



## **King's Research Portal**

DOI: 10.3389/fphy.2020.00126

Document Version Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA):

Blower, J. E., Bordoloi, J. K., Rigby, A., Farleigh, M., Kim, J., O'Brien, H., Jackson, J., Poyiatzis, C., Bezer, J., Sunassee, K., Blower, P. J., & Livieratos, L. (2020). Protocols for Dual Tracer PET/SPECT Preclinical Imaging. *Frontiers in Physics*, *8*, Article 126. https://doi.org/10.3389/fphy.2020.00126

#### Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

#### General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

•Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research. •You may not further distribute the material or use it for any profit-making activity or commercial gain •You may freely distribute the URL identifying the publication in the Research Portal

#### Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

## Protocols for dual tracer PET/SPECT preclinical imaging

J. E. Blower, J. K. Bordoloi, A. Rigby, M. Farleigh, J. Kim, H. O'Brien, J. Jackson, C. Poyiatzis, J. Bezer, K. Sunassee, P. J. Blower, L. Livieratos\*

School of Biomedical Engineering and Imaging Sciences, King's College London, 4<sup>th</sup> Floor
 Lambeth Wing, St Thomas' Hospital, London SE1 7EH, UK

#### 8 9 \*Correspondence

## 10 Lefteris Livieratos

11 Lefteris.livieratos@kcl.ac.uk

12

15

1 2

3

4 5

# Keywords: SPECT, PET, radionuclide, phantom, multi-modality, dual-radionuclide, dead-time, scatter

#### 16 Abstract

Background: Multi-tracer PET/SPECT imaging enables different modality tracers to be present 17 simultaneously, allowing multiple physiological processes to be imaged in the same subject, 18 within a short time-frame. Fluorine-18 and technetium-99m, two commonly used PET and 19 SPECT radionuclides respectively, possess different emission profiles, offering the potential 20 21 for imaging one in the presence of the other. However, the impact of the presence of each radionuclide on scanning the other could be significant and lead to confounding results. Here 22 23 we use combinations of <sup>18</sup>F and <sup>99m</sup>Tc to explore the challenges posed by dual tracer 24 PET/SPECT imaging, and investigate potential practical ways to overcome them.

Methods: Mixed-radionuclide <sup>18</sup>F/<sup>99m</sup>Tc phantom PET and SPECT imaging experiments were carried out to determine the crossover effects of each radionuclide on the scans using Mediso nanoScan PET/CT and SPECT/CT small animal scanners.

Results: PET scan image quality and quantification were adversely affected by <sup>99m</sup>Tc activities
 higher than 100 MBq due to a high singles rate increasing dead-time of the detectors. Below
 100 MBq <sup>99m</sup>Tc, PET scanner quantification accuracy was preserved. SPECT scan image
 quality and quantification were adversely affected by the presence of <sup>18</sup>F due to Compton
 scattering of 511 keV photons leading to over-estimation of <sup>99m</sup>Tc activity and increased noise.
 However, <sup>99m</sup>Tc: <sup>18</sup>F activity ratios of > 70:1 were found to mitigate this effect completely on
 the SPECT. A method for correcting for Compton scatter was also explored.

35 Conclusion: Suitable combinations of injection sequence and imaging sequence can be devised 36 to meet specific experimental multi-tracer imaging needs, with only minor or insignificant 37 effects of each radionuclide on the scan of the other.

### 39

38

Introduction 40 Individually, PET and SPECT tracers allow us to probe the underlying molecular 41 42 characteristics of physiological processes, one mechanism at a time. The ability to image one tracer in the presence of another - dual radionuclide PET/SPECT imaging - enables different 43 modality tracers to be present simultaneously, thus allowing multiple processes to be imaged 44 in the same subject, within a much shorter period of time (removing the need to wait for tracer 45 decay). For example, radionuclides fluorine-18 (PET) and technetium-99m (SPECT) each 46 possess different emission profiles and, in theory, can be imaged in the presence of the other, 47 48 but the impact of each on scanning of the other (i.e. the SPECT and PET scans respectively),

49 could be significant and lead to confounding results.

