Purpose: Cone beam CT (CBCT) on-board new small animal radiation research platforms (SARRPs) is inadequate for guiding focal irradiation of targets in soft tissue. In this study, we examined the setup uncertainty using the more sensitive bioluminescence imaging (BLI) and BL tomography (BLT) for off-line guided irradiation.

Methods: A small glass bulb containing BL cells was implanted in six euthanized mice These were handled free-hand while five others were immobilized. Surface BLI was acquired and BLT reconstructed of each carcass using a Caliper BLI system. An ink-dot marked the "visual" centroid of the surface BL image. The carcasses were then transported to a SARRP for CBCT localization. In the visual setup, the animal was positioned to align the ink-dot with the central axis of the SARRP. In the CBCT-aided setup, the pose of each carcass was adjusted to recapitulate its pose at the time of BLI. The 2D radial offset of the "true" bulb centroid in CBCT from the central axis of the SARRP was measured. In the 3D setup, the 3D contours of CBCT and BLT contours were registered and the 3D radial offset between the 2 centroids was calculated.

Results: The results are virtually identical between free-hand and immobilized setup, as well as using visual and CBCT-aided 2D setup. The onset of rigor mortis rendered immobilization un-necessary. The 2D offset from the "true" position of the BL bulb was 2.3 + -1.3 mm. The 3D radial offset was 1.5 + -0.9 mm, approaching the documented uncertainty in BLT reconstruction.

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