Published in final edited form as:

Opt Lett. 2019 October 01; 44(19): 4769–4772. doi:10.1364/OL.44.004769.

X-ray-induced shortwave infrared luminescence computed tomography

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Abstract

X-ray luminescence computed tomography (XLCT) based on x-ray-excitable nanophosphors has been proposed as a new modality for molecular imaging. The technique has two main advantages compared to other modalities. First, autofluorescence, which is problematic for fluorescence imaging, can be substantially reduced. Second, deep-tissue *in vivo* imaging with high optical contrast and spatial resolution becomes achievable. Here, we extend the novel XLCT modality from the visible or infrared region to a shortwave infrared wavelength by developing an x-ray-induced shortwave infrared luminescence computed tomography (SWIR-XLCT). For this application, rare-earth nanophosphors (RENPs) were synthesized as core/shell structures consisting of a Ho-doped NaYbF₄ core surrounded by a NaYF₄ shell that emit light efficiently in the shortwave infrared spectral region under x-ray excitation. Through numerical simulations and phantom experiments, we showed the feasibility of SWIR-XLCT and demonstrated its potential for x-ray luminescence imaging with high spatial resolution and deep depth.

X-ray luminescence computed tomography (XLCT) is emerging as a new molecular imaging modality through detection of photon emissions from nanophosphors excited by an x-ray [1,2]. XLCT holds promise for high-resolution and high-sensitivity whole-body *in vivo* imaging by extending the advantage of traditional optical molecular imaging such as fluorescence molecular tomography (FMT) [3,4] and bioluminescence tomography (BLT) [5], while mitigating the limitations of the existing optical imaging techniques (e.g., imaging depth, spatial resolution). XLCT was originally investigated using a narrow beam of x-ray which is raster scanned and rotated around the sample to obtain a volumetric image [2]. Owing to its long data acquisition time, the practical application of the narrow beam XLCT system may be limited. Different geometries of XLCT have been investigated including cone beam [6,7], limited angle [8], multiple narrow beams [9], and coded aperture compressive XLCT [10]. Up to now, XLCT has been focused on light emissions in the visible or conventional near-infrared (NIR, 700–1000 nm) spectral region where high photon absorption and scattering deteriorate the spatial resolution and imaging depth. In a previous study [11] our group developed novel x-ray excitable rare-earth nanophosphors (RENPs) with emission in a shortwave infrared (SWIR, alternatively referred to as NIR-II) spectral region, which is intensively investigated for deep tissue imaging [12, 13]. SWIR imaging offers improved spatial resolution in deeper tissue due to greatly reduced photon scattering,

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absorption, and tissue autofluorescence. In this Letter, we investigate an x-ray-induced shortwave infrared luminescence computed tomography (SWIR-XLCT) strategy.

Erbium-doped rare-earth nanophosphors emitting at the wavelength of 1500 nm have been developed recently for *in vivo* SWIR imaging with a high spatiotemporal resolution [11, 14]. Even though the light scattering is suppressed in a longer emission wavelength over 1500 nm [15], the exponential absorption of the emission light in this range of water absorption adversely affects the resolution quality of luminescence tomography imaging of deep tissues and organs. To reduce the adverse interference with water absorption, we tuned the emission wavelength of RENPs by doping lanthanide element Holmium (Ho). Ho-doped RENPs were prepared through solvothermal decomposition of rare-earth trifluoroacetate precursors [Yttrium, Ytterbium, and Holmium trifluoroacetate, Rare Earth Products, Inc. (Beverly, Massachusetts)] in 1-octadecene (ODE) according to previous publications with a slight modification [11]. Briefly, a two-step synthetic procedure was involved in the synthesis of core/shell RENPs. In the first step, the monodisperse cores were obtained by decomposing the rare-earth trifluoroacetate precursors in presence of surfactants at the high temperature (325°C). The second step is similar to the first one. Only yttrium trifluoroacetate was used as a precursor for formation of the shell instead of a mixture of various metal precursors during the solvothermal decomposition reaction. The resultant core/shell RENPs consist of a Ho-doped NaYbF₄ core surrounded by a NaYF₄ shell [16]. As seen in Fig. 1(a), the Ho-doped RENP cores show uniform spherical morphology and narrow size distributions (~17 nm in diameter). To prevent the luminescence from the quenching effect originating from surface ligands and solvent molecules, the NaYbF₄ cores were coated with a 5 nm NaYF₄ passive shell to create a gap between lanthanide ions and surface quenchers, which was confirmed by transmission electron microscopy (TEM) micrographs [Fig. 1(b)]. An x-ray diffraction (XRD) pattern was performed to confirm the hexagonal crystalline structure of RENPs was not influenced by Ho doping in Fig. 1(e). Upon x-ray irradiation, all elements including Na, Yb, Y, F, and Ho in core/shell NaYb_{0.98}F₄:Ho_{0.02}@NaYF₄ RENPs were excited to generate the secondary electrons, which further excite the luminescent centers of Ho. Such a resonant transfer of excitation energy from the sensitizers (i.e., Yb) to activator dopants (Ho) in RENPs resulted in the generation of SWIR emission at ca. 1180 nm with 30 nm of full width at half-maximum (FWHM), which is unique to the specific rare-earth activator (Ho) [Fig. 1(c)]. The photoluminescence excitation mapping further confirmed that Ho-doped RENPs are capable of generating SWIR emissions with a large Stokes shift (~300 nm). Moreover, the characteristic SWIR emission of the Ho dopant at 1180 nm was also observed from an x-ray luminescence measurement using a spectrometer coupled with a SWIR detector (NIRvana 640) [Fig. 1(d)]. The luminescence emission of the RENP upon x-ray irradiation corresponded to those observed using conventional laser excitation. The yttrium and ytterbium in the host of the RENPs serve as x-ray absorbers, which transfer energy to the luminescent dopant Ho, thereby resulting in the emission corresponding to the characteristic Ho peak at 1180 nm observed from the $^5I_6 \rightarrow ^5I_8$ transition.

