

Purpose: Investigating the advantage of in-room PET imaging for *in-vivo* range verification in proton therapy in comparison with an off-line protocol. Considering in-room data acquisition, the signal from short-lived ^{15}O ($t_{1/2}=2.03$ min) isotopes, which are dominant in soft tissues, can be measured and ^{11}C ($t_{1/2}=20.33$ min) signal is increased. Furthermore, the movement of the patient during the PET scanning as well as the biological washout is minimized. **Method and Materials:** Two patients were scanned with a mobile PET scanner designed for neurological studies, NeuroPET, 2-3 minutes after the irradiation of a single field fraction (2Gy(RBE)) with passive scattering proton therapy. For each patient, two PET scans were acquired after two treatment sessions ~1 week apart, with the same treatment plan. The scanner was positioned into the treatment room and for the PET scan the patient remained on the treatment couch for the PET scan, which had to be rotated and shifted from the treatment position. Measured and calculated (GEANT4 simulation of the nozzle and patient delivery) PET images were compared to evaluate the range verification accuracy. **Results:** A very similar number of coincidences were obtained for the in-room (2 min delay, 5 min PET scan) and off-line (17 min delay, 30 min scan) measurements. A ten-fold increase of the count rate at the beginning of the acquisition on in-room measurements has been observed due to the contribution of the ^{15}O . A mean difference up to 5 mm has been observed between falloff positions of in-room and off-line images. **Conclusions:** We have identified an optimal timeframe for compromising signal strength versus noise. Experimental and calculational uncertainties are being reduced for future patient studies by means of a better co-registration accuracy, attenuation correction, washout model, and conversion from CT numbers into tissue materials.