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X-Ray Luminescence and X-Ray Fluorescence Computed Tomography: New Molecular Imaging Modalities

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ABSTRACT X-ray luminescence and X-ray fluorescence computed tomography (CT) are two emerging technologies in X-ray imaging that provide functional and molecular imaging capability. Both emission-type tomographic imaging modalities use external X-rays to stimulate secondary emissions, either light or secondary X-rays, which are then acquired for tomographic reconstruction. These modalities surpass the limits of sensitivity in current X-ray imaging and have the potential of enabling X-ray imaging to extract molecular imaging information. These new modalities also promise to break through the spatial resolution limits of other *in vivo* molecular imaging modalities. This paper reviews the development of X-ray luminescence and X-ray fluorescence CT and their relative merits. The discussion includes current problems and future research directions and the role of these modalities in future molecular imaging applications.

INDEX TERMS Biomedical imaging, molecular imaging, computed tomography, x-ray tomography.

I. INTRODUCTION

X-ray imaging is the oldest internal medical imaging modality and has long been the workhorse of radiology. Today, x-ray based imaging examinations remain the most commonly indicated medical imaging procedures. The main advantages of x-ray imaging include versatility and good image quality for many diagnostic procedures, fast imaging times, and relatively low equipment expense. Among the x-ray imaging modalities, computed tomography (CT), in particular, has been particularly valuable because of dramatically improved image quality over previous x-ray projection imaging and the ability to provide volumetric image information. Continuous research efforts have been made in improving image quality and reducing radiation dose since the advent of CT imaging [1]. These improvements are generally achieved through more efficient x-ray production and usage, more efficient and higher resolution x-ray detectors, better image reconstruction algorithms, and removal of artifacts.

Conventional CT relies on the physical mechanism of x-ray attenuation to generate image contrast. However, this modality falls short of providing sufficient contrast for probing the molecular features that are the basis of diseases. This is because x-ray attenuation occurs in all tissues in the subject leading to low sensitivity to molecular features. Adding molecular imaging information on top of anatomical CT images is of fundamental importance for screening, diagnosis, staging, treatment planning and therapeutic assessment of various diseases. Therefore, much research is focused on developing CT imaging technologies that rely on physical contrast mechanisms fundamentally different from x-ray attenuation. These new imaging technologies would provide new complementary information to attenuation CT to facilitate clinical decision-making.

There are emerging CT technologies based on x-ray stimulated emissions, in which externally applied x-rays interact with either exogenous or endogenous contrast agent leading to the emission of a secondary signal. The secondary emitted signal can be visible light (x-ray luminescence) or secondary x-rays (x-ray fluorescence). Typically, only a specific exogenous material (imaging probe) introduced within the subject emits this signal, setting the basis for highly sensitive imaging. Acquisition of the emitted signal and subsequent reconstruction of the distribution of the imaging probes provide critically needed functional or biological information about the imaged subject. Another x-ray stimulated emissions imaging method that was recently demonstrated is x-ray acoustics [2], in which ultrasonic imaging signals are generated by

differential absorption of short intense x-ray pulses. Though intriguing, the article will not discuss this promising imaging method further due to the scarce amount of literature on the topic. Regardless of which emission, the main advantages of x-ray stimulated emission imaging are high resolution, high sensitivity and the capability of multiplexed imaging.

This article reviews the emerging modalities in x-rays stimulated emissions imaging and highlights their strengths and weaknesses. We review x-ray luminescence CT (XLCT) in Section II and x-ray fluorescence CT (XFCT) in Section III. For each modality we present the basic imaging principles, a review of the current literature, and future directions and applications. We note that most of the research in these emerging modalities is in the context of molecular imaging applications. In Section IV, we discuss the scope of XFCT and XLCT in molecular imaging and compare them with the more established nuclear and optical modalities.

II. X-RAY LUMINESCENCE CT (XLCT)

A. XLCT BACKGROUND

X-ray luminescence CT is tomography imaging based on x-ray stimulated optical emissions (also called x-ray luminescence, radioluminescence, or scintillation). Scintillation is the conversion of x-ray energy into (visible) light energy. X-rays deposit their energy in a target material by ionizing atoms and releasing secondary electrons. High-energy electrons produce other downstream ionization events, resulting in a shower of ionized low energy electrons. This is the first step in the energy transfer from x-rays to the target material. Typically, this excess energy of the secondary electrons is deposited as heat. However, in a scintillating material, the secondary electrons migrate to a luminescent center, an ion dopant in the bulk scintillator material, to create a temporarily-stable excited electronic state. This state eventually decays by the emission of an optical photon. For more detail on the physical process of scintillation after radiation exposure, see Ref [3]. Scintillation has long been used in radiation detection and to detect x-rays and gamma rays in radiographic imaging. Whereas scintillating bulk materials are routinely used in radiation detection instrumentation, microscopic scintillating nanoparticles are used as imaging probes in x-ray luminescence imaging. An external beam of x-rays excites the nanophosphors and the resulting scintillation light emissions from these nanoparticles are detected by an optical camera outside the imaged subject. The premise of x-ray luminescence imaging in tissue was demonstrated by the Anker's group in which they imaged trace phosphor concentrations on a thin surface [4], [5].

