Published in final edited form as:
Proc SPIE. 2012 ; 8313: 83130T.

Investigations on X-ray luminescence CT for small animal imaging

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Abstract

X-ray Luminescence CT (XLCT) is a hybrid imaging modality combining x-ray and optical imaging in which x-ray luminescent nanophosphors (NPs) are used as emissive imaging probes. NPs are easily excited using common CT energy x-ray beams, and the NP luminescence is efficiently collected using sensitive light based detection systems. XLCT can be recognized as a close analog to fluorescence diffuse optical tomography (FDOT). However, XLCT has remarkable advantages over FDOT due to the substantial excitation penetration depths provided by x-rays relative to laser light sources, long term photo-stability of NPs, and the ability to tune NP emission within the NIR spectral window. Since XLCT uses an x-ray pencil beam excitation, the emitted light can be measured and back-projected along the x-ray path during reconstruction, where the size of the X-ray pencil beam determines the resolution for XLCT. In addition, no background signal competes with NP luminescence (i.e., no auto fluorescence) in XLCT. Currently, no small animal XLCT system has been proposed or tested. This paper investigates an XLCT system built and integrated with a dual source micro-CT system. Two novel sampling paradigms that result in more efficient scanning are proposed and tested via simulations. Our preliminary experimental results in phantoms indicate that a basic CT-like reconstruction is able to recover a map of the NP locations and differences in NP concentrations. With the proposed dual source system and faster scanning approaches, XLCT has the potential to revolutionize molecular imaging in preclinical studies.

Keywords

Micro-CT; dual energy; small animal imaging; nanophosphor

1. INTRODUCTION

Preclinical imaging is dramatically altering the way researchers utilize animal models to study mechanisms of disease, its progression, and its response to a therapeutic intervention. Among the preclinical imaging modalities, in vivo optical tomography (fluorescence, bioluminescence) can provide information at both cellular and molecular levels due to its high sensitivity and specificity, but its application is limited by low spatial resolution for deep targets; due to light scattering in turbid media, the current spatial resolution is in the range of a few millimeters, at best. X-ray-based micro-CT scanners on the other hand, can provide very high spatial resolution on the order of 80 to 100 microns for in vivo studies[1].

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But, the application of micro-CT in molecular imaging is limited by its very poor sensitivity ($\times 10^3$ – 10^4 mg/ml range).

Recently, X-ray luminescence CT (XLCT), a hybrid of x-ray CT and optical tomographic methods that exploits nanophosphor (NP) probes has been introduced[2]. When excited by x-ray irradiation, these nanoscale structures emit light at discrete wavelengths over the visible-to-near infrared (vis-to-NIR) spectral domain. Notable advantages of XLCT include increased tissue excitation penetration depths, and complete circumvention of tissue autofluorescence, a problem in imaging modalities that rely on vis/NIR excitation sources. If the x-ray excitation is performed using narrowly collimated pencil beams, the spatial resolution of XLCT can be made similar to pencil beam sizes (<1 mm), while enabling detection sensitivities comparable to fluorescence tomography[3]. Hence, XLCT shows promise for preclinical imaging, but proofs-of-principle extending beyond either simulations or using phantom studies have yet to be demonstrated. To the best of our knowledge, no XLCT system for small animal imaging has yet been developed. We see great opportunity for XLCT as a preclinical imaging modality that will enable tracking of biological processes that range from metabolism to cell-surface receptor-target interactions, gene-expression, disease progression, and drug activity/delivery, with unprecedented structural resolution, while providing high-detection sensitivity. The goal of this work is to present our implementation of XLCT and two new sampling approaches with potential for time reduction.

2. METHODS

2.1 XLCT system

In an attempt to test the feasibility of XLCT with our in-house developed dual source small animal micro-CT system[4], we replaced one of our x-ray imaging cameras with a CCD optical camera (QE Imager, Lavision, Germany) for light detection. Unlike Pratz et al.[2], in this work, we have used translatable pinholes placed in front of the object and not a translated object. This had the advantage of easier modifications on our dual source micro-CT system. We have added a pinhole aperture to generate the pencil x-ray beam by drilling a 2 mm diameter hole in a lead plate. The pinhole was translated horizontally during scanning using a motorized translation stage. All system components were controlled using LabVIEW (National Instruments) applications to perform sampling as in a 1st generation CT scanning (i.e., acquiring light projections pixel by pixel as the pencil beam intersects the object and then rotating to the next angle and repeating the procedure, Fig. 1). The XLCT instrument also required the development of a light-tight black box to shield detectors from ambient light during sampling.

