TLD assessment of mouse dosimetry during microCT imaging

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Advances in laboratory animal imaging have provided new resources for noninvasive biomedical research. Among these technologies is microcomputed tomography (microCT) which is widely used to obtain high resolution anatomic images of small animals. Because microCT utilizes ionizing radiation for image formation, radiation exposure during imaging is a concern. The objective of this study was to quantify the radiation dose delivered during a standard microCT scan. Radiation dose was measured using thermoluminescent dosimeters (TLDs), which were irradiated employing an 80 kVp x-ray source, with 0.5 mm Al filtration and a total of 54 mA s for a full 360 deg rotation of the unit. The TLD data were validated using a 3.2 cm³ CT ion chamber probe. TLD results showed a single microCT scan air kerma of 78.0 ± 5.0 mGy when using a poly(methylmethacrylate) (PMMA) anesthesia support module and an air kerma of 92.0 ± 6.0 mGy without the use of the anesthesia module. The validation CT ion chamber study provided a measured radiation air kerma of 81.0 ± 4.0 mGy and 97.0 ± 5.0 mGy with and without the PMMA anesthesia module, respectively. Internal TLD analysis demonstrated an average mouse organ radiation absorbed dose of 76.0 ± 5.0 mGy. The author's results have defined x-ray exposure for a routine microCT study which must be taken into consideration when performing serial molecular imaging studies involving the microCT imaging modality. © 2008 American Association of Physicists in Medicine. [DOI: [10.1118/1.2959847](https://doi.org/10.1118/1.2959847)]

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I. INTRODUCTION

The investigation of novel radiopharmaceuticals in nuclear medicine and their diagnostic/therapeutic kinetics on human malignant neoplastic tissues requires animal models that can mimic human diseases. Multiple studies have demonstrated that small laboratory animals, such as genetically engineered mice or rats, can serve as *in vivo* models for genetic studies, disease investigation, and pharmaceutical development.^{1–6} However, typical human clinical imaging systems cannot readily provide the high spatial resolution needed for rodent imaging without the use of specialized hardware and software.⁷ This limitation and the rapidly evolving field of molecular/anatomical imaging of animal models have increased the need for dedicated high resolution and high throughput small animal imaging systems. Microimaging modalities, such as microCT, microPET, microSPECT, microMRI, and optical imaging are providing new useful methods and *in vivo* applications for longitudinal laboratory ani-

mal imaging.^{2,4,8–15} However, concerns regarding radiation exposure of the animal have emerged, predominantly with microCT.^{13,16–21} MicroCT analysis has been primarily used for skeletal imaging studies due to the normal physiologic differences in tissue x-ray densities between bone and soft tissue.^{9,20,22} MicroCT provides quantitative data with high density tissue measurements and is also effective for soft tissue contrast-enhanced and high contrast imaging, such as microCT techniques employed for *in vivo* lung tissue imaging analysis.^{9,13,18,23} MicroCT is widely used to obtain high resolution anatomical images, and in order to achieve an acceptable signal-to-noise ratio in images with comparatively small voxels (e.g., 100 μm^3), relatively large radiation doses are required.¹⁶ MicroCT radiation doses ranging from 35 to 300 mGy have been reported depending on the microCT imaging protocol used.^{13,16–21} Previous reports have suggested that cumulative radiation doses in longitudinal microCT studies could potentially produce tumor therapeutic effects leading to tumor growth inhibition.^{13,19–21}

Thermoluminescent dosimeters (TLDs) are commonly employed in the ionizing radiation field to verify absorbed dose calculations at any given location of interest, either in a phantom or directly on a subject (e.g., a patient).²⁴ Their frequent use is due in part to the TLD's low effective atomic number which is only slightly greater than that of soft tissue.²⁵ In general, thermoluminescence (TL) phosphors provide optimal performance as dosimeters if they receive uniform, reproducible, and optimal heat treatment before and after use.²⁵ Extensive literature exists on TL dosimetry and a concise review on TLD behavior and properties provide the basis for the use of TLDs in these studies.^{26–34}