Figure 1(f) shows the diagram of the SWIR-XLCT imaging system, which includes an x-ray source, SWIR detector (NIRvana 640, Princeton Instruments) and its data acquisition (DAQ), sample rotary stage and its controller, and cabinet lead shielding. In this study, a commercial x-ray irradiator (X-RAD 320, Precision) which can generate an x-ray up to 320

kVp in a compact cabinet was adopted [as shown in Fig. 1(g)]. The SWIR camera is fixed in an adjustable tripod to detect the light emission within the cabinet through a transparent lead glass window sealed with a black lead slice to minimize the interference from environment light. A 3D printed sample stage connected to a motorized rotator is mounted in the cabinet. Through rotating the sample stage, images from multiple different views can be obtained. In each experiment, bright-field and luminescence images were sequentially obtained from 24 different angles covering 0° to 360° of the sample under nature light illumination and x-ray excitation, respectively. The acquired bright-field images [Fig. 2(a)] are then gathered to reconstruct the 3D shape (visual hull) of the testing object using a shape-from-silhouette algorithm [Fig. 2(b)]. The created visual hull is discretized to generate a 3D finite element mesh [Fig. 2(c)]. Multiple-view luminescence images [Fig. 2(d)] are converted from free space to the signal of detectors [red dots in Fig. 2(e)] that are in the boundary of the finite element mesh. The collected luminescence signal from each detector and the spatial information of the finite element mesh are then used for 3D luminescence tomographic image reconstruction [Fig. 2(f)].

The diffuse equation (DE), an approximation of the radiative transfer equation (RTE), has been widely used for optical tomography or XLCT in visible or the conventional NIR spectrum region due to its low computation cost and memory efficiency. However, in the SWIR region, the reduced scattering coefficient is so low that it can be comparable to the absorption coefficient in the biological tissue. Thus, the DE is invalid. Instead of DE, a steady-state RTE (1) with the vacuum boundary condition (2) is used here to model the light propagation in the medium, which can be numerically solved by a multigrid solver [17]. Equations 1 and 2 are as follows:

$$\hat{s} \cdot \nabla \Psi(\vec{r}, \hat{s}) + (\mu_a + \mu_s) \Psi(\vec{r}, \hat{s}) = \mu_s \int_{\hat{s}^{n-1}} f(\hat{s}, \hat{s}') (\vec{r}, \hat{s}') d\hat{s}' + q(\vec{r}, \hat{s}), \quad \vec{r} \in \Omega, \hat{s} \in \hat{s}^{n-1},$$

(1)

$$\Psi(\vec{r}, \hat{s}) = 0, \quad \vec{r} \in \partial\Omega, \hat{s} \cdot \hat{n} < 0,$$

(2)

where $\Psi(\vec{r}, \hat{s})$ is the photon flux that depends on space $\vec{r}$ and angle $\hat{s}$, $q(\vec{r}, \hat{s})$ is the luminescent source, $f(\hat{s}, \hat{s}')$ is the Henyey–Greenstein scattering function, μ_a and μ_s are the absorption and scattering coefficient, respectively, and $\hat{n}$ is the outer normal at boundary Ω of domain Ω and the angular space $\hat{s}^{n-1}$. Under the assumption that the luminescent source is isotropic, the solution of RTE (1) is

$$\Psi(\vec{r}, \hat{s}) = \int G(\vec{r}, \hat{s}; \vec{r}') q(\vec{r}') d\vec{r}',$$

(3)

where $G(\vec{r}, \hat{s}; \vec{r}')$ is the Green's function of RTE (1).