Rare-earth nanophosphors have so far been the most studied type of scintillating nanoparticles, due to their versatile optical properties. By selecting the proper dopant element, these nanoparticles can be used for a variety of cellular and small-animal fluorescence applications, including up-conversion, down-conversion, and second-window infrared imaging [6]. Since near-infrared light travels better through tissue than other frequencies of light, the design of near-infrared range luminescent probes is of great interest. With dopants such as Tb and Eu, the rare-earth phosphor nanoparticles can be used for XLCT. It should be emphasized that nanophosphors are not the only type of nanoparticle suitable for XLCT: in the last decade, x-ray luminescence has been demonstrated for quantum dots [7], gold nanoclusters [8], and X-ray-excitable polymer dots [9].

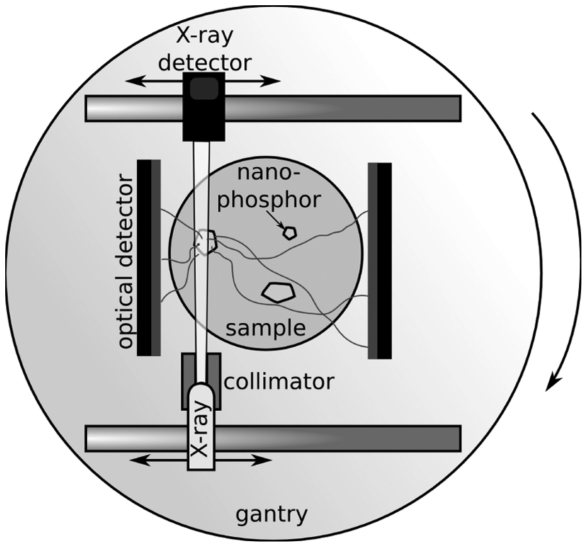

FIGURE 1. System schematic of XLCT imaging system. An x-ray pencil beam scans the subject in translation/rotation steps (first generation CT geometry). Reprinted from Pratx (2010) [10].

XLCT is the result of combining the x-ray luminescence phenomenon with the principles of tomography. The XLCT imaging method is illustrated in Figure 1. A sample is irradiated by a sequence of narrow x-ray beams at predefined translation and rotation positions. This is similar to first-generation x-ray attenuation CT geometry. The scintillating contrast agents within the excited volume emit visible light that is detected by an external optical detector. The total measured flux of the optical emissions is a summation of the amount of nanoparticles in the path of the x-ray beam. By acquiring many such measurements from various rotated views and translation motion steps, a sinogram of projection data is constructed similarly to x-ray attenuation CT. At this point, the cross-section image of nanophosphor distribution is reconstructed using the techniques of CT reconstruction.

One major advantage of XLCT is high spatial resolution, especially compared to optical fluorescence imaging, which is the most widely used modality in pre-clinical molecular imaging. The advantage of XLCT over fluorescence is high spatial resolution at depth in tissue. The resolution of optical fluorescence imaging is limited by the scattering of optical photons in tissue. Since the amount of scattering increases with sample depth, there is a tradeoff between depth and resolution. On the other hand, the emission signal in XLCT originates from the selectively irradiated volume, so it is possible to locate the emission sources despite any scattering

during emission. The image resolution is limited mainly by the x-ray beam collimation. Each individual optical photon carries localized information when it is produced from selective excitation, and therefore, spatial information can be resolved from a limited number of optical photons. On the other hand, optical fluorescence imaging requires many photons and statistical averaging to resolve spatial information.

Pratx et al. recently demonstrated the feasibility of high-resolution XLCT at depth [10]. This simulation study found that small volumes of picomolar nanophosphor concentrations can be detected in a mouse-sized subject using pencil-beam XLCT. This was followed by an experimental demonstration of XLCT [11], although much lower sensitivity was achieved compared to the simulation study (mg/mL vs. μg/mL). The limitation on sensitivity came from the non-negligible x-ray scatter in tissue and from the non-optimal optical imaging geometry. Although x-rays have a low scattering cross-section (compared to optical photons), the non-negligible x-ray scatter excites nanophosphors outside the primary x-ray excitation beam. This effect reduces image contrast, and thus limits the sensitivity. Cong *et al.* proposed using a scatter-estimating forward model in image reconstruction to mitigate the x-ray scattering problem and showed image quality improvement in simulations [12]. At the same time, they also simulated multiplexing with multiple phosphor types simultaneously. The ability to image multiple molecular targets with multiple probes simultaneously is of great interest in high-specificity molecular imaging [13]. Carpenter *et al.* provided an in vivo demonstration of multiplexed x-ray luminescence by imaging with two different types of nanophosphors simultaneously [14]. Another advantage is that x-rays can be focused in tissue through a polycapillary lens, leading to highly selective x-ray illumination [15]. Last, one of the advantages of XLCT is that it does not require the optical signals to be spatially resolved. Thus, a non-imaging endoscopic fiber may be used to collect the light from within the subject. The feasibility of the endoscopic approach has been investigated in a series of simulations [16].