MicroCT is routinely employed in our investigations as a quantitative and qualitative screening tool to assess tumor progression pre- and post-targeted radiotherapy. Acute radiation effects resulting from doses of whole body irradiation, including prodromal and lethal effects, limit the number of small animal microCT imaging studies that can be performed on the same subject.³⁵ The LD_{50/30}, which is defined as the total-body lethal radiation dose that would produce lethality in 50% of the subjects within 30 days of exposure, is a determinant factor that has to be considered when performing preclinical investigations involving radiation exposure. For example, mice LD_{50/30} doses are reported to range between 5.0 and 9.0 Gy depending on the mouse strain, health status, age at exposure, and other factors.^{20,23,35–37} Thus, it is necessary to determine the x-ray radiation dose that is absorbed by rodent tissues during microCT imaging. Defining the sequential microCT cumulative doses to which the animal is exposed during x-ray imaging will assist investigators in effectively planning the number of imaging scans that can safely be performed per experiment. The objectives of this study were to quantify and assess the radiation absorbed dose a mouse receives during a typical whole body microCT scan.

II. MATERIALS AND METHODS

II.A. Thermoluminescent dosimeters

Lithium fluoride (LiF) TLDs (LiF:Mg:Ti), TLD-100® supplied by Harshaw/Bicron (Newbury, OH), were employed. A group of 90 TLD chips with sensitive volumes of $0.3175 \times 0.3175 \times 0.0889$ cm, equal to ~ 24 mg were obtained. Approximately 5% TLD-100 fading in a 12 week period has been reported.²⁵ TLD-100 chips have demonstrated $\pm 15\%$ sample-to-sample uniformity and within 2% repeatability.²⁵ TLD-100 chips have a broad practical linear dose range, from a few cGy to approximately 10 Gy.²⁵

Each TLD-100 chip was assigned an identification number and was individually calibrated, relative to the average response of the entire TLD batch. The relative dosimeter response, termed chip factor, was measured by irradiating the 90 chips individually using a broad beam of Cs-137 gamma rays from a Gammacell 40 Exactor irradiator source ($\gamma = 662$ keV), (MDS Nordion, Kanata, ON, Canada). The irradiator beam flatness across the chip was verified six different times by simultaneous irradiation of a 15 cm³ probe (96035 model) ion chamber Triad model 35050A, (Keithley Instruments, Inc., Cleveland, OH). Experimental acquired doses

were in agreement with the expected measurement uncertainty according to annual calibration data and instrument documentation from the irradiator manufacturer. A 2.5% error was achieved when comparing both expected and experimental findings.

A Harshaw model 2000A thermoluminescent detector and its associated model 2000B Automatic Integrating Picoammeter (Newbury, OH) were used to measure TLD output (coulombs). The Harshaw TLD reader collects total charge integrating the total area under the TLD glow peak. The temperature profile used for the reading cycle of the TLD utilized a 4.3 °C/s linear ramp rate. The reading cycle of 30 s was set to begin reading at 120 °C (T_1) up to 250 °C (T_2). For annealing purposes, two ovens were used, one providing 400 °C for one hour immediately followed by a cool-down to 100 °C for 2 h.²⁵ The TLD crystals were annealed prior to every irradiation at the same conditions. The TLD crystals were protected from exposure to light until they were used and protective latex gloves were always employed when handling the TLDs.

II.B. TLD linearity and uncertainties

Individual TLD output linearity and measurement reproducibility were investigated. The annealed batch of 90 dosimeters was irradiated in the Cs-137 irradiator source, which produced a dose rate of 103.4 mGy/min. The TLDs were exposed individually to 70 and 1000 mGy of dose, respectively. These doses were selected to validate the linear response of the TLDs at expected microCT doses.^{13,16–21} After five annealing and consecutive individual irradiations of the same 90 TLDs at both doses, the TLD output was analyzed and only crystals responding within 8% sample-to-sample uniformity and 7% response variability were selected, resulting in a total of 24 TLDs for the study. Response variability was analyzed postirradiation by comparing individual dosimeter output to the group mean of the selected TLD batch.

The calibrated TLD measurements were analyzed to define the absolute uncertainty associated with the derived radiation doses. The combined standard uncertainties associated with the TLD measurements were determined with standard formulas of error propagation.³⁴ Uncertainties for each group of selected TLD readings ($n=5$) included analysis of the means (μ), standard deviations (σ), and coefficients of variation ($100 \times \sigma / \mu$).

