

Purpose: We commissioned a novel microCT-based kilovoltage 3D conformal radiotherapy system. A two-stage, variable-aperture collimator has been installed between the X-ray source and the CT isocenter to confine the X-ray beam, so that the system can be used for both imaging and treatment. Commissioning included alignment of the axes of the collimator and the X-ray beam, measurement of the beam penumbra, measurement of the X-ray beam dose rate in water, and measurement of the effective aperture size at isocenter. **Method and Materials:** Images projected to gafchromic films placed at the isocenter and the microCT detector were analyzed by software developed for this commissioning, which provided quick and precise guidance for mechanical and control-software adjustments. The penumbra was measured by fitting the beam profile to piece-wise linear functions. The collimator-beam alignment, the aperture ratio of the two stages, and the absolute aperture calibration were measured with known image pixel sizes. The attenuation was measured by comparing the signals in the shielded and the exposed areas in the images. Measurement of the dose rate in water was accomplished using solid water phantoms and film, calibrated using parallel ion chamber measurements. **Results:** The measured penumbra width was 0.5 mm, dictated primarily by the finite x-ray source spot size. Alignment of the collimator and X-ray beam alignment was achieved within 0.1 mm. The beam width precision was less than 0.05 mm, guaranteed by the stage-aperture ratio and absolute beam-width calibration. The measured attenuation was better than 99.85%. **Conclusion:** The 3D-conformal-animal-radiation-system commissioning has achieved the design goals that ensure the precise delivery of x-ray beam to deep-seated targets in experimental animals. The techniques developed for the commissioning also provided reliable methods for future system quality assurance.