B. CURRENT AND FUTURE DIRECTIONS IN XLCT

One current problem in XLCT is long imaging time, especially with the scanning pencil-beam methods. One approach to reducing the scan time is to reduce the number of projection views. A limited angle tomography approach was taken by Carpenter *et al.*, in which they scanned a phantom with a beam from only one projection angle but reconstructed tomographic cross-sections [17], and by Li et al., in which they scanned the beam from two projection angles [18]. Both reconstruction methods use a model of optical scatter and propagation in tissue to localize the emission sources; thus they represent an approach that is halfway between fully-sampled XLCT and diffuse optical tomography [19]–[22]. Reducing the number of x-ray projection view reduces the imaging system mechanical complexity and scan time. Another method of reducing scan time is cone-beam XLCT, introduced by D. Chen *et al.* [23] and validated *in vivo* by Liu *et al.* [24]. Image results from the in vivo XLCT study are shown in Figure 2, where contrast from nanophosphors is apparent with virtually no background intensity in tissue. This single-view method avoids time-intensive pencil beam scanning, but also reduces image contrast and resolution due to the ill-posed nature of the reconstruction problem. Chen's simulation results suggested that some of the image quality can be recovered with compressed sensing reconstruction and *a priori* anatomical knowledge.

One distinguishing feature of XLCT over other x-ray imaging modalities is that chemistry is much more critical to the imaging signal generation. An active area of research is in engineering nanophosphors with greater scintillation output. Recently, a $NaGdF_4:Eu^{3+}$ nanophosphor formulation was found to have one of the highest x-ray luminescence efficiency among nanoparticles [25]. Other promising nanoparticle candidates are metal-organic frameworks (MOFs) [8]. MOFs combine the high x-ray absorption of metals with the efficient optical production of organic scintillators resulting in efficient x-ray luminescent agents. Other research efforts include nanoparticles whose luminescence efficiency is sensitive to their micro-environment conditions. Chen *et al.* presented a pH-sensing nanoparticle that becomes luminescent in acidic environments [26]. This capability may be quite useful, for instance, in imaging the acidic environment of tumors. The same group has also developed magnetic radioluminescent probes that can be guided by external magnetic fields (magnetophoresis) and can also be imaged using MRI [27]. A review of radioluminescent probes is provided in Ref. [28].

An active research area is XLCT image reconstruction methods. XLCT image reconstruction is a problem of reconstructing the distribution of internal luminescent sources given optical emissions measurements at the surface of the imaged subject. As discussed already, the location of these sources is well-determined due to the selective x-ray excitation. However, their relative intensities have uncertainties due to unknown absorption and scattering of the optical emissions. Consequently, the imaging sensitivity is limited by uncertainties in the physical model of light propagation in the subject. The physical model may be obtained from prior image knowledge or from a concurrent secondary imaging modality like conventional CT or MRI. To our knowledge, this has not been done in XLCT, but it has been shown that concurrent MRI imaging provides a subject-specific physical model that improves image reconstruction in diffuse optical tomography (DOT) [29]. (DOT suffers especially from optical scattering and absorption.) As XLCT develops with improved probes and instrumentation hardware and moves from phantom to *in vivo* imaging, there will be an increased demand on image reconstruction to accurately account for optical scattering and absorption.

A modality similar to XLCT is Stored Luminescent Computed Tomography (SLCT) [30]. In SLCT, the imaging probe does not immediately scintillate in response to