In XLCT imaging, the luminescent source $q(\vec{r}')$ can be expressed as (4)

$$q(\vec{r}') = \eta X(\vec{r}') n(\vec{r}'),$$

(4)

where $X(\vec{r}')$ is the x-ray excitation intensity in the imaging domain which can be calculated using Beer–Lambert law [6], $n(\vec{r}')$ is the nanophosphor distribution, and η is the quantum yield.

Use the finite element method (FEM) to discretize Eqs. (1)–(4) into a matrix equation which links the luminescence fluence and nanophosphor distribution as follows [18]:

$$\Phi = \mathbf{W}\mathbf{n},$$

(5)

where Φ is the fluence vector, $\mathbf{W} = \eta \mathbf{J} \mathbf{X} \mathbf{n}$, η is the quantum yield, $\mathbf{J}$ is Jacobian or sensitivity matrix, and $\mathbf{X}$ and $\mathbf{n}$ refer to the x-ray excitation field and nanophosphor distribution in the discretized imaging domain, respectively.

In XLCT imaging, 3D nanophosphor distribution $\mathbf{n}$ is obtained through solving the inverse problem in Eq. (5) given the boundary measurements Φ^m , typically formulated as a least-square minimization problem:

$$\min_{\mathbf{n}} \|\Phi^m - \mathbf{W}\mathbf{n}\|_2^2.$$

(6)

Owing to the limited measurements obtained in the boundary of the imaging domain, solving Eq. (5) for $\mathbf{n}$ is generally an ill-conditioned problem; thus, regularization methods incorporated to least-square minimization, Eq. (6), are typically essential for obtaining a relevant solution [10]. And generally, the nanophosphor is sparsely distributed in the imaging domain, for example, the nanophosphor solution injected into a small animal such as a mouse for targeted tumor imaging; therefore, l1 regularization is a natural and viable selection for obtaining a good approximate sparse solution for Eq. (5), yielding

$$\min_{\mathbf{n}} \|\Phi^m - \mathbf{W}\mathbf{n}\|_2^2 + \alpha \|\mathbf{n}\|_1.$$

(7)

In this study, Eq. (7) is solved by an efficient l2-l1 solver called glmnet [19].

Numerical simulations were first carried out to evaluate the performance of the proposed SWIR-XLCT imaging. A Digimouse atlas [20] was partially extracted to construct a 3D simulation model. The partial mouse model was discretized using a mesh generation toolbox called iso2mesh [21] [as shown in Fig. 3(a)]. Different optical properties were attributed to background tissue (indicated in blue color in Fig. 3(a), absorption coefficient $\mu_a = 0.08 \text{ mm}^{-1}$, reduced scattering coefficient $\mu'_s = 0.7 \text{ mm}^{-1}$), liver (red, $\mu_a = 0.1 \text{ mm}^{-1}$, $\mu'_s = 0.9 \text{ mm}^{-1}$), and kidney (green, $\mu_a = 0.12 \text{ mm}^{-1}$, $\mu'_s = 0.95 \text{ mm}^{-1}$) [22,23]. Two cylinders with a size of 2 mm in diameter and 2 mm in height sealed with constant nanophosphor concentration were inserted into the mouse model as the targets [as shown in Fig. 3(b)]. In each simulation, the measured data for reconstruction consisting of all the signals from the optical detectors [as indicated in magenta color in Fig. 3(c)] in the boundary of the partial mouse model was obtained in two steps: first, solving the forward problem described in Eqs. (1) to (5) given the nanophosphor distribution to get the light fluence in each detector position; second, 15% random Gaussian noise was superimposed on the computed detector measurement. Three cases with different edge-to-edge distance (d) of the two targets [Fig. 3(b)] were investigated, 1, 2, and 3 mm, respectively.

Figures 4(a), 4(d), and 4(g) show the representative slices of the reconstructed images with the target edge-to-edge distance of 1 mm, 2 mm, and 3 mm, respectively. As shown in Figs. 4(b), 4(e), and 4(h), the reconstructed images were also spatially overlaid onto the 3D x-ray computed tomography (CT) provided by the Digimouse atlas [20]. For a quantitative comparison, the simulated nanophosphor distribution (ground truth) was indicated with black circles in these images [Figs. 4(a), 4(b), 4(d), 4(e), 4(g), and 4(h)]. The profiles along the center of the targets were plotted (blue) together with the ground truth (red) as shown in Figs. 4(c), 4(f), and 4(i), respectively.