2.2 Nanophosphore probe

Europium-doped yttrium oxide, [Y₂O₃; Eu], was utilized as the NP imaging probe for this initial work [I. N. Stanton, T.T. Yoshizumi, M.J. Therien, Manuscript in preparation]. This NP provides: (i) high quantum yield scintillation emission at 611 nm, (ii) robust thermal, chemical, and radiation stability, and (iii) an oxide surface for facile surface conjugation of targetable probes. These NPs can be fabricated to provide homogeneous sizes ranging from 10 – 100 nm; varying NP dopant ion concentrations modulates the dominant NP emission band over the vis/NIR spectral domain. We have imaged a phantom with two NP inserts of different concentration, where only 14 angles over 180 ° rotation have been used for sampling.

2.3 Battleship sampling paradigm

One of the problems associated with this initial XLCT prototype was sampling inefficiency if single x-ray pencil beams are used to scan each 2D (x-y plane). We aimed to address this issue by designing faster scanning solutions. In this way, multiple axial planes can be sampled faster, and a fully 3D XLCT image can be obtained by stacking the 2D XLCT slices in the z-dimension. Steering two X-ray pencil beams can reduce the sampling time for each 2D plane. In a first unsophisticated sampling protocol, the two x-ray pencil beams can be used to provide (10 ms apart) two orthogonal angular light projections, reducing the sampling time by half with respect to a single x-ray beam method. Likewise, additional techniques can be used to further increase the sampling efficiency and reduce sampling time. An idea is to mix sampling and reconstruction in a “Battleship-like” approach [5]. In the Battleship game, the players are discovering the empty cells and not “sampling” them in further stages of the game. This thought process can be applied to XLCT image sampling. Molecular imaging is essentially based on targeted probes and typically results in sparse images after reconstruction. Many of the molecular imaging techniques such as PET/SPECT/FDOT provide images with high contrast to background ratios for regions where the probe has high affinity. Consequently, finding these regions containing the probe can be similar to the Battleship game strategy.

For example, Fig. 2 illustrates that sampling at the first two orthogonal angles not only provides measurements for optical projection data, but also gives a map of the empty regions (cells) of the object. As in the Battleship game, these empty cells do not require sampling at the next set of sequential angles, and this information can be used to restrict sampling, making the process adaptive and more efficient in both time and dose. The underlying assumption for this method is that non-zero cells (i.e. the cells of the imaging matrix containing NP probe signal), generate a signal above the background that can be measured by the light detection system. This assumption is obviously linked with the sensitivity of light collection and the detection efficiency.

2.4 Sampling using multiple pinholes

A different approach to increase sampling efficiency, involves simultaneous excitations with more than one x-ray pencil beams at each angle. This is somewhat similar to the approach of a multipixel x-ray source, using the carbon nanotubes [6]. But by using more than one x-ray pencil beam for simultaneous excitation (i.e. the same exposure of the x-ray tube) may result in loss of selectivity and potential image artifacts due to ambiguities in the back-projection of the integrated light signal. However, we have discovered that this is not the case if the number of simultaneous x-ray pencil beams is kept relatively reduced (e.g. 5). The XLCT sampling operation represents a dot product between the unknown image and x-ray pencil beams. If the same pinholes' distribution would be used at each angle, only concentric small regions would be sampled during a rotation. But by using randomness in the distribution of these multiple x-ray pencil beams from one angle to another, one could ensure a relatively uniform sampling over the whole image. Randomness is indeed expected to provide the best strategy for compressed sensing in sampling. This has also been shown in the Rice single pixel camera project, in which using random sampling strategies, the number of measurements were dramatically reduced[7]. In practice, randomness can be mimicked by having a large set of lead plates with multiple drilled pinholes. A different lead plate would be used at each angle. To facilitate a more practical realization of the multiple pinhole sampling, we have also considered the case of one single pattern of 5 pinholes but with offset translations applied from one angle to the next. The translations could be optimized to maximize the uniformity of density in sampling over a central disk-like region on the image where the object has been positioned. This uniformity can be ensured by searching at each

angle, the optimal translation that would minimize the standard deviation of an image obtained by summing all sampled pencil beams.