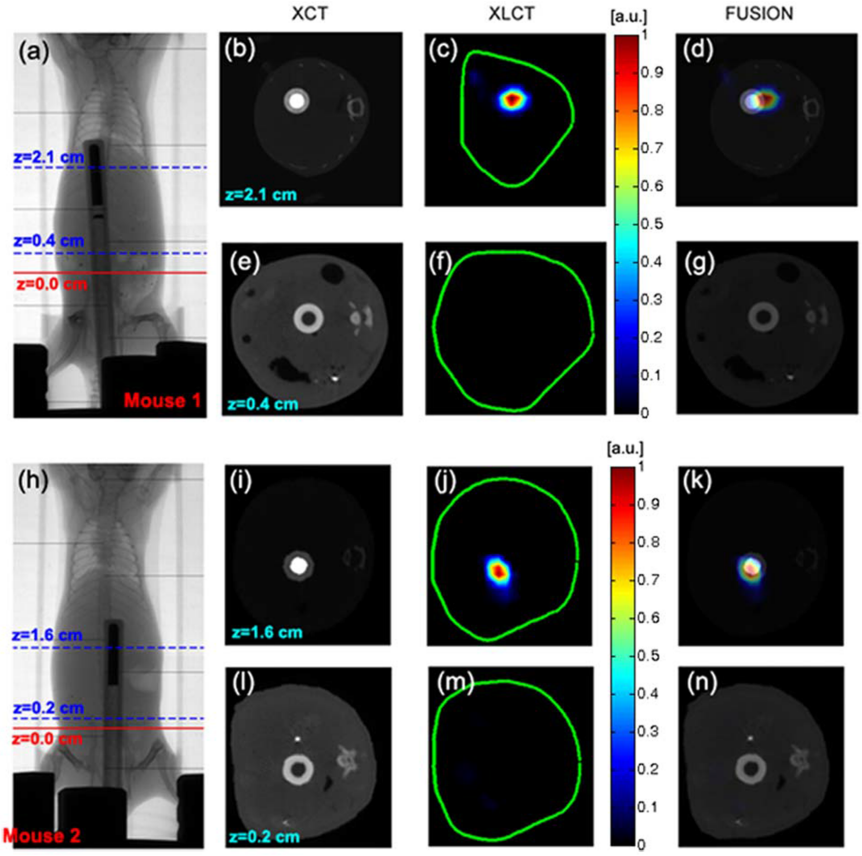

FIGURE 2. In vivo XLCT imaging demonstration of a tube loaded with $Gd_2O_3:Eu^{3+}$ nanophosphors inside two mice. The XLCT shows good agreement with conventional CT. No background signal is visible in XLCT. Reprinted from Liu (2013) [24] with permission from Optical Society of America.

x-ray excitation. Instead, the nanophosphors store the x-ray excitation energy until stimulated by light. This optical excitation is also spatially selective like the initial x-ray excitation, although selective optical excitation is much more limited than selective x-ray excitation. Incorporating a second spatially-selective excitation further localizes each output luminescence photon: i.e. the emitted signal comes from the intersection of the x-ray and optical excitation beams. This strategy may therefore achieve higher sensitivity and resolution, especially when a zone plate is used to focus the X-ray [31]. The disadvantage is that two sequential x-ray and optical scanning steps are required resulting in long imaging times.

While XLCT has much lower levels of tissue autofluorescence than fluorescence imaging, it is not without any sources of intrinsic background luminescence. One well-known source of background is Cherenkov radiation, which occurs for X-ray energies above 260 keV in tissue. As a form of x-ray-stimulated emission, Cherenkov radiation may have application in radiation therapy, where it may be used to monitor radiation dose to tissue [32], [33]. For XLCT, Cherenkov luminescence can be easily avoided by using a lower-energy X-ray beam. Another source of background is air scintillation from excited nitrogen in the atmosphere. As was shown in a previous study, this effect can yield significant luminescence and does not have an energy threshold [34]. This luminescence phenomenon occurs mostly in the ultraviolet, and can be blocked with a suitable long-pass filter. Last, a number of endogenous compounds luminesce under the action of X-rays. Water is particularly problematic for XLCT, because although it is approximately 10^{-5} less efficient than a bulk inorganic scintillator, it is very abundant in tissue. Furthermore, the radioluminescence of water has a broad spectrum, which makes it difficult to block it entirely [35]. Another major source of radioluminescence is the aromatic amino acids such as tryptophan [36]. These amino acids are the building block of proteins and are also abundant in living tissue. Further progress toward improving the sensitivity of XLCT and thus achieve theoretically possible sub-microgram/mL concentration will require novel strategies to mitigate and block background contributions from endogenous substances.

Finally, a very important problem is the toxicity of XLCT probes, particularly nanophosphors [37] and quantum dots [38], [39]. Although x-ray luminescence molecular imaging has been performed in mice [24], the toxicity problem represents a major obstacle to clinical implementation. We refer the reader to the following article for a more detail on nanoparticle toxicities [40].

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The potential applications of XLCT will take advantage of its high-resolution due to selective x-ray excitation. This will better localize signal to specific organs and tissues. Moreover, concurrent anatomic x-ray imaging is possible since the same x-ray beam used for luminescence excitation can also be used for traditional x-ray transmission imaging. XLCT and CT images are then inherently co-registered. The potential high resolution of XLCT will improve the characterization and treatment response of small tumors. In turn, this will advance early interventions in disease.