From these results, we can see that nanophosphor targets were accurately localized and mapped in submillimeter spatial resolution. In Figs. 4(a), 4(d), and 4(g), besides the

nanophosphor targets, there are artifacts, which were from the inverse reconstruction with the noisy boundary measurements.

The feasibility of the proposed SWIR-XLCT imaging was further investigated through experiments using tissue mimicking phantoms. A cylindrical phantom with a size of 26 mm in diameter and 40 mm in height was made of TiO_2 as scatter, India ink as absorber, and 2% agar as solidification material with the absorption coefficient of 0.12 mm^{-1} and the reduced scattering coefficient of 0.7 mm^{-1} [23]. Two pieces of transparent glass tubing with a diameter of 2.2 mm and a length of 4 mm filled with nanophosphors were embedded in the center of the phantom as the targets [Fig. 5(a)]. The phantom was then fixed on the sample stage of the SWIR-XLCT imaging system. A different edge-to-edge distance of the two targets were investigated: (1) 1 mm and (2) 3.5 mm. During the experiment, the voltage and current of the x-ray source in the irradiator (X-RAD 320) were set as 320 kVp and 12.5 mA. The phantom was rotated 360° , and 24 luminescence projections were acquired by the SWIR camera (InGaAs camera, NIRvana 640) with an angular increment of 15° . The exposure time of the SWIR camera was set to be 30 seconds. Figure 5(b) shows one of the projections directly acquired by the SWIR camera under inducing the phantom consists of two nanophosphor targets with an edge-to-edge distance of 1 mm and 3.5 mm, respectively.

The phantom was discretized into 52,816 tetrahedron elements with 19,310 nodes for 3D image reconstruction. Figure 6(a) shows the reconstructed images in the case that the edge-to-edge distance between the two targets is 1 mm, while the distance in Fig. 6(c) is 3.5 mm. Here, slices in X–Y and Y–Z cross-sectional planes are shown, respectively.

For quantitative comparison, we also plotted the profile crossing the two targets in X–Y plane together with the ground truth in Figs. 6(b) and 6(d), in which, the blue curve is the profile of the reconstructed targets, and the red line represents the real target position and size. Again, in Figs. 6(a) and 6(c), the regions included in the black-dotted lines are the real target position and size. From these experimental results, we can see that nanophosphor distribution can be mapped with high spatial resolution.

In summary, a novel SWIR-XLCT imaging strategy has been investigated. Moreover, to realize the potential of SWIR-XLCT imaging, x-ray excitable RENPs have been synthesized as core/shell structures consisting of a Ho-doped NaYbF_4 core surrounded by a NaYF_4 shell emitting light at the peak wavelength of 1180 nm with 30 nm of FWHM. Both numerical simulations and tissue-mimicking phantom experiments were carried out to demonstrate the feasibility of SWIR-XLCT imaging. The results show that the proposed SWIR-XLCT has the strong potential of improving spatial resolution and depth imaging performance.

Funding.

American Association of Physicists in Medicine (Research Seed Grant); National Cancer Institute (1R01CA223667, 1R01CA227713).

