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COMMENTARY

The potential of L-shell X-ray fluorescence CT (XFCT) for molecular imaging

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ABSTRACT

X-ray fluorescence CT (XFCT), a novel modality proposed for high-sensitivity high-resolution molecular imaging of probes labelled with a high atomic-number element, has been performed with high-energy K-shell X-rays. XFCT performed with low-energy L-shell X-rays could, in principle, result in an increase of XFCT imaging sensitivity; however, the significant L-shell X-ray attenuation limits its use for imaging of small objects. This commentary discusses the advantages and drawbacks of L-shell XFCT imaging.

INTRODUCTION

X-ray fluorescence CT (XFCT) has been recently investigated as a novel modality for high-sensitivity molecular imaging of probes and pharmaceutical compounds labelled with a non-radioactive high atomic-number (*Z*) element. For example, molecularly targeted gold nanoparticles (AuNP) can be imaged using fluorescence X-rays with an energy characteristic to gold emitted from the probe following its atomic excitation by kilovoltage X-ray beams. Iodine and gadolinium compounds, commonly used in diagnostic imaging, are other examples of imaging probes suitable for XFCT imaging thanks to their high-*Z* components. Through XFCT imaging, relative and absolute concentrations of imaging probes can be measured, which can be indicative of disease extent and severity. Additionally, XFCT images of chemotherapy drug cisplatin can be used to relate tumour drug concentration to treatment outcomes.

To date, mostly higher energy 60- to 70-keV K-shell X-rays have been considered for XFCT imaging owing to their deeper tissue penetration than that of higher-shell fluorescence X-rays. Idealized Monte Carlo simulations have demonstrated that K-shell XFCT could detect AuNP with concentrations down to 0.6 mgAu ml^{-1} in a 5-cm object.¹ Experiments, on the other hand, have shown that sensitivity limits of K-shell XFCT imaging of similar-sized objects were in the order of 10 mgAu ml^{-1} .² Concentrations of AuNP and other suitable XFCT imaging agents of up to three orders of magnitude lower than the latter value have been detected in small animals and are expected in human studies. Therefore, in order to apply XFCT imaging clinically and even pre-

clinically (*i.e.* in small animal laboratory studies), these sensitivity limits must be improved.

ADVANTAGES OF L-SHELL XFCT COMPARED WITH K-SHELL XFCT IMAGING

An alternative approach to increase XFCT imaging sensitivity is to image with lower energy approximately 10-keV L-shell X-rays. These characteristic X-rays are excited by lower-energy X-ray beams ideally just above the probe's L-edge and have approximately 20 times higher interaction probability but 3 times lower fluorescence yield than K-shell X-rays. As a result, L-shell XFCT imaging could improve K-shell XFCT sensitivity limits by nearly a factor of 7.

Due to the higher energy of K-shell fluorescence X-rays and their associated deeper penetration, K-shell XFCT could theoretically compete with clinical K-edge CT or even dual-energy CT imaging of iodine. XFCT images of iodine could be used to display vessels without calcium-containing structures to assess the carotid and vertebral arteries in the skull base and the cervical spine.³ In order to perform K-shell XFCT images on a clinical CT scanner, the X-ray beam would have to be heavily filtered requiring an extremely high-power X-ray tube. Additionally, to achieve clinically acceptable imaging times, a large array of energy-resolving collimated detectors would have to be used. Unfortunately, the prohibitive cost of such system makes clinical K-shell XFCT currently impractical.

We have recently presented a Monte Carlo study demonstrating that, under idealized conditions, L-shell XFCT was capable of imaging clinically relevant concentrations of

cisplatin of $10 \mu\text{gPt ml}^{-1}$ in a small animal.⁴ Additionally, Manohar et al⁵ were able to quantify AuNP concentrations down to $20 \mu\text{gAu ml}^{-1}$ in planar images of a 1.2-cm diameter phantom using a bench-top L-shell X-ray fluorescence imaging system. The feasibility of L-shell XFCT has therefore been demonstrated both in silica and experimentally, however, a number of challenges prevent us from introducing L-shell molecular imaging into even the pre-clinical settings.

LIMITATIONS AND ALTERNATIVE SOLUTIONS OF L-SHELL X-RAY FLUORESCENCE CT IMAGING

X-ray attenuation

The main drawback of L-shell XFCT is attenuation of both the excitation and fluorescence X-rays, which will most likely limit the imaging technique to pre-clinical applications. Gold and platinum L-shell fluorescence X-rays attenuate to 4% of their original intensity after passing through only 5 mm of soft tissue. Similar to positron emission tomography (PET) and single-photon emission CT (SPECT) imaging, attenuation correction based on transmission CT images must be applied to accurately quantify concentrations of the imaging probe. Unlike in PET and SPECT imaging, XFCT does not require the acquisition of an additional CT scan, because transmission CT data can be acquired simultaneously with XFCT data.

Imaging dose

The fast attenuation of the L-shell excitation X-ray beam results in higher dose to the periphery of the scanned subject, which might result in excessive skin dose, especially when scanning large objects. For example, the dose to the centre is 33% and 7% of the peripheral dose for 2-cm and 4-cm sized objects imaged with a 15-keV beam, respectively. The need for attenuation correction and the significant dose fall-off most likely mean that L-shell XFCT will be most likely limited to imaging of small animals. Furthermore, only elements with $Z \geq 70$, such as gold and platinum, have L-shell fluorescence energies above 10 keV and are therefore more suitable for L-shell XFCT than iodine and gadolinium.

