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Accuracy of Off-Line Bioluminescence Imaging to Localize Targets in Preclinical Radiation Research

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Abstract

In this study, we investigated the accuracy of using off-line bioluminescence imaging (BLI) and tomography (BLT) to guide irradiation of small soft tissue targets on a small animal radiation research platform (SARRP) with on-board cone beam CT (CBCT) capability. A small glass bulb containing BL cells was implanted as a BL source in the abdomen of 11 mouse carcasses. Bioluminescence imaging and tomography were acquired for each carcass. Six carcasses were setup visually without immobilization and 5 were restrained in position with tape. All carcasses were setup in treatment position on the SARRP where the centroid position of the bulb on CBCT was taken as “truth”. In the 2D visual setup, the carcass was setup by aligning the point of brightest luminescence with the vertical beam axis. In the CBCT assisted setup, the pose of the carcass on CBCT was aligned with that on the 2D BL image for setup. For both 2D setup methods, the offset of the bulb centroid on CBCT from the vertical beam axis was measured. In the BLT-CBCT fusion method, the 3D torso on BLT and CBCT was registered and the 3D offset of the respective source centroids was calculated. The setup results were independent of the carcass being immobilized or not due to the onset of rigor mortis. The 2D offset of the perceived BL source position from the CBCT bulb position was $2.3 \text{ mm} \pm 1.3 \text{ mm}$. The 3D offset between BLT and CBCT was $1.5 \text{ mm} \pm 0.9 \text{ mm}$. Given the rigidity of the carcasses, the setup results represent the *best* that can be achieved with off-line 2D BLI and 3D BLT. The setup uncertainty would require the use of undesirably large margin of 4–5 mm. The results compel the implementation of on-board BLT capability on the SARRP to eliminate setup error and to improve BLT accuracy.

INTRODUCTION

Preclinical methods of delivering focal radiation to biological targets have made significant advances in recent years with the construction of a number of instruments specifically designed for this purpose. Our group has constructed and employed a device, the Small Animal Radiation Research Platform (SARRP) (1–3), capable of using X-ray cone-beam CT (CBCT) to guide highly conformal radiation beams with great accuracy. The SARRP is able to identify and irradiate substructures of the brain (4, 5), skeleton (6) and other regions (7) of the mouse and rat anatomy. However, with a nonlethal imaging dose, X-ray CBCT is deficient in localizing small sub-palpable (less than 5 mm) orthotopic soft-tissue tumors of pancreatic, liver and prostate cancer, among other regions of great research interest. As an alternative solution, the SARRP imports off-line data from other preclinical imaging modalities, such as MR, PET and optical imaging that are more sensitive for detecting different attributes of the tumor. Optical imaging, in particular bioluminescent imaging (BLI), is attractive for its affordability, accessibility and its sensitivity in detecting small pre-palpable orthotopic tumors in animal models. Off-line image guidance, however, is associated with inherent setup uncertainty because the procedure requires the transport and repositioning of the animal between the imaging and irradiation sessions. In this study, we evaluate the setup accuracy of using off-line BLI as a means to guide focal irradiation of small tumor volumes.

METHODS AND MATERIALS

SARRP and CBCT Imaging

The SARRP is capable of delivering radiation to a predetermined volume accurately under CBCT guidance. It consists of a dual-focus X-ray source mounted on a gantry that can rotate 120°, from 0° at vertical to 30° below the horizon. To facilitate tumor localization and targeting, the SARRP is equipped with a 20 × 20 cm digital X-ray flat panel detector mounted opposite the X-ray source when in the horizontal position. Projection images are acquired of the imaging subject as it is continuously rotated 360° on a horizontal platform between the detector and X-ray source in their fixed positions. In this study, the SARRP was employed solely for imaging with CBCT using a 65 kVp, 1.0 mA X-ray beam with 1 mm aluminum filtration. One projection image was acquired per degree of rotation, yielding 360 projection images for CBCT reconstructions. CBCT volumes were reconstructed with a 0.2 mm isotropic voxel size. The system employs the XVI CT navigation software from the Netherlands Cancer Institute (8). The XVI software facilitates image-guided radiation procedures as well as multimodality (MRI, CT, PET, etc.) image fusion.

