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Abstract. Thanks to the current advances in nanoscience, molecular biochemistry, and x-ray detector technology, x-ray fluorescence computed tomography (XFCT) has been considered for molecular imaging of probes containing high atomic number elements, such as gold nanoparticles. The commonly used XFCT imaging performed with K-shell x rays appears to have insufficient imaging sensitivity to detect the low gold concentrations observed in small animal studies. Low energy fluorescence L-shell x rays have exhibited higher signal-to-background ratio and appeared as a promising XFCT mode with greatly enhanced sensitivity. The aim of this work was to experimentally demonstrate the feasibility of L-shell XFCT imaging and to assess its achievable sensitivity. We built an experimental L-shell XFCT imaging system consisting of a miniature x-ray tube and two spectrometers, a silicon drift detector (SDD), and a CdTe detector placed at $\pm 120^\circ$ deg with respect to the excitation beam. We imaged a 28-mm-diameter water phantom with 4-mm-diameter Eppendorf tubes containing gold solutions with concentrations of 0.06 to 0.1% Au. While all Au vials were detectable in the SDD L-shell XFCT image, none of the vials were visible in the CdTe L-shell XFCT image. The detectability limit of the presented L-shell XFCT SDD imaging setup was 0.007% Au, a concentration observed in small animal studies. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JMI.2.4.043501]

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1 Introduction

X-ray fluorescence computed tomography (XFCT) presents itself as an attractive biomedical imaging modality due to a good balance of imaging depth, resolution, and imaging sensitivity.¹ XFCT is based on x-ray excitation of imaging probes containing high atomic number (Z) elements followed by fluorescence x-ray emission. The energy of the fluorescence x-ray is characteristic of the high-Z element, and it is measured with a photon-counting detector. Three-dimensional XFCT images of the distribution of the molecular probe are then reconstructed from multiple projections acquired with rotation of the x-ray source.

XFCT is commonly performed with higher-energy K-shell x rays enabling imaging at larger depths in tissue. Several researchers have studied XFCT by means of computer simulations,^{2–6} and several groups have demonstrated the feasibility of XFCT imaging of gold nanoparticles in phantoms experimentally, measuring concentrations of 5 to 20 mgAu/mL.^{7–10} While XFCT has the potential to become a molecular imaging modality with a high spatial resolution, its sensitivity limits have lagged behind other imaging modalities, such as positron emission tomography and single photon emission computed tomography. In order to perform *in vivo* XFCT imaging, gold nanoparticle concentrations in the range of 1 to 60 μ gAu/ml, as measured in small animal studies,¹¹ must be detectable.

One approach to increase XFCT sensitivity is to image with lower-energy L-shell x rays, because the x-ray cross-section of gold at the L-shell energy is about 21 times higher than that at the K-shell energy.¹² Even though the L-shell yield is approximately three times lower compared to the K-shell yield, gold L-shell XFCT imaging could theoretically result in seven times more intense x-ray fluorescence signal compared to K-shell XFCT imaging. Additionally, the amount of interference contributed by Compton scattering is greatly reduced at lower energies. Taken together, these two features lead to much greater sensitivity in L-shell compared to K-shell XFCT.³ The drawback of L-shell XFCT is the high attenuation of the excitation and fluorescence x rays, which will, therefore, likely limit the approach to imaging small (<4 cm) objects.

L-shell x-ray fluorescence (XRF) has already been considered for detecting gold nanoparticles. Manohar et al. used a scanning pencil beam to generate a projection image of gold nanoparticles injected into a 12-mm-diameter phantom, and they were able to detect gold nanoparticle concentrations of 0.35 μ g per imaged voxel (1.73×10^{-2} cm³), or 20 μ gAu/mL.¹³ Our group used Monte Carlo simulations to demonstrate that under idealized conditions, platinum in Cisplatin can be imaged with L-shell XFCT in concentrations of 12 and 19 μ gPt/ml in 2- and 4-cm-diameter phantoms, respectively.³ We also showed that L-shell XFCT sensitivity limits were higher than those of K-shell XFCT by a factor of 3.0 to 4.4.