II.C. TLD calibration

To ensure accurate calibration of the selected annealed TLDs two different irradiation sources were utilized, including the Cs-137 irradiator and a clinical x-ray source. The irradiation chamber has a sample cavity with a plastic tray, which holds the object that needs to be irradiated. Each TLD was placed in a thin plastic container which was positioned at the center of the plastic tray using a top and bottom symmetrical foam piece for reproducibility purposes. The time of irradiation of the TLD chips was calculated to give 69, 999, 2512, and 4507 mGy of dose. These doses were selected to

validate the TLD dose response linearity. A group of ten annealed dosimeters were chosen from the selected batch and exposed to the defined Cs-137 irradiator doses (mGy) on every other day during the same week of the study. Using the TLD output (μC), a calibration plot of exposure (C/kg) versus microCoulomb (μC) output was created. Since the photon energies are different between the irradiator ($\gamma=662$ keV) and the microCT (average x-ray energy = 28 keV), energy correction factors were applied when the Cs-137 dose calibration plot was employed for the microCT dose data analysis.²⁵ Literature predicts a 40% over response of LiF TLD when exposed to 28 keV x rays.^{25,34,38}

The x-ray source was a standard calibrated clinical radiographic unit MVP series generator (General Electric, USA), which has a three phase 150 kVp x-ray tube. The radiographic unit was set at 80 kVp with 1 mm Al filtering to match the kVp and Al filtration employed by the microCT system. An external Keithley model 35050A x-ray dosimeter with a 15 cm³ probe (96035 model) was used to measure exposure. The 15 cm³ air vented ion chamber has an energy range of 20–150 kVp with a nominal sensitivity of 2.0×10^8 R/C with a diameter of 4 cm and a depth of 1.2 cm. The kVp of the beam was measured using a calibrated kVp divider (Keithley 35080A) along with the x-ray dosimeter 35050A, accurate to $\pm 2\%$. The x-ray unit was set at 800 mA for 400 ms. TLDs were placed at a distance of 40 cm from the focal spot and exposed to 2.8×10^{-3} C/kg to validate the dose calibration plot generated by the Cs-137 irradiator and our technique to convert exposure to delivered doses. The TLDs and a Keithley model 35050A x-ray ion chamber were irradiated simultaneously. The TLD output from the x-ray clinical unit was compared to the results at 107.2 mGy from the Cs-137 irradiator.

II.D. MicroCT system

Our research laboratory is equipped with an *in vivo* x-ray microCT (MicroCAT IITM CT/SPECT, Siemens Pre-Clinical Solutions, Knoxville, TN) scanner that provides an isotropic voxel size down to 15 microns. The scanner is a third generation “step and shoot” system in which the low-power x-ray source and charge coupled dence (CCD) detector rotate about the specimen. Fan beam and cone beam (Feldkamp) filtered backprojection algorithms are used for reconstructing images using the Cobra 5.0 reconstruction engine (Exxim Computing Corporation, CA). The system consists of a fully shielded cabinet x-ray system with a maximum voltage of 80 kVp, maximum current of 500 μA , and a tungsten anode. To reduce beam-hardening artifacts, a beam filter module is attached in front of the x-ray source exit window with aluminum filters (4 cm in diameter) of thicknesses ranging from 0.25 to 3.5 mm. The x-ray detector has a 4096×4096 pixel CCD array that is coupled to a phosphor screen via a fiber-optic taper. A 26.88 cm source to isocenter distance was constant throughout this investigation which provided a microCT field of view (FOV) (along *z* axis) of 11 cm. All microCT irradiation experiments were conducted employing an 80 kVp x-ray spectrum filtered with 0.5 mm of Al, with a

0.15 mAs per 1 deg step motion to provide 360 microCT projections acquired in a full 360 degree rotation of the gantry.

II.E. MicroCT geometry

The tungsten enclosed x-ray source houses an x-ray tube that is bathed in dielectric oil for dissipation of the generated heat during x-ray production. The x-ray source exit window is composed of thin carbon material (2 cm in diameter and 0.8 mm in thickness) and is approximately located at a distance of 2.6 mm from the x-ray tube. The x-ray low power source block (model SB-80-500, Source-Ray, Inc., NY) is composed of a 1.4 cm thick tungsten rectangular enclosure ($5 \times 5 \times 11$ cm). Contained within the source block is a cylindrical x-ray tube (model PN90507, Oxford Instruments, CA) of approximate weight and density of 114 g and 0.885 g/cm³, respectively. The tungsten cylinder anode target (1.5 cm $\times$ 1.5 cm) is tilted 20 deg with respect to the electron beam and the electron gun is located approximately 2 cm away from the target center. The x-ray tube glass enclosure has a thickness of 1.4 mm with overall dimensions of 8 cm height and a diameter of 3 cm. Taschereau *et al.* provide extensive details regarding material composition contained within the x-ray source block.²⁰