Bioluminescent Source Implantation

The accuracy with which off-line BLI can be used to localize targets for irradiation was evaluated using a sealed bioluminescent glass bulb source implanted into the abdomen of a mouse carcass to mimic an orthotopic pancreatic tumor model. Athymic nude mice (4 weeks old; Harlan Laboratories, Madison, WI) were used in accordance with institutional guidelines under institutionally approved protocols. Prior to luminescent source implantation and imaging, mice were humanely euthanized by CO₂ inhalation per institutional protocol.

The bioluminescent source was based on a MiaPaCa-2 human pancreatic carcinoma cell line stably transfected with the luciferase aminoglycoside phosphotransferase fusion gene under the control of the elongation factor-1 α promoter. The cell line was kindly provided by Dr. Ralph Graeser, ProQinase GMBH, Freiburg, Germany (9). MiaPaCa-2 cells were routinely cultured and expanded in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin. MiaPaCa-2 cell pellets were generated by adapting an established protocol (10). Pellets were maintained in serum-free culture medium at 37°C for 4 days. For bioluminescence localization experiments, culture medium was aspirated, pellets were gently washed in phosphate-buffered saline and completely resuspended into a 70 μ L cell solution with Luciferin-D (30 mg/ml; Gold Biotechnology, St. Louis, MO). This cell suspension was injected into a spherical glass bulb of 4.4 mm inner diameter (5 mm outer diameter), sealed and sutured in place under the left lobe of the carcass liver to a depth between 5–10 mm from the anterior abdominal surface so as to minimize any motion of the bulb. Given that the volumetric capacity of the bulbs was predetermined, they were completely filled with the cell suspension. A concerted effort was made to make sure the cells were suspended uniformly. Each carcass was then imaged for bioluminescent signal. The entire implantation and imaging procedure took about 10 min and was repeated one carcass at a time.

Animal Positioning and Transport

Using an “off-line” procedure, the carcasses underwent BLI and were subsequently transported and repositioned on the SARRP for CBCT imaging. Two methods of animal repositioning were employed during this process in which the animals were either unrestrained or restrained. Each method of setup was carried out separately. In the unrestrained setup of six carcasses, we simulated a scenario where the animal was transported and repositioned freehand for CBCT after BLI. In the restrained setup of 5 carcasses, we simulated a scenario where an animal support jig was used for both BLI and CBCT in an attempt to minimize repositioning error. To accomplish this, all 4 limbs of the mouse carcass were extended and taped down using Micropore™ tape (3M, St. Paul, MN) on a rigid cardboard sheet covered in black construction paper to minimize spurious optical signals from the cardboard itself. The entire assembly of the taped carcass was transported intact between the imaging and irradiation session, representing an idealized condition of minimal repositioning error in animal transport.

Bioluminescent Imaging and Localization

Two-dimensional (2D) BLI and 3D bioluminescent tomography (BLT) were acquired of each mouse carcass using the IVIS Spectrum Imaging System (Caliper LS, Hopkinton, MA). Carcasses were positioned individually on the imaging platform. In both unrestrained and restrained setups, carcasses were imaged in the supine position. In each case, the 2D BLI was performed first followed immediately by 3D BLT, with no interval perturbation of the carcass. The Diffused Luminescent Imaging Tomography (DLIT, Caliper LS) algorithm was available on the IVIS Spectrum for 3D BLT reconstruction, which produces the 3D location of the luminescence source within the 3D surface of the study animal. After BLI, the carcasses were transported to the SARRP and similarly positioned for CBCT. For each carcass, about 15 min were required for source implantation and BLI. An additional 15 min

were required to transport the carcasses from the facility housing the BLI to that housing the SARRP. After repositioning, X-ray CBCT was acquired for each carcass to determine the “true” 3D position of the implanted bulb for comparison with the setup using BLI or BLT (Fig. 1).