The aim of this work is to experimentally demonstrate the feasibility of benchtop L-shell XFCT imaging of small animal

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sized subjects. Adding tomographic scanning and image reconstruction to L-shell XRF should improve contrast and sensitivity, just as conventional tomographic x-ray CT improves image contrast over x-ray projection radiography.

We built an L-shell XFCT imaging system composed of a miniature x-ray tube, x-ray photon-counting detectors, and a translation/rotation stage. We tested two different x-ray detector types for their feasibility in L-shell XFCT. We imaged vials with gold concentrations down to 600 $\mu\text{gAu/ml}$ in a 28-mm-diameter phantom with L-shell XFCT. Additionally, we demonstrated that the L-shell XFCT detectability limit of the presented nonoptimized experimental setup suitable for imaging of small animal sized subjects was 70 $\mu\text{gAu/mL}$. The novelty of this work lies in the fact that we employed tomographic data acquisition of L-shell XRF and that we were able to detect low gold concentrations of tens of $\mu\text{gAu/ml}$ in a small animal sized phantom.

2 Materials and Methods

2.1 L-Shell X-Ray Fluorescence Computed Tomography Imaging System

We constructed a prototype L-shell XFCT imaging system consisting of one miniature x-ray tube and two x-ray photon-counting detectors. The system was designed to image a 28-mm-diameter phantom with gold solutions using L-shell XFCT (Fig. 1). A 4-W Mini-X x-ray tube (Amptek, Bedford, Massachusetts) with a 0.75- μm -thick silver anode and a focal spot of 2 mm was positioned 2.0 cm from the center of the phantom and run at 50 kV and 12 μA . The 50 kV beam was chosen because it generated the highest gold XRF signal compared to 30 and 40 kV beams. After passing through a 127- μm -thick beryllium window, the 50-kV x-ray beam was collimated with a 1-mm brass collimator, but it was not filtered further. For tomographic data acquisition, the phantom was placed on a translation/rotation stage (Velmex, Bloomfield, New York) controlled by a custom program for the LabView control platform software (National Instruments, Austin, Texas). A (5 $\times$ 5) mm^2 active area single-pixel silicon drift detector (SDD, X-123SSD, Amptek, Bedford) was placed 8 cm from the isocenter at +120 deg with respect to the excitation beam [Fig. 1(a)]. Thanks to the thin 12.5- μm beryllium window, the SSD was suitable for detecting low-energy L-shell x rays

with a 130-eV energy resolution at 5.9 keV and a maximum count rate of 1×10^5 cps, as quoted by the manufacturer. The detection efficiency of the 500- μm -thick SDD operating at a bias voltage of -120 V was $\sim 100\%$ at 5 to 10 keV x rays. A second Amptek (3 $\times$ 3) mm^2 active area single-pixel CdTe detector of 1 mm thickness (X-123CdTe, Amptek, Bedford) was placed 10 cm from the isocenter on the other side of the x-ray tube at -120 deg with respect to the excitation beam [Fig. 1(a)]. According to the manufacturer's specifications, the CdTe detector had a 530-eV energy resolution at 14.4 keV and a maximum count rate of 2×10^5 cps. The efficiency of the CdTe detector to count 10 keV x rays was $\sim 100\%$ for a detector bias voltage of 500 V. The distance of the spectrometers to the imaging isocenter could not be further reduced, because it would result in dead times $> 5\%$. The spectrometer gains were set to detect x rays up to energies of ~ 15.5 keV in 2048 channels, i.e., each energy bin was 7.6 eV wide. The x-ray spectra were calibrated with the 14.41 keV peak of a ^{57}Co source.

The ^{57}Co spectra used for detector energy calibration are presented in Fig. 2(a). As demonstrated in Fig. 2(a), the energy resolution of the SDD (220 eV at 14.4 keV) was three times higher than the energy resolution of the CdTe detector (660 eV at 14.4 keV).