2.5 Simulations

We have performed simulation to understand the advantages and the limits of the Battleship and multiple pinholes approaches. The algorithms were implemented in MATLAB using a phantom shown by Figs. 3 A,B. The phantom was circular in section with a 3 cm diameter (mouse-like in size). Its x-ray attenuation and optical properties were of a homogeneous tissue (muscle). This body section contained 3 other round structures of 5.6, 3.75, 2.8 mm in diameter and with NP concentration in of 0.125 0.25, 0.5, in arbitrary units. Sampling was performed with one or multiple pinholes each equal in size to 4 pixels i.e. 1.87 mm. The optical detector covered only a partial side of the phantom surface, similar to the case of using a CCD detector with a focusing lens. The simulations involved the creation of a sensitivity matrix that combined the pencil beam x-ray excitations with optical sensitivity following a mathematical formalism described in[3]. For optical sensitivity we modeled the light attenuation as a function of distance from the center (see Fig. 3 C). In a complete XLCT acquisition protocol, the phantom was sampled using single pencil beam excitations at 61 positions for each angle and 61 angles over 360° rotation. Following each exposure the optical signal has been summed in a single number. For complete single pencil beam sampling, the integrated optical signal can be displayed as a sinogram (Fig. 3 D).

In the simulations of the Battleship sampling, the number and positions of single X-ray pencil beam excitations were adaptively reduced to sample only the regions of the image producing light.

To investigate sampling strategies using multiple pinholes, we first considered the case of using a set of 19 patterns of 5 randomly distributed pinholes. At each angle, one of these patterns has been selected in a cyclical way and only one exposure was performed. We have also simulated the situation of a single pattern but with optimal translations from one angle to another. At each angle, the optical signal has been integrated in a single number and used for XLCT reconstruction. In both cases we used 360 angles over a complete rotation. The multiple pinholes approaches may require the simultaneously acquired x-ray images to accurately determine the geometric footprints of each x-ray pencil beams but this information is readily available in a combined micro-CT/XLCT system.

XLCT reconstruction has been performed both using a filtered back-projection and maximum likelihood expectation maximization (MLEM) [8] using the combined x-ray and light sensitivity matrix.

We have used these simulations to assess the accuracy of the reconstruction and the potential sampling time reductions associated with the Battleship and multiple pinholes approaches. Root mean squared error (RMSE) has been used as a measure of accuracy for the reconstruction. To estimate the reduction in sampling time we have plotted the RMSE in reconstructed images after 100 MLEM iterations versus the total number of measurements for two situations i.e.: 1) when we have a complete sampling in which all 61 x-rays pencil beams and 61 angles are used and 2) when the number of x-ray pencil beams is reduced using the Battleship approach. In order to speed-up the convergence of the MLEM algorithm we have ordered the angles to alternate orthogonally. The total number of measurements in the two cases is equal to the product between the number of angles and the number of sampled rays. We have also measured RMSE for the multiple pinholes approach and compared with the Battleship approach.

3. RESULTS

The results for the real phantom experiment are shown by Fig. 4 and underscore the potential ability of XLCT techniques to provide accurate imaging. A simple FBP based CT-like reconstruction was able to recover a map of the NP locations and discriminate between localized NPs of two different concentrations. However, FBP reconstruction is noisy.

The more sophisticated MLEM reconstruction results are shown in Fig. 5. We have also simulated a Battleship-like sampling approach by applying a detection threshold on the sinogram and the result is shown in (Fig 5B). The estimated sampling time with Battleship was 3 times shorter than for a normal scan. No apparent differences in image quality are seen between the complete sampling and Battleship results. We note that this reduction would have been larger if more angles would have been sampled.

