

**Purpose:** To treat small animal disease models with clinically-relevant radiotherapy strategies, an image-guided 3D conformal radiotherapy system based on a microCT imaging device has been constructed. **Method and Materials:** A variable-aperture collimator was installed in a GE RS120 microCT, so that the beam width could be adjusted between 0 to 100 mm at the CT isocenter. The collimator was aligned with the X-ray beam axis through film and detector measurements. A two-dimensional translation stage was integrated with the existing z-stage of the scanner to allow 3D positioning of a subject within the CT bore. Radiation treatment planning software is based on the RT\_Image application, including tools to specify treatment parameters based on the CT image and an EGSnrc-based Monte Carlo dose calculation package. To test this system, two mice with spontaneous lung tumors have been irradiated to a dose of 2 Gy. **Results:** Automation of the system has been realized through high-precision mechanical design and assembling of the collimator and stage, the calibration, and the control software. Geometric and dosimetric calibration of the system has resulted in beam width and position precisions better than 0.1 mm, and dose precision better than 3%. The treatment planning software development can specify treatment parameters within the coordinate frame of the CT image, which can then be used to control the device hardware. Mice with lung tumors were irradiated with the system. Post-mortem immunohistochemical assays demonstrated the presence of DNA double strand breaks within the radiation target. **Conclusion:** We have demonstrated the accuracy and utility of a novel microCT-based image-guided 3D conformal radiotherapy system. Clinically-similar image-guided radiation therapy has been applied to small animals, facilitating future investigations of this technology in the laboratory.