2.2 Phantom

The 28-mm-diameter imaging phantom [Fig. 1(a)] consisted of three mini Eppendorf vials (800 μL volume and 4 mm in diameter) filled with water solutions of gold chloride (AuCl_3 , Salt Lake Metals, Salt Lake City, Utah) with 0.06, 0.08, and 0.1% Au by weight (or 0.6, 0.8, and 1.0 mgAu/ml). The different concentrations were prepared by serial dilutions. All vials were placed equidistantly at 10 mm distance from the center of the phantom using a three-dimensional printed plastic holder and were fully immersed in water. In other words, the vials were placed at 4 mm depth, the shortest distance from the edge of the vials to the edge of the phantom was 2 mm, and the distance between the vials was 17.3 mm.

2.3 Data Acquisition

First, the gold L-shell XRF signal as a function of Au concentration was evaluated for the SDD and CdTe detectors. The

Fig. 1 (a) Schematics and (b) a photograph of the experimental setup for L-shell x-ray fluorescence computed tomography (XFCT) imaging. The red protective caps were removed during data acquisition.

Fig. 2 (a) ^{57}Co spectra of the silicon drift detector (SDD) and CdTe detectors with noted energy resolution of the 14.4 keV peak. X-ray fluorescence (XRF) spectra for 0.06, 0.08, and 0.1% Au vials acquired with the (b) SDD and (c) CdTe detector during a 2-min data acquisition.

800 μL Eppendorf vials with 0.06, 0.08, and 0.1% Au solutions were taped directly on the x-ray tube and irradiated for 2 min with the x-ray source operated at 50 kV and 12 μA . SDD and CdTe fluorescence x-ray spectra were acquired with the detectors placed 8 and 10 cm away from the center of the rotation

Fig. 3 XRF signal as a function of Au concentration for the SDD (squares) and CdTe (circles) detectors.

stage at +120 and -120° (Fig. 1), respectively, and analyzed, as described in Sec. 2.4.

Second, tomographic imaging was performed with both detectors, which was achieved by translating and rotating the phantom using first-generation CT scanning. The phantom was first carefully centered on the rotation/translation stage and then translated by -15 mm to the scanning start position. Next, the continuous x-ray exposure, phantom translation, and detector acquisition were all started simultaneously. The stage translation consisted of a $+30$ mm continuous motion covering the extent of the phantom with a speed of 2 mm/min. The detectors were programmed to save spectra every 30 s, resulting in the acquisition of 30 translation steps spaced by 1 mm. After each translation, the rotation stage was rotated by 10° and the phantom translated by -30 mm to the next starting position. The scanning procedure was repeated for each angular position of the phantom. XFCT data were, therefore, acquired over 360° in 10° intervals resulting in an imaging time of 9 h. The x-ray detectors remained stationary relative to the x-ray source in this setup. The low-energy excitation fluorescence x rays resulted in asymmetric counts at the -120° and $+120^\circ$ detector positions. Therefore, the detectors were swapped (the SDD was placed at -120° and the CdTe detector at $+120^\circ$ [Fig. 1(a)] and a second dataset with identical scanning parameters was acquired. The total XFCT data acquisition time was 18 h.

2.4 L-Shell X-Ray Fluorescence Analysis

L-shell XRF analysis was performed with Au L_α and L_β lines; L_γ lines were excluded from the analysis due to their low yield. Thanks to the proximity of the individual α and β lines (Table 1), the $L_{\alpha 1}$ and $L_{\alpha 2}$ as well as $L_{\beta 1}$ and $L_{\beta 2}$ could not be distinguished in the x-ray energy spectra of either detector and were combined for data analysis. Figures 2(b) and 2(c) represent x-ray spectra for the 0.06, 0.08, and 0.1% Au vials acquired during a 2-min irradiation with the SDD and CdTe detector, respectively. The Au L_α and L_β lines are clearly visible in the high-energy resolution and larger detection volume SDD spectra, but they are less apparent in the CdTe spectra.

The total XRF signal was calculated as the sum of the net number of XRF counts in both L_α and L_β peaks. The net number of XRF counts was calculated as the total number of peak counts minus the number of background counts. The number of background counts [dashed lines in Figs. 2(b) and 2(c)] was estimated through second-order polynomial interpolation based on the neighboring 1 keV bins (i.e., 130 energy bins) for each L_α and L_β peak. Due to the different energy resolutions of the SDD and CdTe detectors, the net number of counts were calculated in 400 and 600 eV energy windows, respectively, centered around 9.7 keV for the L_α lines and around 11.6 keV for the L_β lines.