Optimizing Image Guidance

Visual (2D) setup—Three methods of image guidance were applied in an attempt to optimally position the implanted source in each of the unrestrained and restrained carcasses on the SARRP. Using the 2D BLI as a reference guide, the brightest point of visually observed bioluminescent signal intensity was marked on the corresponding area of the animal’s skin using a black marking pen (Fisher Scientific, Waltham, MA). Each carcass was then transported and repositioned on the SARRP using laser crosshairs with the skin mark centered at the system isocenter, which also represented the vertical beam axis. The procedure mimicked one that would be employed using an irradiator without on-board X-ray projection or CBCT imaging capabilities, such as are available on the SARRP. Once the carcass was in position, CBCT was obtained. The XVI navigation software was used to measure the offset on the X-Y horizontal plane between the center of the glass bulb on 3D CBCT and the central axis of the vertical beam (Fig. 2).

BLI-CBCT (2D) fusion—The second method of image guidance compared the actual 2D bioluminescent and CBCT images to enhance setup of the carcasses. Using the image fusion utility of the XVI navigation software, the coronal bioluminescent surface image without skin mark was imported and laid over the 2D silhouette of a corresponding coronal slice of the CBCT image (Fig. 3). All fusions were manually performed by a single user and special attention was paid to align the two image sets based on the outline of the carcass, ignoring the region containing the bioluminescent signal and glass bulb. After alignment, the SARRP vertical beam axis was centered to the point of highest bioluminescent signal intensity. The X-Y horizontal distance to the center of the glass bulb on coronal CBCT was then measured. This alignment method mimicked the present availability of a stand alone 2D BLI system and a SARRP-like irradiator with on-board CBCT, where the latter might be used to improve reproducibility of carcass position for irradiation.

BLT-CBCT (3D) fusion—The final method of image guidance mimicked a scenario where the carcass was setup directly based on the 3D BL source position estimated by the BLT utility available on the IVIS Spectrum system, which provides both 3D animal surface information and internal 3D location of the BL source. The center of mass of the BL source in 3D was then compared with that of the glass bulb as determined by X-ray CBCT. As a common reference, the 3D outline of the carcass torso was registered with the torso as determined by CBCT (Fig. 4). The resultant offset in the 3D position of the BL source and that of the glass bulb was determined. The comparison thus evaluates the accuracy of the source reconstruction using BLT, assuming that the bulb position in X-ray CBCT is the “truth,” albeit given some uncertainty in the uniform distribution of the injected BL cells. It is noteworthy that this 3D comparison would yield the radial distance between centroids identified using BLT and CBCT. The offset in the horizontal X-Y plane was also calculated by simply removing the depth (Z) component for fair comparison with the previous two 2D

methods, as the latter did not include depth information of the BL source. The positioning uncertainty for a 2D setup would apply for irradiation with a single vertical beam, insensitive to the lack of depth information.

RESULTS

Results for the different methods of image guidance and carcass positioning are shown in Table 1. In the visual method, the 2D BL image was used to estimate the location of the glass bead source on the skin surface of the mouse carcass. The mean 2D offset between the isocenter of the associated vertical beam and the centroid of the bulb was $2.2 \text{ mm} \pm 1.2 \text{ mm}$ for the 6 unrestrained carcasses. In the separate run of 5 restrained carcasses, the mean offset was measured to be $2.5 \text{ mm} \pm 1.5 \text{ mm}$. The results for restrained and unrestrained carcasses were not statistically different.

In the 2D BLI-CBCT setup, the central axis of the vertical beam was arranged on the position of highest bioluminescent signal intensity on the carcass torso after fusion of the coronal 2D BL image to the corresponding coronal CBCT image. The resulting mean 2D offset distance in a single plane was measured to be $2.2 \text{ mm} \pm 1.2 \text{ mm}$, which was not significantly different compared to the offset measured using the visual setup method. Restraining the mice led to a similar mean offset of $2.4 \text{ mm} \pm 1.1 \text{ mm}$.