The simulation results of the Battleship versus normal sampling are shown by Fig. 6. For a number of measurements (number of x-ray exposures in an experiment) around 1000, if the Battleship approach is used, the number of rotational angles is complete i.e. 61 and the RMSE error is as low as in the case where all x-rays pencil beams at all angles are used as in the complete sampling set-up. Without Battleship sampling and performing only 1000 measurements we can sample only 16 angles if all (i.e. 61) x-ray pencil beams were used. The RMSE error in this angular under sampled case, is however is much higher. Therefore, for similar RMSE errors, using a Battleship approach we have reduced the number of measurements 3.45 times compared with the normal sampling.

Finally, Fig. 7 presents results of the simulations on multiple pinholes sampling using a set of random patterns (A) or a single pattern with optimal translations (B) applied at each angle. For an objective comparison with the Battleship approach we have maintained to 100 the number of iterations of MLEM reconstruction. Note, that as shown by the comparisons to the true phantom line profiles (Figs. 7 B, D), these multiple pinholes reconstructions are recovering the correct values in concentrations but are more noisy than the Battleship versions. The noise could be potentially reduced by increasing the number iterations in MLEM reconstruction and/or including regularization. The RMSE values for multiple pinholes with 100 iterations reconstruction has been 0.01 (for both cases Figs 7 A, C) which is approximately two times higher compared with the Battleship with 61 angles. However, the number of measurements (i.e. the number of exposures) and the sampling time has been reduced 2.66 times relative to Battleship with 61 angles.

In fact, using multiple pinholes sampling and one exposure at each angle, the acquisition time is expected to be equal to that of a normal micro-CT scan. Some other variations of this sampling approach are possible. For example, the number of angles could be reduced but more translations could be performed at each angle. The number of x-ray pencil beams and their distributions will impact the image quality in the multi pinholes approach.

Since all these approaches are based on the use of a sensitivity matrix, we could optimize the sampling to minimize the condition number of the sensitivity matrix. The condition number of a matrix measures the sensitivity of the solution of a system of linear equations to errors in the data. It gives an indication of the accuracy of the results from matrix inversion and the linear equation solution. Using simulations, we have confirmed that an increase in the number of pinholes from 5 to 20, would increase the condition number from 12.95 to 26.32 and will result in more inaccuracies in the reconstruction. A singular value analysis of the optimal number of x-ray pencil beams, their optimal translation and the number of angles is not the focus of this study but could be performed as described before in FDOT imaging[9].

4. DISCUSSION

Our unique dual source micro-CT offers an excellent starting point for developing an XLCT instrument. In a first unsophisticated sampling protocol, the two x-ray pencil beams can be used to provide two orthogonal angular light projections, reducing the sampling time by half with respect to a single x-ray beam sampling. To maintain the selectivity of excitation, the two x-ray beams could be alternatively pulsed every 10 ms. Our x-ray tubes have large focal spots of 0.6/1 mm (Varian, G256) and were specifically selected to provide instantaneous flux with very short exposures (e.g. 10 ms) for dynamic cardiopulmonary imaging. We still obtain a very high flux by compensating with large tube current (up to 300 mA). In the XLCT-proposed implementation [2], the authors use a micro-focus x-ray tube with very low currents (0.5 mA) and exposures times of 1 sec to provide the x-ray excitation. The light produced by NP is directly proportional to the radiation dose. But, 1-sec excitations for each pencil beam increase the sampling time. Therefore, with our short-pulsed but high mA x-ray excitations, we can provide a faster XLCT sampling solution. The previously proposed XLCT system [2] used one or two EMCCD cameras as optical detectors placed on opposite sides of the animal. While EMCCDs are an excellent choice in terms of sensitivity and quantum efficiency (QE=90%), they might not be justifiable for XLCT, since they are only used to integrate light over the 2D area in a single pixel value. Relative to cameras, photon multiplier tubes (PMT) are more cost-effective and are typically used as single pixel light detectors. Furthermore, with more and uniformly distributed detectors (e.g., a configuration with $n=4$ detectors arranged to cover every quadrant), the sensitivity of light collection is maintained with the rotation angle. PMTs have also a small footprint that allows their placement in closer proximity to the animal. We have identified multiple PMT models from Hamamatsu, such as the R3896 and R928P that have very high gain (approx. 10^7) and can be used both in integration and single photon counting modes to optimize detection efficiency. Their active areas are large (8 mm $\times$ 24 mm); consequently they will cover a quite large view of the animal similar to a camera. Light collection can be maximized by lenses placed in front of PMTs.