2.5 L-Shell X-Ray Fluorescence Computed Tomography Image Reconstruction

Two XFCT sinograms for each detector position (+120 and -120 deg) were created based on the projections acquired at 36 projection angles and 30 translation steps. For each of the detectors, the +120 and -120 deg sinograms were simply added together, since the detectors were positioned symmetrically with respect to the excitation beam for the two data acquisitions.

We reconstructed SDD and CdTe XFCT images for each of the raw sinogram dataset using the well-known maximum-likelihood expectation-maximization (ML-EM) algorithm.¹⁵ The image reconstruction problem is summarized by

$$\mathbf{A}\vec{x} = \vec{N}_{xf}, \quad (1)$$

where $\vec{N}_{xf}$ is a vectorized list of all XRF counts in the sinogram, $\vec{x}$ is a vectorized list of all the reconstruction pixel values, and $\mathbf{A}$ is the system matrix representing the physical model of the imaging system. Element a_{ij} of system matrix $\mathbf{A}$ is the probability that a fluorescent x ray is emitted in pixel j for excitation beam i . In our implementation, we chose a_{ij} to be the length of the intersection between excitation beam i and reconstructed pixel j . We assumed that this intersection length is proportional to the actual probability of XRF emission. a_{ij} is equal to 0 in those cases

where the excitation beam does not intersect a reconstructed pixel, representing a zero probability of XRF emission. We also assume that fluorescent x rays are equally likely to be detected for all pixels. These two assumptions mean that we ignore attenuation of the excitation beam and the fluorescent x rays. The ML-EM algorithm solves Eq. (1) iteratively for the image $\vec{x}$ using the following updated equation:

$$x_j^{(k+1)} = x_j^{(k)} \sum_i \frac{(N_{xf}^i) a_{ij}}{\bar{a}_i \cdot \vec{x}^{(k)}}, \quad j = 1, 2, \dots, M, \quad (2)$$

where a_i is the i 'th row of system matrix $\mathbf{A}$. Integers k and $k + 1$ denote sequential estimates of $\vec{x}$. The initial estimate was chosen as all 1's (i.e., $x_j = 1$, $j = 1, 2, \dots, M$). The L-shell XFCT images were reconstructed at an initial resolution of 30×30 pixels with a pixel size of 1 mm (30 mm field of view).

2.6 L-Shell X-Ray Fluorescence Computed Tomography Image Analysis

The reconstructed L-shell XFCT images were normalized to the mean signal of the 0.1% Au vial. The XFCT signal in the three 0.06 to 0.1% Au vials and in water (0% Au) was then plotted as a function of the true Au concentration. Additionally, the contrast-to-noise ratio (CNR) was calculated using

$$\text{CNR} = \frac{\mu_{\text{vial}} - \mu_{\text{water}}}{\sigma_{\text{water}}}, \quad (3)$$

where μ_{vial} is the mean signal in a vial, and μ_{water} and σ_{water} are the mean and standard deviation of a region of interest in water, respectively. We used the Rose criterion¹⁶ of $\text{CNR} = 4$ to estimate the minimum detection limit of the presented XFCT system for both the SDD and CdTe detectors.

3 Results

L-shell XRF linearity, XFCT raw data sinograms, and reconstructed images together with XFCT image analyses for both SDD and CdTe detectors are presented in the following sections.

3.1 L-Shell X-Ray Fluorescence Signal Linearity

L-shell XRF signal linearity as a function of Au concentration for both detectors is plotted in Fig. 3. During a 2-min data acquisition, ~650 and 50 L_α and L_β XRF counts were acquired with the SDD and CdTe detectors for the highest concentration 0.1% Au vial, respectively. The high-energy resolution larger sensitive volume SDD resulting in 13 times higher XRF signal was undoubtedly the more suitable detector for L-shell XFCT data acquisition compared to the CdTe detector. The signal was linear with respect to Au concentration for both detectors with $R^2 > 0.98$, which demonstrated the feasibility of quantitative L-shell XFCT imaging.