Finally, BLT images were fused with those of the 3D CBCT. The offset between the center of the mass of the BL source and the CBCT glass bead image was calculated to be $1.5 \text{ mm} \pm 0.9 \text{ mm}$, which represented a 3D radial distance measurement. When carcasses were restrained and all other conditions were maintained, the offset was $1.6 \text{ mm} \pm 0.8 \text{ mm}$, where the difference from the unrestrained results was again not significant. To allow for appropriate comparison to 2D methods of image guidance and localization, the depth (z) coordinate was removed and the 2D offset in the horizontal X-Y plane was calculated. For the unrestrained and restrained carcasses, the mean 2D offsets were $0.8 \text{ mm} \pm 0.5 \text{ mm}$ and $1.0 \text{ mm} \pm 0.6 \text{ mm}$, respectively.

DISCUSSION

Using the centroid of the bulb on CBCT as the “truth”, the offsets in the setup of the carcasses using the visual method were virtually identical to those using the 2D BLI-CBCT method, regardless of whether or not restraint was applied. The seemingly surprising results were most likely due to the onset of rigor mortis during the entire procedure that lasted over 60 min. It was observed that by the time CBCT was acquired, all carcasses had stiffened. The carcasses were thus representative of rigid phantoms, rendering minimal difference between the restrained and unrestrained setups. For the same reason, there would be minimal difference between a visual setup and one that is augmented with CBCT information, as observed. With the idealized carcass geometry, our setup results using 3D BLT are slightly larger than the average 1 mm uncertainty reported by Virostko *et al.* (11) on their CT validation of the reconstructed BLT position of an implanted small light source in mice. Our larger deviation may be due to the nonuniform distribution of BL cells in our larger bulbs, which were used to mimic a 3–5 mm orthotopic bioluminescent tumor.

Our results suggest that setup uncertainty using off-line BLI with animal restraint will be relatively large. The setup uncertainty of a rigid carcass was no better than 2.5 mm. Adopting the recipe of van Herk (12) to calculate a target margin expansion that ensures adequate target coverage, the diameter of the radiation aperture required to cover a small 3 mm orthotopic tumor would need to be more than 15 mm. With 3D BLT reconstruction, the uncertainty in 2D target localization is reduced to about 1 mm and allows the use of a smaller beam aperture of about 10 mm. It should be noted that these estimations are only appropriate for the 2D setup, which does not include depth information. For example, the calculated aperture is only valid for a vertical beam. When beams from multiple angles are employed for more advanced focal irradiation, the target position in 3D is required. The BLT algorithm from the IVIS Spectrum system offers such utility. Our results show that the 3D localization uncertainty is about $1.5 \text{ mm} \pm 1 \text{ mm}$, reflecting the additional and larger uncertainty in the depth direction. For a spherical target of 3 mm diameter, an aperture of about 12 mm would be required and can be delivered from any 3D orientation.

The beam aperture required to adequately cover a small 3 mm diameter internal target would be about half the torso diameter of the mouse and is disappointingly large. Unfortunately, our results indicate that this may be the *best* that can be achieved using present methods of 2D surface BLI and 3D BLT for off-line localization of a soft tissue target. Our idealized rigid carcass model minimized the potentially large repositioning error of a deformable live animal. Correspondingly, the results are suggestive of the setup accuracy that can be achieved on the SARRP equipped with on-board BLI guidance capabilities, where the anesthetized animal would be stationary throughout the entire procedure of CBCT and BL imaging and irradiation. Indeed, BLT on-board the SARRP would be most desirable as it completely avoids any transport and repositioning error, as well as the additional uncertainty associated with registration of the CBCT with the off-line BLI or BLT information. Furthermore, with anatomic information (or priors) available from X-ray CBCT, the opportunity exists to significantly improve the accuracy of BLT. Naser *et al.* have shown recently that for a phantom with an optical inhomogeneity, the incorporation of anatomic and optical priors, as well as additional BLI information from more than a single view of the IVIS Spectrum system, would enable BLT localization of an internal light source to well within 1 mm (13). This level of localization accuracy would rival that of the micro-PET system.