III. X-RAY FLUORESCENCE CT (XFCT)

A. XFCT BACKGROUND

X-ray fluorescence CT (XFCT) is tomographic imaging based on x-ray stimulated x-ray emissions (x-ray fluorescence or XRF, for short). X-ray fluorescence is the physical process in which externally applied x-rays produce secondary x-ray emissions in a target material. In this process, an x-ray ejects an inner shell electron from an atom in the target material via the photoelectric effect. The vacancy created in the inner electron shell is then filled by a second electron from one of the outer electron shells. Since the inner electron shell has a lower energy than the outer shell, the excess energy in this transition is emitted in the form of a characteristic x-ray. The energy of this characteristic x-ray is, as the name implies, a characteristic signature of an atomic element in the target material and is always less than the energy of the primary x-ray causing the excitation. Because the input x-rays produce secondary x-rays, the process is called x-ray fluorescence (XRF). This is analogous to optical fluorescence in which optical excitation produces secondary optical emissions. The term fluorescence itself was named after fluorite which emits blue light when excited by ultra-violet light. However, unlike optical fluorescence, in which the optical emissions have a range of energies, XRF has specific discrete energies and thus has been used for spectroscopic materials analysis which determines a material's elemental composition.

X-ray fluorescence CT combines the techniques of XRF material analysis and tomographic imaging. In XFCT, a pencil-beam of x-rays scans the imaged object at discrete translation and rotation steps, similar to that in first-generation x-ray transmission CT. This is the same setup as XLCT illustrated in Fig. 1, except that visible light detectors are replaced by energy-resolving x-ray detectors. In conventional CT, the detector is placed opposite the x-ray source to acquire the x-rays transmitted through the subject. In XFCT, however, detectors are placed outside the x-ray beam to acquire the x-rays emitted from the subject. These detectors are photon-counting x-ray detectors that measure the energy of each detected x-ray. XFCT detectors are different from conventional CT detectors which operate in an integrating mode measuring total x-ray exposure with little or no discrimination for x-ray photon energies. The XFCT detectors acquire a spectrum of counts over x-ray energies. This spectrum contains peaks corresponding to different elements in the imaged subject. A single spectrum is acquired for each
x-ray pencil beam projection, and these projections are sequentially acquired by translation/rotation scanning. Cross-section images of elemental composition of the subject are then reconstructed using the mathematical framework of tomographic reconstruction.

Generally speaking, the x-ray absorption for any material decreases with higher energies, as the x-rays become more penetrating. However, the exceptions to this rule are discrete, discontinuous *increases* in x-ray absorption at specific energies. This abrupt increase in x-ray absorptionb is called the K-edge and tuning the x-ray energy to the K-edge can maximize the x-ray absorption efficiency of the imaging probe. High atomic number (high-Z) elements are efficient as imaging probes in XRF signal production in biological subjects because the K-edge of these elements matches well with the energy required to penetrate tissue. In particular, stabilized gold nanoparticles (AuNPs) [41] have been demonstrated as biocompatible contrast agents due to their low cytotoxicity [42]. In XFCT-based molecular imaging, a high-Z nanoparticle is designed to target a specific biological process. For example, gold nanoparticles have been conjugated to ligands that bind specifically to the epidermal growth factor receptor (EGFR), which is over-expressed in cancer cells [43]. The EGFR is an important part of the signaling mechanism that cancer uses to grow itself. In this example, a high XFCT signal from the AuNPs is a molecular imaging biomarker of cancer.

The initial XFCT research was performed in the late 1980's at synchrotron facilities, where high intensity x-ray beams were available for imaging applications [44], [45]. One important advantage of synchrotrons is the availability of mono-energetic x-rays. Mono-energetic x-rays provide the maximum efficiency in converting primary x-rays into fluorescence x-rays, especially if the synchrotron x-ray energy is tuned to the K-edge (maximizing the excitation) of a particular imaging probe. Leveraging these advantages, synchrotron XFCT has provided spectroscopic microscopy imaging. Takeda *et al.* performed *in vivo* perfusion imaging of the mouse brain [46]. These experiences demonstrate the proof-of-concept and potential of XFCT in biomedical imaging. The major drawback of synchrotron XFCT imaging is the high-cost and space requirement of synchrotron facilities. Access to synchrotrons is prohibitive and rules out routine biomedical imaging.