3.2 L-Shell Sinograms and X-Ray Fluorescence Computed Tomography Images

L-shell XFCT sinograms acquired with the SDD and CdTe detectors are presented in Figs. 4(a) and 4(b), respectively. L-shell sinograms acquired with the SDD placed at +120 and -120 deg with respect to the excitation beam as well as

Table 1 L-shell x-ray transition energies and intensities for gold from Ref. 14.

	$L_{\alpha 1}$	$L_{\alpha 2}$	$L_{\beta 1}$	$L_{\beta 2}$	$L_{\gamma 1}$
Energy (keV)	9.72	9.63	11.42	11.59	13.38
Intensity w (%)	100	11	67	23	13

the combined sinogram generated by addition of the two sinograms are presented in Fig. 4(a). The top and middle sinograms demonstrate the effect of beam attenuation that is diminished in the sum sinogram. The corresponding CdTe sinograms with lower numbers of counts and considerably higher noise compared to the SDD sinograms are shown in Fig. 4(b).

The SDD and CdTe L-shell XFCT images of the phantom with vials containing 0.06, 0.08, and 0.1% Au solutions are presented in Figs. 5(a) and 5(b), respectively. All vials were clearly detectable in the SDD XFCT image. The respective distance of the vials measured in the XFCT image was (17.2 ± 0.4) mm, which was close to the actual distance of 17.3 mm. On the other hand, the CdTe XFCT image was noisy and none of the vials were visible according to our quantitative criterion.

3.3 L-Shell X-Ray Fluorescence Computed Tomography Image Analysis

The reconstructed Au concentration calculated in the SDD and CdTe L-shell XFCT image as a function of the true

concentration is plotted in Fig. 6(a). The SDD XFCT image demonstrated a higher degree of XFCT signal linearity as a function of Au concentration with $R^2 > 0.99$. The CdTe XFCT image linearity with $R^2 > 0.90$ was slightly inferior compared to the SDD data due to its lower energy resolution and smaller sensitive volume.

The CNR of the 0.06, 0.08, and 0.1% Au vials calculated in both XFCT images is presented in Fig. 6(b). The CNR was approximately 27 times higher in the SDD XFCT image than the CNR in the CdTe XFCT image. The CNR of the 0.1% Au vial was 87.1 and 3.2 in the SDD and CdTe XFCT image, respectively. The XFCT detection limit (defined by $\text{CNR} = 4$) was calculated to be 0.007% Au and 0.126% Au for XFCT images acquired with the SDD and CdTe detector, respectively.

4 Discussion

In the presented work, we reconstructed L-shell XFCT images of 0.06 to 0.1% gold concentrations in 4-mm-diameter vials located at 4 mm depth within a 28-mm-diameter phantom.

Fig. 4 L-shell XFCT sinograms for (a) SDD and (b) CdTe detectors placed at +120 deg (top), -120 deg (middle), and their sum (bottom).

Fig. 5 L-shell XFCT image of the phantom acquired with the (a) SDD and (b) CdTe detectors.

Fig. 6 (a) Reconstructed Au concentration as a function of true Au concentration calculated from the SDD and CdTe XFCT images of the phantom. (b) Contrast-to-noise ratio of Au vials in the SDD and CdTe XFCT images as a function of Au concentration.

Although anticipated XFCT applications will use nanoparticle probes, we used ionic gold solution instead of gold nanoparticles in this work. Nevertheless, the results here are equally valid since x-ray fluorescence is independent of particle formulation.