While our experimental results define proof-of-concept, they also reveal challenges associated with the development of XLCT. While selective excitation using a single x-ray pencil beam promises to improve XLCT resolution, it is also a very slow and inefficient process. Using the Battleship or multiple pinholes sampling approaches we could achieve a higher efficiency. In the Battleship sampling, a detection threshold will be required to decide between signal/no signal for each excitation. We expect this threshold to influence the sensitivity of the method. Since light signal is proportional to x-ray dose, repeated exposures in the same location with signal integration can increase the sensitivity at suspected NP. Sampling can be speed-up even more in XLCT with the use of multiple pinholes. While we have tried this approach via simulations only, its implementation especially using a single pattern and optimized translations should not be very difficult. Its attractiveness comes also from the possibility of extending the distribution of multiple pinholes over two dimensions in the lead plates to allow a 3D scanning mode. Further work will concentrate on the implementations of these novel sampling approaches.

5. CONCLUSIONS

These proof-of-concept results utilizing [Y2O3; Eu]-based x-ray luminescent nanoparticles underscore the potential ability of XLCT techniques to provide high sensitivity in vivo imaging, and indicate that basic CT-like reconstruction is able to recover a map of the NP locations and discriminate between localized NPs of different concentration. At this early investigational level, we have not measured the sensitivity limits for XLCT, but have provided foundation measurements to pursue this technology for high resolution, high

sensitivity deep tissue imaging. Two novel sampling solutions were proposed with potential for speeding up the sampling time. With faster scanning approaches, XLCT has the potential to revolutionize molecular imaging in preclinical studies.

Acknowledgments

All work was performed at the Duke Center for In Vivo Microscopy, an NIH/NCRR National Biomedical Technology Resource Center (P41 RR005959), with additional support from NCI (U24 CA092656).

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Fig. 1.

(A) The XLCT set-up using a CCD camera and translatable pinhole. (B) A phantom containing 2 inserts immersed in body-like optical properties fluid (C) The object and cameras protected from ambient light by a black box.

Fig. 2.

An example of a sparse image (A) containing 3 cells with NP probes. After sampling at 2 orthogonal angles (B) and using an AND operation only 6 cells are possible candidates for containing NP (marked as 1's). The total number of variables has been reduced from 36 to 6 for the next sampling angles based on the binary map of NP locations. At the next sampling angle (C), three more "false" non-zero variables (yellow circle) are eliminated due to lack of signal.

Fig. 3.

(A) A phantom containing 3 structures with various NP concentration (A) included in a circular container with homogeneous x-ray and optical properties similar to soft tissue and on which we mark the position of the light detection (B). The optical sensitivity of the optical detection system as a function of source position and distance from the center (C). The XLCT simulated sampling results in a sinogram like optical projection data (D).

Fig. 4.

(A, B) Oblique and axial CT slices showing the NP inserts. (C) Sinograms for the optical signals over 14 angles obtained by integration of the light on the camera for each x-ray pencil beam excitation. (D) XLCT reconstruction via FBP. (E) XLCT reconstruction via MLEM after 10 iterations.

Fig. 5.
The phantom reconstructed using 100 MLEM iterations using all sampled data (A) and a Battleship-like sampling (B).

Fig. 6.

The phantom reconstructed using around a 1000 measurements for the normal sampling (A) in which all x-ray pencil beams are used but only 16 angles are sampled and (B) with Battleship sampling approach where all 61 angles are sampled.;(C) shows a comparisons of line profiles relative to the true phantom values; (D) a plot of the RMSE for normal and Battleship for various total number of measurements.

Fig. 7.

The phantom reconstructed using 5 pinholes and 360 angles using 19 patterns selected cyclically (A) and a single pattern with optimized translations to ensure uniformity of sampling (C) In (B) and (D) we shows a comparison of line profiles relative to the true phantom values for the two situations