In order to characterize the quality of the microCT x-ray beam the half value layer (HVL) was determined. The HVL is defined as the material thickness required to reduce the intensity of an x-ray beam to one-half of its initial value.³⁹ Aluminum (Al) slabs ranging in thickness from 0.25 to 3.5 mm were employed along with an external Keithley (model 35050A) x-ray dosimeter equipped with a 15 cm³ probe (96035 model). The 15 cm³ probe (6.4 cm diameter with a 13 mm height) was centrally placed in the holder imaging table and exposure measurements were taken of the incident beam with, and with out consecutive side by side Al slabs until 50% intensity reduction of the incident beam was achieved.³⁹ The ion chamber was placed perpendicular to the incident beam at a distance of 26.88 cm. The focal spot of the microCT X-ray source was approximately 45 μm . The HVL was used to estimate the microCT effective energy at 80 kVp.³⁹

II.F. MicroCT radiation exposure

MicroCT radiation exposure at the FOV isocenter was measured employing a Keithley 35050A dosimeter with a 3.2 cm³ CT probe. The CT probe was a air vented polystyrene ion chamber (500-100 CT model) that had an inside diameter of 6.4 mm with a chamber wall thickness of 54 mg/cm² and a sensitive length of 10 cm. Radiation exposure was measured as a function of kVp (other scanning parameters fixed) to assess the delivered FOV isocenter exposure as a function of x-ray tube potential from 45 to 80 kVp in the microCT unit. Exposure as a function of radiograph angle during a full 360 deg CT acquisition with the use of the microCT poly(methyl methacrylate) (PMMA)

FIG. 1. MicroCT PMMA anesthesia support module. Radiation exposures were defined for microCT scans when conducted with and without the use of the microCT PMMA anesthesia support module. The anesthesia module provides gas anesthesia under a HEPA filtered pathogen-free environment.

anesthesia support module was evaluated to assess the quality of the x-ray beam around the rodent during a typical microCT acquisition.

Exposures were defined for a microCT scan when conducted with the use of the PMMA anesthesia (Fig. 1) support module (wall thickness of 1.5875 mm, diameter of 54 mm, and length of 203 mm) as well as a scan without the use of this module. The anesthesia module provides gas anesthesia under a HEPA filtered sterile environment. TLD chips were placed with plastic tweezers in a matrix formation with 12 chips on a foam bed separated by approximately 2 mm in each direction and scanned with, and without, the PMMA anesthesia support module. This procedure was repeated with the remaining 12 chips on a second microCT acquisition. TLDs were held in place with a narrow piece of plastic tape on the animal bed. TLDs were exposed using a microCT imaging protocol described above. Alignment of the TLD chips was confirmed with the gantry alignment lasers, and a single radiograph acquired to assure that the batch was in the FOV. After performing the microCT scans, TLDs were removed from the foam bed and analyzed with the TLD reader. The TLD output (μC) was inserted in the exposure calibration plot generated from the Cs-137 irradiator and an exposure output (C/kg) corresponding to the TLD output was generated. The exposure generated from the exposure calibration plot was converted to dose in air and water (mGy) with the use of standard formulas. Uncertainties associated with each measurement were propagated accordingly.³⁴

II.G. Mouse internal radiation absorbed dose measurements

To determine the internal absorbed dose delivered during microCT imaging, multiple TLDs were implanted into selected anatomical locations within three euthanized 24 week old C57BL/6 female mice (body weight ~ 35 g). The TLD crystals were annealed prior to every microCT irradiation at the same conditions previously discussed.²⁵ TLDs were wrapped in single layer Saran plastic wrap (S.C. Johnson, WI). Mice were euthanized by CO_2 gas inhalation, and then TLDs were surgically implanted post euthanasia into multiple body locations with minimal organ disruption by a laboratory