No attenuation correction was applied in the image reconstruction due to location of the vials at the same depth in the phantom. We anticipate that in a small animal study, however, attenuation correction would be necessary. This was presented in our previous work³ and has been extensively studied by others.¹⁷

The imaging dose was not measured in the presented work, but it was estimated based on the x-ray tube specifications stating the approximate dose rate of 1 Sv/h at 30 cm distance and on our experimental setup to be 0.4 Gy. Such a high imaging dose would damage the subjects and preclude the use of XFCT imaging in many studies. A 100- μ m aluminum filter could be added to the x-ray beam in order to filter out the low-energy component in the x-ray spectrum, which would result in a lower imaging dose without sacrificing the XRF signal. Additionally, implementing an XFCT system with an array of photon-counting detectors can significantly decrease the imaging dose. For example, in an XFCT system consisting of three rings of tightly packed SDD detectors equivalent to those used in this study, i.e., with a detection area consisting of a 15-mm cylindrical surface, the 0.4 Gy imaging dose and the 18-h imaging time would reduce to 1.3 mGy and 3.6 min, respectively. Admittedly, the cost of such a system would be prohibitively high, especially if the detectors are to be placed farther from the subject/phantom to minimize the dead time. Additionally, the imaging time could be further reduced by replacing the low-power x-ray source with a readily available higher-power x-ray source. However, the maximum counting rate of the detector cannot be exceeded and might become the limiting factor when XFCT imaging with a high-power x-ray tube is employed.

Our study demonstrated that the SDD was a more suitable detector for L-shell XFCT imaging compared to the CdTe detector due to its higher energy resolution at low x-ray energies. Additionally, the area of the SDD is 2.8 times larger than the area of the CdTe detector, and the SDD was placed 20% closer to the imaging isocenter compared to the CdTe detector, which contributed to higher XRF collection efficiency. These two

factors had a significant contribution to the better L-shell XFCT image quality.

In this work, we were able to image gold contrast solutions placed at 4 mm depth within a water phantom. We note that L-shell XFCT imaging sensitivity for objects at larger depths will be compromised. At first approximation, the detection sensitivity of gold contrast located at 1 cm depth will decrease due to the exponential attenuation of the excitation and fluorescence beams. Assuming the average excitation beam energy of 15 keV (the corresponding linear attenuation coefficient for water is $\mu_{15 \text{ keV}} = 1.672 \text{ cm}^{-1}$) and the average fluorescence x-ray energy of 10 keV ($\mu_{10 \text{ keV}} = 5.330 \text{ cm}^{-1}$), the imaging sensitivity compared to the 4 mm depth contrast would decrease by a factor of 67 as follows from $(e^{-0.4 \text{ cm} \times \mu_{15 \text{ keV}}} \times e^{-0.4 \text{ cm} \times \mu_{10 \text{ keV}}}) / (e^{-1 \text{ cm} \times \mu_{15 \text{ keV}}} \times e^{-1 \text{ cm} \times \mu_{10 \text{ keV}}})$. In other words, L-shell XFCT imaging sensitivity of gold at 1 cm depth would decrease from 70 $\mu\text{gAu/mL}$ to 4.7 $\mu\text{gAu/mL}$ in the presented experimental setup. Consequently, we recommend that only subjects with contrast agents at shallow depths (<1 cm) be imaged with L-shell XFCT.

No attenuation corrections of the excitation and fluorescence beam were applied in this work mainly due to the shallow depth of the contrast vials. For L-shell XFCT imaging of gold at deeper depths, however, attenuation corrections will be crucial and the subject of our future research directions.

5 Conclusions

We have presented the first experimental XFCT study performed with L-shell x rays, in which gold inside a small animal sized phantom was imaged. We have shown that gold with concentrations down to 0.007% Au (or 70 $\mu\text{gAu/mL}$) can be detected in a 28-mm-diameter phantom by means of L-shell XFCT acquired with a high energy resolution SDD. The imaging and sensitivity indicate the feasibility of precisely localizing gold nanoparticle uptake in mice and other small animals. We also found the SDD technology to be appropriate for this application. When the CdTe detector with a lower energy resolution was used for L-shell XFCT data acquisition, the sensitivity dropped significantly to 0.126% Au (or 1260 $\mu\text{gAu/mL}$).

The presented long imaging time and high imaging dose restrict the use of L-shell XFCT imaging to small subjects. We anticipate, however, that the imaging time could be

decreased to 3.6 min and the imaging dose to 1.4 mGy if an L-shell XFCT system equipped with an array of SDDs is designed. Such an imaging system would render L-shell XFCT imaging suitable for *in vivo* applications.

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