Given the challenges of soft tissue target localization, integrating on-board X-ray CBCT-BLT to guide focal irradiation represents the next milestone for the SARRP technology. Figure 5 shows a CAD drawing of our current effort on such integration. In this design, BL images from the mouse are directed to a CCD camera via a 45° mirror. The mirror and camera subsystem are attached to the gantry of the SARRP and can be rotated 90° . In combination with the rotation support stage of the SARRP, BLI can be obtained over a 2π hemisphere. Focal irradiation will be directed through the mirror with Beam's eye view perspective from BLI. We are hopeful that the new CBCT-BLI capabilities will achieve sub-mm localization and allow halving the current 3D margin expansion of 5 mm or so using off-line BLI methods.

In summary, we have investigated the utility of off-line BLI to help guide focal irradiation of small soft tissue targets in a low contrast environment. Our study using a mouse carcass model shows that the setup uncertainty using current off-line BL imaging methods would require the use of an undesirably large beam aperture to ensure adequate coverage of the soft tissue target. Our results compel the integration of BLT and X-ray CBCT capabilities on-board the SARRP or similar systems to attain focal irradiation of small soft tissue targets.

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FIG. 1. Implant procedure and materials used. A glass bulb (panel A) containing luciferase-expressing MiaPaCa-2 cells was surgically implanted under the left lobe of the liver of a nude mouse carcass (panel B). Bioluminescent imaging was accomplished using the IVIS Spectrum (not pictured) and subsequent CBCT images were acquired on the SARRP (panel C), which employs a novel rotating stage CBCT geometry.

FIG. 2.

Visual setup. Panel A: Coronal BL imaging of mouse carcass with implanted BL bulb with red indicating the most intense signal. An ink marking (circled) was made to visually correspond with the signal seen on the BL image (panel B). The mouse was then positioned on the SARRP stage utilizing mounted guidance lasers to align to the skin mark (panel B). The lasers intersect at the beam's isocenter. Coronal CBCT image indicated the true position of the glass bulb (panel C). The offset between the localization methods was measured and recorded.

FIG. 3. BLI-CBCT (2D) fusion. Coronal external animal contours of 2D BLI (panel A) and a CBCT image (panel B) were aligned using a composite fusion (panel C). Magnified inset of panel C shows the localization of the BLI signal (open arrow) and the glass bulb identified on CBCT (closed arrow).

FIG. 4. BLT-CBCT (3D) fusion. Coronal, sagittal and axial imaging performed using CBCT (panel A) and BLT (panel B) were fused and aligned using external anatomy, as well as the glass bulb and corresponding BL signal (panel C). Magnified insets show the highest intensity BL signal (closed arrow) relative to the glass bead contour.

FIG. 5.

A CAD drawing of a BLI/BLT imaging system mounted on-board the SARRP. Images from the mouse are directed to a CCD camera by a 45° mirror. Collimated focal radiation will be directed through the mirror (panel A). The mirror and camera subsystem can be rotated 90° with the SARRP gantry. In combination with the rotation support stage of the SARRP, BLI can be obtained over a 2π hemisphere.

TABLE 1

Image Guidance Offsets with Various Alignment Methods

	Visual fusion (mm)	BLI-CBCT fusion (mm)	BLT-CBCT fusion (mm)	
			3D	2D
Unrestrained carcass	2.2 ± 1.2	2.2 ± 1.2	1.5 ± 0.9	0.8 ± 0.5
Restrained carcass	2.5 ± 1.5	2.4 ± 1.1	1.6 ± 0.8	1.0 ± 0.6