system, which can reveal a quantitatively accurate fluorophore concentration image, has great potential in a number of applications covering a broad range from stem cell to cancer research.

The ultimate goal of this development is to perform *in vivo* preclinical longitudinal studies. In CT imaging performed at high spatial resolution (typically $<50\text{ }\mu\text{m}$) in longitudinal measurements radiation effects can produce effects that can possibly corrupt the outcome of the biological measurement (42). A study measured the dose delivered during one high-resolution CT scan at about 10% of the LD50 dose to mice (x-ray source 40 kVp, 800 μA ; 0.5 s per projection) (43). In addition, the radiation dose produced by CT is roughly inversely proportional to the cube of the pixel dimension (*i.e.*, inversely proportional to voxel volume). The current generation of preclinical CT systems requires approximately 13 cGy for a spatial resolution of $50\text{ }\mu\text{m}$. Within this dose range, CT can change the genetic or functional status of biological tissues, or can cause premature death for a measurable fraction of animals, especially for studies that involve high spatial resolution or involve serial imaging. These issues emphasize the importance of developing methods that reduce the radiation dose needed for micro-CT imaging, and moreover, motivate the need for a highly efficient radiation detector for CT that can reduce both the delivered dose and the radiation-induced effects for these studies. A highly efficient detector would reduce image noise when operated with equivalent imaging parameters as those used with current CT detectors. Finally, a highly efficient radiation CT detector could be operated at lower x-ray tube power, thereby increasing the number of scans and improving scanner throughput before tube loading limits are reached. These important goals all motivate the need for development of a small CT x-ray detector that has optimized dose efficiency and a design compatible with (and ideally optimized for) preclinical CT imaging applications.

In this study, we have used a commercially available Hamamatsu flat panel detector. However, this detector converts incident x-ray to fluorescence, which then enters the photodiode array where electric charge is accumulated according to the light intensity. This indirect conversion decreases the sensitivity of the detector. To create an optimized preclinical CT x-ray detector we are developing a method to grow polycrystalline mercuric iodide (HgI_2) thin films directly onto CMOS readout arrays. With direct conversion the combination of higher efficiency energy transfer, higher stopping power for x-rays, and lower lateral spreading of signal can lead to significant improvements in sensitivity and detective quantum efficiency, particularly at lower incident x-ray intensities (44-45). Future plans include fabricating 100 cm^2 ($10\text{ cm} \times 10\text{ cm}$) CMOS arrays completely coated with HgI_2 for a sufficient field of view to replace the existing x-ray detector in the combined FT/CT system. We anticipate an order of magnitude dose

reduction to 0.13 cGy for high 50 μm spatial resolution CT imaging from the increased sensitivity of the polycrystalline HgI_2 films as compared to commercially available flat panel pre-clinical CT detectors.

In addition to dose reduction, throughput is important for large-scale longitudinal studies and scan time becomes a limiting factor. The CT data acquisition can be completed in as little as two minutes however the FT data acquisition can take up to 20 minutes with the current optical components. The combined FT/CT demonstrated in this work contains sufficient space for additional components. Future plans to decrease scan time may include adding an additional CCD camera and corresponding laser arrays.

In summary, a multi-modality FT/XCT system for quantitative molecular imaging was built. The XCT image was used as the anatomical *a priori* information to guide and constrain the FT reconstruction, while the background optical property maps were reconstructed from DOT and used as functional *a priori* information. Phantom studies were carried out to evaluate the performance of the system. The results demonstrated that the fluorophore concentration can only be obtained accurately when guided by the *a priori* information provided by the x-ray CT. ICG was used as the fluorophore in this study. This very system, however, can be used to image any infrared fluorophores by changing to proper filters.

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Figure 1.

Pictures of the prototype combined FT/CT system. The image on the left shows an axial view of the rotating gantry (trans axial image) where the optical components are seen and the x-ray components lie behind. The image on the right shows a side view (coronal or sagittal projection) where the x-ray components are to the left and the optical components to the right.

Figure 2.

A schematic drawing of the combined FT/CT system design showing the configuration of the optical and x-ray components. The view is along the axial direction and P represents the phantom/patient position.

Figure 3.

The first phantom study. The schematic of the phantom design is shown in (a). The image in the middle (b) contains CT derived map of x-ray attenuation coefficients (anatomical) and the image on the right (c) contains DOT derived absorption coefficients (optical morphological).

Figure 4. Reconstructed ICG concentration map without (a) and with (b) *a priori* information. Without any *a priori* information, the recovered ICG concentration has 86% error, and the reconstructed image shows many artifacts. However, the ICG concentration can be recovered within 5% error when both XCT structural *a priori* information and DOT functional *a priori* information are used. The color bars all have the units of μM .

Figure 5.

The results for the second phantom study. (a) The CT image of the irregular-shaped phantom. The reconstructed ICG concentration maps without (b) and with (c) the XCT *a priori* information are also shown. As seen from the images, the recovered ICG concentration value is underestimated significantly without the *structural a priori* information (b). However, the ICG concentration can be recovered within 15% error when XCT structural *a priori* information is used (c). The color bars all have the units of nM.