MATERIALS AND METHODS

Calibration of PET/MRI Scanner Images

PET/MRI scanner images of syringe phantoms were decay corrected and normalized for scan duration prior to analysis. The first linearity phantom image acquired during a given study session was used to convert image intensities to units of absolute activity concentration (kBq/mL). Rod image intensities from the PET/MRI image, obtained from cylindrical volumes of interest axially centered on each rod (diameter = 40% of the rod diameter, length = 8.3 mm), were plotted against activity concentrations measured by well counting of samples taken from the phantom. Plots were fitted by linear regression, taking into account the random variability of both the measured independent and dependent variables (J). The resulting regression coefficients were used to calculate activity concentrations for all images acquired during the corresponding study session. This calibration procedure incorporates phantom-specific compensation for attenuation, scatter and partial volume effects into the calibration coefficients.

Cross-comparison of PET/MRI Scanner and microPET Linearity with Autoradiography and Direct Assay

To enable quantitative comparison of images across PET and QAR systems, it was necessary to ensure that all three systems had a linear response. Two phantoms were used for this purpose. A linearity phantom was made by solidifying a 10% gelatin solution (solid state density = 1.01 g/mL) in a cylindrical mold made from a 50 mL Falcon tube (BD). While the gel was liquid, 4 rods, each 4 mm in diameter, were evenly spaced in the mold. Once the gel had set, the rods were removed, and the holes were filled with gelatin mixed with $^{64}$CuCl$_2$ solution (Washington University School of Medicine) containing Prohance (Bracco; 0.1 mM) and Isovue 300 (Bracco; 1mM). Initial mixture activity concentrations were 3.7 MBq/mL, 1.9 MBq/mL, 0.93 MBq/mL and 0.56 MBq/mL. The 12.7 h half-life of $^{64}$Cu enabled the phantom to be imaged on the same day with the PET/MRI scanner, commercial small-animal PET and QAR. A second “hot-rod” gelatin phantom was made in the same manner as the linearity phantom, except that the 4 rods each contained an initial activity concentration of 1.9 MBq/mL. The rods were surrounded with gelatin containing 0.19 MBq/mL of $^{64}$Cu.

Phantoms were cut to 15 mm length to remove gelatin spillover at the ends. The removed sections of the phantom were sampled with a biopsy punch (inner diameter = 2 mm, Miltex), and
the samples were placed in pre-tared Eppendorf tubes for gamma counting. Each region of
interest (background and rod regions) was sampled twice. The phantoms were imaged
simultaneously with PET/MRI (PET: 2700 s; MRI: FLASH TR/TE = 500/4ms, FOV: 35.4×35.4
mm³, slice thickness = 0.75 mm² matrix size = 128×128, 40 slices). Immediately after imaging,
the phantoms were placed in a dry ice and isopropyl alcohol bath for 5 minutes. The phantoms
were subsequently imaged on a microPET R4 (Concorde Microsystems) (2) for 20 minutes (350-
650 keV energy window, 6 ns timing window).

After microPET imaging, the phantoms were cut in half. One half was sampled for gamma
counting, while the other half was mounted onto a cryomicrotome (Bright 5030/WD/MR, Hacker
Instruments). Five 50μm-thick frozen transaxial sections, spaced 250μm apart, were obtained
and transferred onto a chilled autoradiography cassette containing a storage phosphor screen
(Super Resolution Screen, PerkinElmer). The loaded cassette was stored for 1 day at –20°C.
Screens were read with a laser scanner (Packard Cyclone). Tubes containing phantom samples
were assayed for radioactivity using a calibrated gamma counter (Model 1480 Wizard 3°, Wallac
Oy). Sampling of the phantom was done at each stage of the imaging procedure (PET/MRI
imaging, microPET R4 imaging and autoradiography) to account for diffusion of the
radioactivity and contrast agent as evident in Supplemental Figure 1.

PET images were reconstructed and analyzed as described in the Materials and Methods section
of the main manuscript. Circular ROIs (diameter = 80% of rod diameter) were used for rod
analyses on autoradiography images. Background activity was analyzed with ROIs (diameter =
5.3 mm) placed at five different locations per slice. Although variable slice thickness is a
potential source of error in QAR, it was found to be negligible in our study. The CoVs of activity
concentrations measured across several slices of the gelatin hot-rod phantom (background = 0.19
MBq/mL, rods = 1.9 MBq/mL) were 3.0% and 3.2% for background and hot-rod regions
respectively. These values are lower than observed by Christian et al. (3.5%) after adjustment
for differences in slice thickness (3).