B. CURRENT AND FUTURE DIRECTIONS IN XFCT

Recent XFCT research has shown promising developments in XFCT imaging systems using conventional x-ray tube sources. The groups of S. Cho, L. Xing, and G. Wang in particular have shown with simulations, modeling, and experiments that tabletop XFCT has potential in biomedical imaging applications [47]–[49]. Cheong et al. combined a common clinical radiography x-ray source with a single CdTe photon-

counting detector in a benchtop XFCT system and obtained a tomographic XFCT image of a gold-filled phantom [47]. Two important features in that study were the sensitivity and phantom size. The sensitivity was shown to be 10 mg/mL AuNP concentration, which is roughly the sensitivity of conventional dual-energy CT [50] with the same dose. The phantom size was 5-cm, whereas previous XRF applications have analyzed microscopic samples. Bazalova *et al.* further modeled and compared XFCT and transmission CT in simulations and found that XFCT achieves higher sensitivity for the same radiation dose [48]. An interesting finding was a ''cross-over'' point in concentration. Gold concentrations greater than the cross-over point of 5 mg/mL had better image quality in transmission x-ray imaging than in XFCT. However, concentrations lower than 5 mg/mL had better image quality in XFCT, bolstering the case for XFCT in molecular imaging applications, where high sensitivity is needed. Furthermore, Kuang *et al.* demonstrated multiplexed XFCT, in which multiple probes were imaged simultaneously [51].

Further sensitivity increase is required in XFCT. Although it was shown both theoretically and experimentally that XFCT has higher sensitivity than x-ray transmission CT, adequate sensitivity for molecular imaging applications has not yet been realized. A biologically relevant target is a 10μg/mL sensitivity (based on nanoparticles uptake studies [52], [53]). For imaging subjects smaller than 3 cm, the 10 μg/mL sensitivity is already possible [54]. Although optical imaging modalities have much higher sensitivity at this small scale, XFCT could provide superior resolution. With a 10 μg/mL sensitivity at a 3-cm subject size, XFCT with high resolution should enable new molecular imaging applications in mouse models. Further increases in sensitivity will require improvements in the fundamental x-ray source and detector technology, and the imaging system design.

Based on the successful results of synchrotron XFCT, one approach to high-sensitivity benchtop XFCT is to develop compact x-ray sources that have a mono-energetic or narrow x-ray spectrum. Mono-energetic x-rays beams are feasible at synchrotrons because of their high intensity, and a narrow portion of synchrotron radiation spectrum can be selected by x-ray diffracting crystals. However this approach is not feasible with conventional x-ray tubes due to insufficient x-ray production. Quasi-monoenergetic x-ray sources can be formed from x-ray tubes by filtering beams through materials that pass x-rays within an energy window. Lead and tin filters are relatively efficient filters for x-rays in the 80–90 keV range [55]. Another tabletop x-ray source design uses a liquid-metal-jet x-ray. In contrast to x-ray tubes which have a solid anode that melts when its heat capacity is exceeded, the new x-ray design uses a liquid metal anode as the electron beam target. This design is much less constrained by overheating since the anode material is flown continuously. This x-ray source was used in a recent XFCT imaging study that demonstrated an impressive 100-μm resolution [56] (shown in Figure 3). Other promising high-intensity tabletop x-ray sources include inverse Compton scattering sources [57] and free-electron x-ray lasers [58].

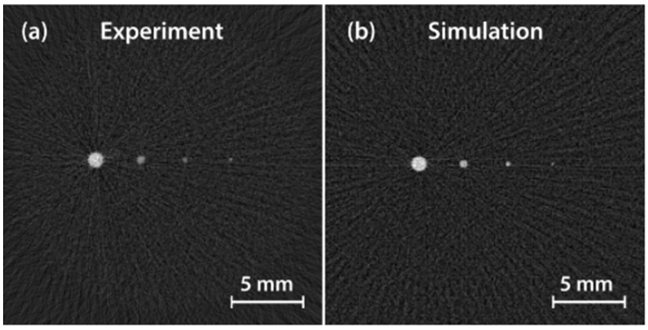

FIGURE 3. XFCT Image of 0.5% concentration molybdenum rods inside a 2-cm diameter plastic cylinder phantom. The smallest 100-μm diameter rod was visualized. The plastic background has very little background signal. Reprinted from Hertz (2014) [56] with permission from Optical Society of America.

X-ray source intensity also impacts imaging resolution in the case of pencil-beam scanning, where resolution is determined by the x-ray pencil beam width. The use of pinhole collimators to form a narrow pencil beam is an inefficient process since most of the x-rays produced are blocked. Therefore, narrower x-ray beams require a higher intensity x-ray source to maintain an equivalent scan time. One research direction seeks to overcome the limitation of x-ray collimation through x-ray optics which focus x-ray power. This is done with x-ray refractive lenses [59], mirrors [56], [60] and internally reflecting capillaries [61]–[63]. A good review of methods in x-ray optics is given by Dhez *et al* [64]. Unfortunately, these methods for focusing x-ray power only work well at energies below 30 keV. At higher energies, coherent x-ray scattering cross-sections become vanishingly small. New technological breakthroughs in focusing high energy x-rays (80–100 keV) are needed for clinical whole-body applications.