animal veterinarian. The same TLDs were implanted in the same organ of each mouse. TLDs were inserted in the following organs: brain, right flank, left flank, bladder, left kidney, right kidney, left lower GI, right lower GI, left upper GI, right upper GI, stomach, left liver lobe, right liver lobe, left lung, and in the left ventricle of the heart. After implanting the dosimeters, the skin and other organs were carefully repositioned into their original location before scanning on the microCT. The mouse cadaver containing TLDs was secured on the scanning bed and the position and height adjusted using laser positioning beams so that the field of exposure approximated the sagittal and axial center of the mouse. The mouse was imaged using the standard microCT imaging protocol described above for a single microCT acquisition. TLDs were removed postscan and analyzed. Internal exposures were converted to dose in water, which mimics tissue equivalent properties. The internal absorbed doses for the C57BL/6 murine model were determined and doses in air were converted to absorbed doses in water, assuming charged particle equilibrium conditions, unchanged exposure to the volume analyzed, negligible bremsstrahlung losses, and maximum x-ray energies of 80 keV. The mass energy-absorption coefficients at this photon energy range were 0.0262 and 0.0243 cm^2/g for water and air, respectively.²⁵ Radiation exposure, as measured by the TLD and ion chamber measurements, were converted to radiation doses in air and subsequently converted to radiation absorbed doses in water with standard formulas.

III. RESULTS

III.A. TLD calibration

The calibration experiments using the Cs-137 irradiator produced a plot of TLD light output versus absolute exposure from the standard source, which was used to create an exposure response curve. From this curve, a calibration factor [in exposure units of C/kg per microcoulomb (C/kg/ μC)] was employed for calculating exposure for all microCT measurements.

Figure 2 shows the TLD response to the Cs-137 irradiator. The figure highlights exposure (0.002, 0.026, 0.065, and 0.117 C/kg) versus TLD output (μC). As can be seen, the response is linear with exposure. The calibration factor is the slope of this line as determined by a linear least square fit of the data which gives a value of 0.0076 C/kg/ μC and a correlation coefficient (r^2) value of 0.9906. Since photon energies are different between the irradiator and the microCT, energy correction factors were needed in order to use the Cs-137 irradiator exposure response curve (Fig. 2). The calibration difference between the x-ray source at 80 kVp and Cs-137 is due to the energy responses of LiF:Mg:Ti . The x-ray source produces photons with an average energy of approximately 28 keV compared to the 662 keV energy of Cs-137. Published data predicts a 40% LiF TLD over response when exposed to x rays (28 keV).^{25,34,38} Dividing the Cs-137 dose calibrations by 1.4 (i.e., a 40% reduction) results in a derived 80 kVp x-ray exposure response from the Cs-137 data of 0.00542 C/kg/ μC . The microCT energy cor-

FIG. 2. Calibration curve to convert TLD output into radiation dose. We used this calibration plot to determine the exposure response of the TLD chips which was $0.00542 \text{ C/kg}/\mu\text{C}$. This calculation allowed us to convert TLD output into radiation dose for the remainder of the experiments.

rection of $0.00542 \text{ C/kg}/\mu\text{C}$ was employed throughout this investigation for all microCT dose measurements generated from the Cs-137 dose response curve.

The accuracy of the x-ray energy corrected Cs-137 response curve in defining x-ray exposure was validated by exposing 12 TLDs in a clinical radiographic unit. An average exposure of $3.0 \times 10^{-3} \text{ C/kg}$ was reported by the Cs-137 calibration plot as compared to a measurement of $2.8 \times 10^{-3} \text{ C/kg}$ that was obtained using the ion chamber measurements. The coefficient of variation of 3.8% was achieved among the analyzed TLDs. A 7.9% experimental error was noted among the analyzed crystals as compared to the ion chamber readings.

III.B. MicroCT radiation exposure

Based on our investigation to determine the quality of the microCT x-ray beam, the HVL of the x-ray beam at 80 kVp was 2.5 mm Al equivalent providing an estimated x-ray effective energy of 32 keV.³⁸

Exposure as a function of radiograph angle during a full 360° microCT acquisition with the use of the anesthesia module was evaluated. Noticeable reduction of the x-ray beam occurring between 200° and 340° was noted due to further attenuation of the photons from the presence of the plastic animal bed within the anesthesia module (Fig. 3). Since our microCT uses a polyenergetic x-ray spectrum, the lower energy x rays are attenuated to a greater extent than higher energy x rays when interacting with the PMMA bed material. Based upon exposure versus kVp experiments, we found that the microCT scan radiation exposure increases linearly by $0.000234 \pm 0.000018 \text{ C/kg}$ per 5 kVp increment when holding all other imaging protocol parameters constant (Fig. 4).