Imaging time

In the published L-shell XFCT work, images have been acquired with a pencil beam.^{4,5} The size of the pencil beam and the transversal scanning parameters define the spatial resolution of XFCT images. Small beam size and fine scanning steps result in high spatial resolution down to $150 \mu\text{m}$, with the expense of long imaging times. For example, the $20 \mu\text{gAu ml}^{-1}$ concentration measured with a single collimated detector by Manohar et al⁵ required an imaging time of more than 8 h. Long imaging times are impractical for pre-clinical imaging, as duration of animal anaesthesia should not exceed 1.5 h. Takeda et al⁶ were able to generate synchrotron pencil beam K-shell XFCT images of iodine in mice brains with imaging times of 1.5 h.

Imaging times can be decreased by using an array of detectors as demonstrated in the simulation work by Bazalova et al.⁴ Alternatively, a fan or cone beam in conjunction with a collimated detector array, similar to SPECT imaging, could be used to speed up the data acquisition process. Such a set-up has been studied for K-shell XFCT imaging, in which Monte Carlo simulations

demonstrated that accurate AuNP concentrations down to 1 mgAu ml^{-1} could be imaged with a cone beam geometry and two banks of parallel pinhole collimators and a dose of 30 cGy.⁷ It is therefore reasonable to assume that the long imaging times in L-shell XFCT can be shortened with more sophisticated imaging geometries employing wider beams and multiple detectors.

L-SHELL X-RAY FLUORESCENCE CT INSTRUMENTATION

As for any imaging modality, instrumentation for L-shell XFCT is an important aspect determining the image quality. Similar to K-shell XFCT and CT imaging in general, the highest imaging sensitivity is achieved with monoenergetic X-ray sources typically generated by synchrotrons. Owing to the high cost of synchrotron technology, efforts have been made to approximate monoenergetic beams with highly filtered low-energy X-ray tube beams at the expense of significantly lower X-ray output. The typical output of a synchrotron X-ray source at 25 keV is 4–6 orders of magnitude higher than an output of a 30-kV X-ray pencil beam filtered with a 1-cm thick copper filter. Alternatively, metal-jet anode microfocus X-ray source could be used for L-shell XFCT due to its high brightness. Hertz et al⁸ used 24-keV indium lines generated by a metal-jet microfocus X-ray source to image fluorescence from molybdenum nanoparticles at approximately 17 keV with K-shell XFCT, *i.e.* at energies relevant to L-shell XFCT.

Energy-resolving detectors are a critical component of XFCT imaging systems. L-shell XFCT imaging poses more challenges for detector technology than does K-shell XFCT owing to the requirement to register low-energy X-rays with sub-kiloelectronvolt resolution and a low noise floor. High-cost high-purity germanium (HPGe), silicon doped with lithium [Si (Li)], silicon drift and cadmium telluride (CdTe) detectors are currently the most suitable detectors for L-shell XFCT. Ideally, these detectors should be arranged in 4π geometry to maximize the detection efficiency of isotropically emitted fluorescence X-rays. Increasing the number of detectors would, however, further increase the cost of XFCT imaging systems.

POTENTIAL APPLICATIONS OF XFCT IMAGING AND ITS COMPARISON WITH OTHER IMAGING METHODS

A number of potential pre-clinical and clinical applications of K-shell and L-shell XFCT imaging and its comparison to other imaging methods will be briefly discussed in the present study.

K-shell XFCT imaging of iodine could, for example, be used for diagnosis of breast cancer,⁹ for diagnosis and staging of thyroid cancer,¹⁰ for cancer imaging studies by means of peptide radiolabeling¹¹ and for hypoxia imaging.¹² Additionally, K-shell XFCT imaging of gadolinium could be used for diagnosis of multiple sclerosis¹³ and brain cancer.¹⁴

Extensive developments in the arena of AuNP research have made it possible to use them for a vast variety of potential L-shell XFCT imaging applications through targeting to cancer-specific antibodies, such as antiepidermal growth factor receptor

antibodies¹⁵ or the UM-A9 antibody in squamous cell carcinoma,¹⁶ and through visualization of bare AuNP circulating in microscopic blood vessels indicating leaky vasculature of cancerous tissue.^{17,18} L-shell XFCT images of biodistribution of cisplatin could, on the other hand, be used to link cisplatin concentration in the tumour and healthy tissues to tumour control probability and normal tissue complication probability, respectively, and to guide dose-enhanced radiotherapy.

While higher-sensitivity images could be provided with PET and SPECT for some of the applications above, XFCT offers increased spatial resolution and in bypassing costly and not readily available radiolabelling. Optical imaging, on the other hand, mainly offers two-dimensional images and is limited to shallow 1–2 mm depths in tissue compared with XFCT imaging.

SUMMARY

In my opinion, L-shell XFCT imaging is an attractive imaging modality with the potential to detect clinically relevant concentrations

of probes containing high atomic-number elements. Even though the extreme X-ray beam attenuation will most likely restrict L-shell XFCT for imaging of small animals, it could still be a valuable addition to the existing pre-clinical imaging modalities. Through extensive small animal experiments, pre-clinical XFCT imaging has the potential to accelerate the translation of novel cancer imaging probes into the clinic. Pre-clinical XFCT studies can also be beneficial for the evaluation of cisplatin efficacy for cancer treatment as a function of the biodistribution of the drug, which could have an effect on clinical practice.

Nonetheless, currently available X-ray source and detector technologies and their related high cost prevent us from achieving short imaging times that are required for pre-clinical XFCT imaging. It is crucial, however, that we explore the potential of L-shell XFCT imaging in the laboratory, while X-ray source and detector technologies further advance to allow us to build an L-shell XFCT system for routine pre-clinical imaging.

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