REFERENCES

1. Li C, Di K, Bec J, and Cherry SR, Opt. Lett 38, 2339(2013). [PubMed: 23811921]

2. Pratz G, Carpenter CM, Sun C, and Xing L, IEEE Trans. Med. Imaging 29, 1992(2010). [PubMed: 20615807]
3. Ntziachristos V, Annu. Rev. Biomed. Eng 8, 1(2006). [PubMed: 16834550]
4. Jiang H, Appl. Opt 37, 5337(1998). [PubMed: 18286015]
5. Wang G, Cong W, Durairaj K, Qian X, Shen H, Sinn P, Hoffman E, McLennan G, and Henry M, Opt. Express 14, 7801(2006). [PubMed: 19529149]
6. Chen D, Zhu S, Yi H, Zhang X, Chen D, Liang J, and Tian J, Med. Phys 40, 031111(2013). [PubMed: 23464291]
7. Chen D, Zhu S, Chen X, Chao T, Cao X, Zhao F, Huang L, and Liang J, Appl. Phys. Lett 105, 191104(2014).
8. Carpenter C, Pratz G, Sun C, and Xing L, Phys. Med. Biol 56, 3487(2011). [PubMed: 21606553]
9. Zhang W, Zhu D, Lun M, and Li C, Biomed. Opt. Express 7, 2506(2016). [PubMed: 27446686]
10. Tzoumas S, Vernekohl D, and Xing L, IEEE Trans. Biomed. Eng 65, 1892(2017). [PubMed: 29989958]
11. Naczynski DJ, Sun C, Türkcan S, Jenkins C, Koh AL, Ikeda D, Pratz G, and Xing L, Nano Lett. 15, 96(2014). [PubMed: 25485705]
12. Hong G, Diao S, Chang J, Antaris AL, Chen C, Zhang B, Zhao S, Atochin DN, Huang PL, and Andreasson KI, Nat. Photonics 8, 723(2014). [PubMed: 27642366]
13. Smith AM, Mancini MC, and Nie S, Nat. Nanotechnol 4, 710(2009). [PubMed: 19898521]
14. Franke D, Harris DK, Chen O, Bruns OT, Carr JA, Wilson MW, and Bawendi MG, Nat. Commun 7, 12749(2016). [PubMed: 27834371]
15. Zhong Y, Ma Z, Zhu S, Yue J, Zhang M, Antaris AL, Yuan J, Cui R, Wan H, Zhou Y, Wang W, Huang NF, Luo J, Hu Z, and Dai H, Nat. Commun 8, 737(2017). [PubMed: 28963467]
16. Naczynski D, Tan M, Zevon M, Wall B, Kohl J, Kulesa A, Chen S, Roth C, Riman R, and Moghe P, Nat. Commun 4, 2199(2013). [PubMed: 23873342]
17. Gao H and Zhao H, Transport Theory Statist. Phys 38, 149(2009).
18. Gao H and Zhao H, Opt. Express 18, 1854(2010). [PubMed: 20174013]
19. Friedman J, Hastie T, and Tibshirani R, J. Stat. Softw 33, 1(2010). [PubMed: 20808728]
20. Dogdas B, Stout D, Chatzioannou AF, and Leahy RM, Phys. Med. Biol 52, 577(2007). [PubMed: 17228106]
21. Fang Q and Boas DA, in IEEE International Symposium on Biomedical Imaging: From Nano to Macro (IEEE, 2009), pp. 1142–1145.
22. Jacques SL, Phys. Med. Biol 58, R37(2013). [PubMed: 23666068]
23. Wang K, Wang Q, Luo Q, and Yang X, Opt. Express 23, 12669(2015). [PubMed: 26074521]

Fig. 1.

TEM images of (a) $\text{NaYb}_{0.98}\text{F}_4:\text{Ho}_{0.02}$ and (b) core/shell $\text{NaYb}_{0.98}\text{F}_4:\text{Ho}_{0.02}@\text{NaYF}_4$ nanophosphors, (c) photoluminescence excitation mapping of core/shell $\text{NaYb}_{0.98}\text{F}_4:\text{Ho}_{0.02}@\text{NaYF}_4$ nanophosphors, and (d) x-ray spectra of core/shell $\text{NaYb}_{0.98}\text{F}_4:\text{Ho}_{0.02}@\text{NaYF}_4$ nanophosphors, (e) XRD of core/shell $\text{NaYb}_{0.98}\text{F}_4:\text{Ho}_{0.02}@\text{NaYF}_4$ and standard hexagonal-phase NaYF_4 (black vertical lines), (f) the diagram, and (g) photograph of SWIR_XLCT imaging system.

Fig. 2.

Visual hull and XLCT reconstruction. (a) diagram of obtaining bright-field images, (b) visual hull, (c) 3D finite element mesh, (d) diagram of acquiring luminescence images, (e) finite element mesh with detector positions, (f) XLCT image reconstruction. RTE, radiative transfer equation.

Fig. 3. 3D numerical simulation model. (a) Partial mouse mesh with kidney and liver, (b) two simulated cylindrical targets inserted into the mouse mesh, d, edge-to-edge distance of the two targets, (c) detectors in the boundary of 3D model.

Fig. 4. Numerical simulation results. The representative slices of the reconstructed images with the target edge-to-edge distance of (a) 1 mm, (d) 2 mm, and (g) 3 mm, spatially overlaid onto the corresponding x-ray computed tomography images (b), (e), and (h), respectively, and their corresponding profiles along the center of the targets compared to the ground truth (c), (f), and (i).

Fig. 5.
(a) Tissue-mimicking phantom and (b) projection measurements.

Fig. 6. Tissue-mimicking phantom and experimental results. Reconstructed images of the edge-to-edge distance between two targets of (a) 1 mm and (b) its corresponding profile, and (c) 3.5 mm and (d) its profile.