Calibrated TLDs were then scanned using the microCT imaging protocol. TLD analysis resulted in a single microCT scan air kerma of $78 \pm 5.0 \text{ mGy}$ when measured in a PMMA mouse anesthesia module and $92 \pm 6.0 \text{ mGy}$ without the use of the anesthesia module (experimental error=3.7%). The data were validated with the ion chamber. The ion chamber produced measurements of $81 \pm 4.0 \text{ mGy}$ with and $97 \pm 5.0 \text{ mGy}$ without the anesthesia module (experimental error=5.2%) which are in good agreement with the TLD results.

III.C. Mouse internal radiation absorbed dose measurement

Table I provides the dose measured following a single microCT scan by TLD chips implanted in various organs in mice ($n=3$). The average absorbed dose over all measurements to the organs is $76 \pm 5.0 \text{ mGy}$. This is in good agreement with the TLD air kerma analysis results of $78 \pm 5.0 \text{ mGy}$ measured employing the mouse anesthesia module and with the Keithley ion chamber measurements of $81 \pm 4.0 \text{ mGy}$. Each TLD measurement in Table I has an associated uncertainty that was generated from propagation of errors and also included the standard deviation generated from repeated TLD exposure measurements, and individual TLD variability. Axial, sagittal, and coronal microCT images highlighting the location of implanted TLD are shown in Fig. 5.

IV. DISCUSSION

Table I indicates that the bladder receives the lowest dose ($65 \pm 3.0 \text{ mGy}$) and the left upper gastrointestinal system receives the highest dose ($86 \pm 6.0 \text{ mGy}$) during a typical microCT scan. Results indicate that the right and left flanks

FIG. 3. C/kg delivered per radiograph. This graph shows the charge imparted to the field of view isocenter as a function of radiograph acquisition angle. The radiation dose is relatively constant from 0 to ~ 200 deg angle of acquisition, but then decreases from 200 to ~ 350 deg. This reduction is due to the plastic animal bed within the anesthesia module which absorbs some of the x-ray dose and therefore reduces the radiation exposure measured by the CT probe ion chamber.

received a dose of 71 ± 4.0 mGy and 70 ± 3.0 mGy, respectively. Table I demonstrates that the left and right kidneys received a dose of 76 ± 4.9 mGy and 84 ± 4.0 mGy, respectively. Figure 5 illustrates that the mouse limbs were asymmetrical in positioning during the microCT scan acquisition; however, this position difference is not likely to have affected the resulting dose to nearby organs or the overall dose measurement because when one takes into account the intrinsic uncertainty of each TLD measurement, the specific organ dose is uniform across the whole body of the rodent.

If a laboratory has the necessary instrumentation to process TLDs, this dose assessment procedure could prove to be

cost effective. A substantial disadvantage of the methods employed in this investigation could be the requirement of sending the TLDs off-site for external reading and processing if the necessary instrumentation is not available onsite. A limitation of the study, as compared to computational simulation codes, was the inability of TLDs to provide the associated microCT radiation dose to the skeleton and bone marrow. Reproducibility and reliability of the TLDs for dose assessment are dependant on how thoroughly the methodology for annealing and calibration of the TLD batch is followed.

Carlson *et al.*¹⁹ conducted experiments by inserting TLDs intratumorally and in the midabdominal region in a flank

FIG. 4. C/kg delivered as a function of kVp. This graph shows the linear relationship between dose and kVp setting of the x-ray source. As the kVp increases, the dose increases linearly. For all three conditions, the slopes of the lines are very similar confirming that TLD radiation measurements are comparable to ion chamber measurements.

TABLE I. Internal adsorbed doses of different mouse organs. Table I provides the dose measured following a single microCT scan by TLD chips implanted in various organs in a mouse. Radiation dose was measured employing a typical microCT scan protocol. The average absorbed dose over all measurements to the organs is 76 ± 5.0 mGy ($n=3$).