Another limiting factor in XFCT is Compton scatter. X-rays in the primary excitation beam can simply scatter off the imaged subject toward the x-ray detector and interfere with the desired XRF emissions. This scatter occurs in background tissue as well as the imaging probe, reducing the overall contrast and sensitivity, and can be undistinguishable from fluorescent photons due to limited detector energy resolution. One research direction is to optimize the imaging instrumentation design such that the acquisition of scatter x-rays is minimized. It has been shown that arranging detectors in a back-scatter configuration (such that only XRF emissions back toward the x-ray source are acquired) substantially improved the sensitivity to gold particles [65]. Another system design used Bragg reflection x-ray optics to isolate the XRF signal from scattered x-rays and achieved an iodine concentration sensitivity of 10 μg/mL [66].

Apart from sensitivity, an issue with current tabletop XFCT system design is long image acquisition times. To address this issue, novel imaging system geometries that irradiate large volumes instantaneously have been proposed which eliminate the need for slow pencil-beam scanning [67], [68].

TABLE 1. Comparison among various imaging methods.

	Sensitivity	Specificity	Resolution	Imaging depth
x-ray luminescence	+++	+++	+++	++
x-ray fluorescence	++	++	+++	++++
x-ray attenuation	+	+	++++	++++
nuclear imaging	++++	+++	+	++++
optical imaging	+++	++++	++	+

The decrease in scan time seems to come at the expense of sensitivity, although the quantitative trade-off is not exactly known. The x-ray source strength, collimation, filtration, and detector configuration, composition all have an impact on sensitivity, scan time, radiation exposure, and spatial resolution. Optimization of XFCT system design is therefore an active area of research.

There are also several image reconstruction challenges in XFCT. First, at low x-ray energies, attenuation of both primary and fluorescence x-rays can reduce the detection of objects deep in tissue. Bazalova *et al.* improved image contrast with an attenuation correction model that is calculated prior to image reconstruction [69]. An attenuation correction matrix was included into the system matrix (also known as the forward model) in the well-known iterative maximum-likelihood expectation maximization (MLEM) reconstruction [70]. La Riviere showed that in cases where attenuation is not severe, it can be corrected using an approximate analytic reconstruction algorithm [71]. Other iterative reconstruction approaches include the joint estimation of the image and the attenuation of the raw data [72], [73]. In both algorithms, the attenuation estimate is updated in each iteration and is used to correct the reconstructed image. Secondly, since Compton scatter creates a false background signal and reduces sensitivity, scatter correction strategies are needed in image reconstruction. Since XFCT involves quantification of peaks in noisy XRF spectrum data, any prior knowledge of the shape and intensity of the Compton scatter background can help reduce noise and improve sensitivity. A simulation study showed reconstruction results from XRF spectrum data both with and without gold nanoparticle contrast agent. The XRF spectrum data without contrast agent provided an estimate of Compton scatter that was used to improve reconstruction results and achieve a high sensitivity [65]. An accurate model of Compton scattering can be introduced into reconstruction to mitigate the loss of image contrast resulting from Compton scatter [74]. Finally, a conventional CT can be acquired simultaneously with no additional radiation dose (in both XLCT and XFCT). The CT image may be used to provide a subject-specific physical model of excitation, emission, and Compton scatter background [75].

Future applications will leverage the advantages of XRF: high imaging depth and potentially-high spatial resolution. This makes a XFCT a candidate for whole-body clinical molecular imaging. Although XFCT is not as sensitive as PET and SPECT, which are excellent in cancer detection, its high-resolution may be useful in a number of tumor characterization applications. In surgery and radiotherapy, high-resolution is critical in precise delineation of tumor boundaries. In chemotherapy, the drug molecule itself can act as an imaging contrast agent. A future application may monitor the Cisplatin drug concentration *in vivo* using platinum as a XRF emitter [69]. Other potential applications are in high-resolution small-animal molecular imaging. In particular, the study of tumor genesis and early growth require methods that have both high sensitivity and high resolution. This capability could measure parameters in cancer models such as tumor size, growth rate, heterogeneity, and metastatic growth.

IV. COMPARISON TO OTHER MOLECULAR IMAGING MODALITIES

The goal of most research in XLCT and XFCT is to enable new molecular imaging applications. Therefore we briefly summarize the other common tomographic molecular imaging modalities and compare them with XLCT and XFCT. Molecular imaging is the use of biomedical imaging methods to study molecular processes in living subjects. This is usually taken to be *in vivo* study of molecular processes inside whole organisms (as opposed to *in vitro* or *ex vivo* molecular imaging). Molecular imaging is also commonly described as the marriage of molecular biology and non-invasive *in vivo* imaging. The most widespread example of clinical molecular imaging is positron emission tomography (PET) using radio-labeled fluoro-deoxyglucose (FDG). In this application, PET imaging is used to detect abnormally high glucose uptake, an important molecular biomarker of cancer. Similarly, the hope of the XLCT and XFCT research programs is to provide new imaging tools to study the basic molecular biology underlying disease and other biological processes. We compare the described x-ray stimulated emissions imaging modalities against nuclear and optical imaging, the most commonly-used molecular imaging modalities. MRI [76], ultrasound [77], and photoacoustic imaging [78] are also gaining new applications in molecular imaging, but we will not discuss them here as they are less established in molecular imaging. A comprehensive review of molecular imaging is given in Ref. [79].