Rod and background intensity values from gelatin phantom images were plotted against activity
concentrations measured by direct weighing and gamma counting of relevant samples. Plots
were fitted by linear regression. Pearson correlation coefficients compared to gamma counting
were also calculated.
SUPPLEMENTAL FIGURE 1

Phantoms used in this study. (A) Schematic of the linearity and hot rod phantoms (syringe and gelatin) used in the study. (B-I) Images of gelatin linearity phantoms acquired across modalities. Blue food dye was used to visualize rods (B,F), while MR (Prohance) and CT (Isovue 300) visible contrast agents were mixed with radioactivity to enable visualization in MRI and CT respectively (C,G). A small crack is evident in a frozen test phantom prepared for CT imaging (G); this phantom was not used in the studies. The physical properties of the gelatin phantom (E) enabled sampling for gamma counting as well as thin, frozen sectioning for autoradiography (I). The orange box in (E) outlines a rod that was sampled with a biopsy punch and transferred for weighing and gamma counting.
SUPPLEMENTAL FIGURE 2

Linearity of PET/MR scanner, microPET R4 and autoradiography. Image intensities acquired from the same gelatin-based linearity and hot rod phantoms were plotted against direct assay measurements for (A) PET/MR scanner, (B) microPET R4 and (C) autoradiography. Linear regression was performed for each data set. Pearson correlation coefficients are shown for each data set. Software processing revealed that the linearity phantom rod with the highest activity was over-exposed on the autoradiography phosphor imaging plate (the * data point in C). That data point was excluded from further analysis. (a.u. = arbitrary units)
SUPPLEMENTAL FIGURE 3

Linearity and stability of the PET/MRI scanner across multiple imaging sessions. Measurements were made on syringe “rod” phantoms described in the Materials and Methods section of the main manuscript. (A) Linearity phantom rod image intensities from 3 separate imaging sessions over the course of 10 days are plotted against actual activity concentrations. Fitted linear regression lines used to determine the calibration coefficients are also shown. (B) Slope and intercept calibration coefficients (mean and s.d.) across the 3 sessions. CoVs for the slope and intercept were 6.8% and 11% respectively.
SUPPLEMENTAL FIGURE 4

Scatter plot of simultaneous diffusion-MRI and PET antibody measurements at two different times after injection of $^{64}$Cu-labeled antibody. Quantitative pixel values from the tumor ROIs shown in Figure 5 of the main manuscript show low correlation between antibody uptake and ADC values ($r = 0.21$ and -0.02 at 4 and 20 hours, respectively).
REFERENCES


### SUPPLEMENTAL TABLE 1: Image similarity metrics used to compare PET image quality with autoradiography

<table>
<thead>
<tr>
<th>Metric Name</th>
<th>Equation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Correlation coefficient</td>
<td></td>
<td>Voxel by voxel correlation between two regions of interest. $x$, $y$ refer to the voxel value for the first and second image respectively, $\mu$ and $\sigma$ refer to the mean and standard deviation of the voxel values in images $X$ and $Y$. $E$ denotes expectation value.</td>
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<tr>
<td>Peak signal to noise ratio (PSNR)</td>
<td>$10 \log_{10} \frac{\text{Max voxel value}_i \times \text{Max voxel value}_j}{\text{Mean voxel difference}_j}$</td>
<td>Voxel by voxel measure of SNR within a ROI between images $i$ and $j$ (4).</td>
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<td>Structural similarity index (SSIM)</td>
<td>$\text{SSIM}(x,y) = l(x,y)\alpha c(x,y)\beta s(x,y)\gamma$</td>
<td>Similarity index comparing local image structure. $l$, $c$, $s$ refer to the luminance, contrast and structure of the image, respectively. $\alpha$, $\beta$, $\gamma$ are adjustable parameters. (Default settings from (4) were used.) See reference for a detailed description.</td>
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<tr>
<td>Complex wavelet structural similarity Index (CWSSIM)</td>
<td>Same as SSIM, but with wavelet analysis incorporated (5).</td>
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