Organ	TLD read out (μC)	TLD exposure (R)	Charge imparted (C/kg)	Dose in air (mGy)	Absorbed dose in water (mGy)
Brain	0.4 ± 0.06	8.8 ± 0.6	0.00226 ± 0.0002	76 ± 5.0	82 ± 5.0
Right flank	0.4 ± 0.05	7.6 ± 0.4	0.00196 ± 0.0001	66 ± 4.0	71 ± 4.0
Left flank	0.4 ± 0.04	7.4 ± 0.3	0.00191 ± 0.0001	65 ± 3.0	70 ± 3.0
Bladder	0.3 ± 0.04	7.0 ± 0.3	0.00179 ± 0.0001	60 ± 3.0	65 ± 3.0
Left kidney	0.4 ± 0.05	8.0 ± 0.5	0.00208 ± 0.0001	70 ± 4.0	76 ± 4.0
Right kidney	0.4 ± 0.04	9.0 ± 0.5	0.00231 ± 0.0001	78 ± 4.0	84 ± 4.0
Left lower GI	0.3 ± 0.03	7.3 ± 0.3	0.00188 ± 0.0001	63 ± 3.0	70 ± 3.0
Right lower GI	0.4 ± 0.04	7.5 ± 0.4	0.00191 ± 0.0001	64 ± 3.0	70 ± 3.0
Left upper GI	0.4 ± 0.06	9.2 ± 0.6	0.00236 ± 0.0002	80 ± 5.0	86 ± 6.0
Right upper GI	0.4 ± 0.03	8.0 ± 0.4	0.00209 ± 0.0001	70 ± 3.0	76 ± 3.0
Stomach	0.3 ± 0.04	7.2 ± 0.3	0.00185 ± 0.0001	62 ± 3.0	67 ± 3.0
Left liver lobe	0.4 ± 0.05	7.5 ± 0.4	0.00194 ± 0.0002	65 ± 4.0	70 ± 4.0
Right liver lobe	0.4 ± 0.03	7.5 ± 0.3	0.00192 ± 0.0001	65 ± 3.0	70 ± 3.0
Left lung	0.4 ± 0.04	7.6 ± 0.3	0.00197 ± 0.0001	66 ± 3.0	71 ± 3.0
LV heart	0.4 ± 0.05	7.5 ± 0.4	0.00194 ± 0.0001	65 ± 3.0	70 ± 4.0
Average	0.4 ± 0.05	8.2 ± 0.5	0.00201 ± 0.0001	71 ± 4.0	76 ± 5.0

xenograft tumor model and reported doses ranging from 87 to 134 mGy when employing a similar microCT imaging protocol operated at 0.26 mA and 80 kVp (60 mA s). However, with the microCT operating at an ultrafast mode with 15 mAs, they achieved tumor doses ranging from 14 to 20 mGy per microCT scan.¹⁹ Obenus and Smith employing a microCT imaging protocol operating at 0.8 mA and 50 kVp (155 mA s) have reported an average whole body dose of 35 mGy as measured by TLDs.¹⁷ Obenus and Smith¹⁷ and Carlson *et al.*¹⁹ dose results are comparable to the results achieved in this investigation.

The microCT mouse TLD dosimetry data demonstrated that a single whole body microCT scan results in an air kerma of 78 ± 5.0 mGy when using a PMMA anesthesia sup-

port module and an air kerma of 92 ± 6.0 mGy without the use of the anesthesia module. The TLD data were validated using an ion chamber with a 3.2 cm^3 CT probe, which measured radiation absorbed doses of 81 ± 4.0 mGy and 97 ± 5.0 mGy with and without the anesthesia module, respectively. Different laboratories have reported microCT radiation doses ranging from 35 to 300 mGy depending on the microCT imaging protocol employed.^{13,16–21} Comparing our findings, we demonstrate an approximately 75% reduced radiation dose to the rodents routinely imaged in our laboratory as compared to the highest doses reported and comparable results to the lowest microCT doses cited in the literature. Even at these lower x-ray doses, we are able to achieve satisfactory count statistics to generate acceptable microCT images.

Taschereau *et al.* used a Monte Carlo simulation code (GATE) to calculate the radiation dose associated to the rodent from microCT imaging operated at 0.5 mA and 70 kVp for a total of 90 mA s and reported an average whole body dose of 93 mGy.²⁰ Taschereau *et al.* concluded that for a typical microCT study, the average whole body dose varies from 80 to 160 mGy at 80 and 50 kVp, respectively.²⁰ Boone *et al.*¹⁶ employing Monte Carlo simulations have defined delivered mice radiation doses of ~ 133 mGy when employing similar microCT imaging parameters (0.5 mA, 80 kVp, and 82 mA s) as the ones employed for this investigation. Rodents, in our laboratory, are routinely imaged longitudinally for up to five sequential microCT scans, and in a number of cases, they are imaged further with other modalities such as microSPECT or microPET. Funk *et al.* have demonstrated through computational simulations that the radiation dose associated with microSPECT or microPET can be substantially higher than the radiation dose delivered by microCT to the