Nuclear imaging including PET and single photon emission CT (SPECT) are the workhorse clinical molecular imaging modalities. Nuclear imaging uses radio-labeled molecules (radiopharmaceuticals) to evaluate molecular biomarkers in

clinical exams. Radiopharmaceuticals (e.g. FDG) are synthetic molecules whose biochemistry (systemic circulation, tissue and cellular uptake, and metabolism) allow them to probe specific *in vivo* molecular processes such as glucose uptake. The relative wide variety of radiopharmaceutical probes gives nuclear imaging a high specificity. The radionuclides in these pharmaceuticals emit gamma ray signals that allow imaging systems to determine their locations and quantities. Nuclear imaging methods have high sensitivity and can detect trace quantities of radiopharmaceuticals because background tissue has very low gamma radiation signal. Since gamma radiation from common radionuclides can penetrate 30 cm of tissue, PET and SPECT have useful clinical whole body imaging applications. One disadvantage of nuclear imaging methods is relatively low spatial resolution compared to other modalities, which arises from the physical limits in focusing and capturing emitted high-energy gamma rays. Other drawbacks include the high cost of both instrumentation and imaging probes, and the limited half-lives of probes.

Optical imaging modalities (fluorescence, bioluminescence, optical coherence, and Raman scattering) have been very successful molecular imaging tools in basic science and pre-clinical research. The fundamental reason for this success is that optical imaging unveils bio-molecular processes with high specificity. One example is the use of engineered reporter genes that endogenously produce optically fluorescent proteins or chemiluminescent enzymes wherever there is a high expression of a particular gene [80]. ''Smart'' probe designs are also much more possible in optical imaging. Unlike radiopharmaceuticals, the biochemistry of optical probes is involved in the signal production. Biochemical reactions can switch an optical probe ''on'' or ''off'' [81]. This feature can be used to image a specific reaction and provide excellent contrast against background tissue. Furthermore, optical imaging methods take advantage of mature optics technology (focusing lenses, mirrors, and filters) to enhance signal and achieve high sensitivity. By comparison, radiopharmaceuticals are always ''on'' regardless of the probe chemical state because radioactive decay cannot be turned off. By leveraging the chemistry of optical fluorescence, optical probes have also been used to measure other chemical quantities such as tissue pH or redox status. The wide variety of optical probes available for different enzymatic or genetic expression are not available for nuclear or x-ray imaging methods. Because of the availability of probes with high biochemical specificity, the optical imaging modalities are said to have very high specificity.

On the other hand, the penetrating depth of optical photons is limited, and whole-body human imaging with optical methods is inconceivable. Furthermore, there is a fundamental trade-off between imaging depth and spatial resolution because of increasing optical scattering with increasing depth, and spatial resolution is typically poor in mouse-size subjects. Nevertheless, optical imaging is used heavily in small-animal imaging studies in which high resolution (at tissue and organ scales) is not required, and where imaging depth is not a problem. In these cases, the rich variety of chemical and genetic methods in designing optical probes has provided exquisitely specific imaging of biological processes.

We have summarized the foregoing discussion of molecular imaging modalities in Table 1. We discussed how XLCT and XFCT improve upon the sensitivity and specificity of conventional x-ray attenuation imaging. XFCT has lower sensitivity than XFCT due to Compton scatter. XLCT has potentially high specificity because probes can respond specifically to their microenvironment. As discussed, nuclear imaging modalities have excellent sensitivity and imaging depth, but poor resolution. Optical imaging modalities have good sensitivity, excellent specificity, but poor imaging depth. An ideal imaging modality would combine the excellent specificity of optical imaging, the high imaging depth of nuclear imaging, and the high sensitivity of both methods. In addition, the ideal imaging modality would also have the high resolution of x-ray absorption imaging. Compared to nuclear and optical imaging, the added value of x-ray luminescence and x-ray fluorescence imaging methods will be good spatial resolution deep in tissue, with molecular specificity and sensitivity.

X-ray luminescence and x-ray fluorescence CT therefore have potential roles in molecular imaging because of the unique combination of capabilities including high sensitivity, specificity, resolution and depth. These capabilities will let molecular biologists answer new questions and equip physicians with new diagnostic clinical tests. We envision a future in which XFCT is used for high-precision whole-body clinical molecular imaging. We also anticipate XLCT and XFCT for imaging biological processes non-invasively deep within living animals at the tissue scale, rather than organ scale. The development of x-ray based molecular imaging thus represents an important milestone in medicine and biology.

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