FIG. 5. MicroCT images of implanted TLDs. MicroCT reconstructed images highlighting the precise locations of implanted TLDs. Figure shows (A) a sagittal microCT slice, (B) a coronal microCT slice, and (C) the axial microCT slice corresponding to the intersected lines in (A) and (B).

small animal.²¹ Funk *et al.* estimate that mice whole body dose, associated with microSPECT and microPET imaging, ranges between 60 mGy and 1 Gy, respectively.²¹

MicroCT longitudinal scanning is a screening tool employed frequently for assessing radiopharmaceutical efficacy in preclinical tumor models, however, tumor growth behavior and/or inhibition due to cumulative radiation doses from serial microCT imaging is of considerable interest to scientists.^{13,19–21} Mouse strain, health status, age at exposure, dose delivered, and type of radiation (charged or uncharged particles), among other factors, will define the extent of tissue biological effect when exposed to radiation.^{19,20,23,35–37} In addition, the microCT dose will depend on the mA s employed, imaging time, number of longitudinal scans, desired system resolution, x-ray source settings, total number of projections, and desired image contrast and noise.^{13,19–21,23} Therefore, dose delivered to the rodent will be significantly influenced by the microCT imaging protocol employed. For example, Cavanaugh *et al.*¹³ used a high-resolution respiratory-gated microCT protocol to improve image quality and contrast and reported a delivered rodent radiation dose of 150 mGy per scan, as measured via ion chambers and skin dose monitors, employing a 0.45 mA, 80 kVp, and 81 mA s. In addition, they conclude that by employing this *in vivo* microCT protocol radiation damage is unlikely to occur,¹³ even though they report a radiation dose of approximately twice the dose reported by this investigation (150 mGy vs 78 mGy). To support the Cavanaugh *et al.* conclusion, a single rodent radiation dose of 20 Gy or less to the thorax has been shown to produce minimal histological changes within 3 months postirradiation.⁴⁰ However, Amundson *et al.* have shown that mice do exhibit elevated gene expression for approximately a day after receiving a 200 mGy dose.⁴¹ Therefore, even though large radiation doses (e.g., 20 Gy) do not produce histologic changes in tissue, smaller doses of radiation (e.g., 200 mGy) can induce changes in gene expression. Consequently, although microCT imaging radiation doses should not produce gross tissue histology changes, it could alter gene expression and influence experimental results where the outcome measures are based on gene expression and gene products. Accordingly, it would be prudent to optimize longitudinal microCT imaging to deliver a low, but still sufficient, rodent radiation dose to provide the necessary image quality, and attempt to minimize any untoward effects.¹⁹ Our study suggests that microCT radiation dose cannot be ignored, in view of the fact that a relatively small dose can have a tissue biological impact, and should be considered when designing experiments utilizing microCT imaging.²¹

Our dose results indicate that we are providing low microCT doses and many factors are potentially contributing to this dose reduction as compared to published rodent dose literature (35–300 mGy^{13,16–21}), such as the 0.5 mm equivalent Al intrinsic filtration within the microfocus x-ray source employed. The x-ray spectrum is filtered, even further, with an additional 0.5 mm Al sheet, located external to the x-ray source that will result in further decrease to the low-energy x-ray beam. The internal TLD experimental dose findings

(Table I) of 76 ± 5.0 mGy, are in the low dose range as compared to published literature, however, this behavior could be explained by the fact that many of the soft microCT x rays are attenuated and absorbed by the PMMA anesthesia mouse holder surrounding the rodent during CT acquisition. Low energy x rays are predominantly absorbed in the skin and result in increased skin doses to the rodent. We employ a relatively low x-ray tube exposure, a high tube potential and low currents with added beam filtration, as compared to other microCT imaging protocols reviewed. The combination of these factors could be contributing to a reduction in rodent dose with microCT imaging in our laboratory.

V. CONCLUSIONS

The described methodology demonstrates a simple, precise, and reproducible method for localized tissue dose assessment. This TLD investigation did not require the use of phantoms, hardware, or any software for the determination of the microCT dose. The TLD precision was demonstrated when compared to calibrated clinical standard ion chambers. A 3.7% experimental error was observed when directly comparing the TLD findings with the ion chamber data when both experiments were conducted employing a standard PMMA anesthesia animal module. In summary, we have performed an x-ray dosimetry characterization of a commercial microCT system without the use of commercial phantoms or estimated doses based on computational models. Our results have defined a typical x-ray exposure per routine microCT study which must be taken into consideration when performing longitudinal molecular imaging studies involving microCT analysis.

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