Acquiring a PET image in the presence of a SPECT radionuclide may introduce additional 50 dead-time (the time after each photon is detected by the scanner during which the system is not 51 able to record another event). Photons emitted from decaying 99mTc nuclei are not coincident, 52 and their energy of 140 keV is much lower than the 511 keV PET scanner energy window, so 53 do not contribute to the image data acquired. However, they do interact with the PET detectors, 54 and at high enough flux, can potentially prevent true coincidence events from the positron 55 emitter being recorded. One study in mice showed PET signal loss of 12% due to increased 56 57 dead-time when 99mTc was present in an almost 10-fold higher activity compared to <sup>18</sup>F (1). It 58 is worth noting that this phenomenon is not specific to mixed radionuclide effects, and deadtime is generally recognised as a performance-limiting factor at high concentrations of PET 59 60 tracers (2).

Performing a SPECT scan in the presence of a PET radionuclide can also be problematic. If 61 <sup>18</sup>F is present during a <sup>99m</sup>Tc SPECT scan, a proportion of the photons from <sup>18</sup>F positron 62 annihilation will enter the SPECT <sup>99m</sup>Tc energy window (140.5 keV, ±10% i.e. 20% width) 63 due to Compton scattering – the scattering of a photon by a charged particle, resulting in a 64 65 decrease in energy and change in trajectory of the photon. Previous studies have shown that this down-scatter can generate significant noise and artefacts in the SPECT image (1). A 66 clinical study showed that the simultaneous use of <sup>99m</sup>Tc-sestamibi and [<sup>18</sup>F]FDG (with a 67 <sup>99m</sup>Tc:<sup>18</sup>F ratio of 3.2:1) resulted in a <6 % increase in the apparent <sup>99m</sup>Tc count rate due to 68 down-scatter from <sup>18</sup>F (3). This overestimation can be corrected for on clinical scanners by the 69 70 use of auxiliary energy windows; such methods use the signal in parts of the spectrum outside 71 the photopeak window to estimate an amount of signal to subtract from the imaging window to correct for scatter (4, 5). However, these methods may be difficult to implement in a 72 preclinical setting due to the low volume of the scatter medium (mice, rats etc.) and hardware 73 and software constraints. 74

Here, we explore some of the challenges posed by dual tracer PET/SPECT preclinical imaging using radionuclides <sup>18</sup>F and <sup>99m</sup>Tc as examples, and investigate potential practical ways to overcome these obstacles by appropriate experimental design. Mixed-radionuclide <sup>18</sup>F/<sup>99m</sup>Tc phantom experiments were carried out to determine the crossover effects of each radionuclide on the scans, and ultimately, to help design the optimal protocol for *in vivo* dual radionuclide preclinical imaging using <sup>18</sup>F and <sup>99m</sup>Tc.

## 82

# 83 Methods84 PET scanner phantoms

Plastic syringes (5 mL) were filled with either <sup>18</sup>F only (5 MBq), <sup>99m</sup>Tc only (5 MBq) or 85 mixtures of  ${}^{18}$ F (5 MBq) and increasing amounts of  ${}^{99m}$ Tc (5, 50, 100, 150, 200, 250, 350 MBq). 86 Activities of 5 MBq <sup>18</sup>F and < 200 MBq <sup>99m</sup>Tc reflect routine protocols avoiding count-rate 87 limitations of each modality separately. Volumes were made up to 3 mL by addition of water. 88 89 Radioactivity in each syringe was measured using a dose calibrator (Capintec, Ramsey, NJ, 90 USA), calibrated to the national standard. First the syringe was filled with <sup>18</sup>F only and the activity measured. Then the required activity of <sup>99m</sup>Tc was prepared in a microcentrifuge tube, 91 measured in the dose calibrator, and transferred to the syringe. Residual <sup>99m</sup>Tc activity in the 92 93 needle and microcentrifuge tube was subtracted from the measured activity. Syringes were inverted several times for uniform distribution of radioactivity. Each syringe was placed in the 94 95 pre-calibrated nanoPET/CT (Mediso, Budapest, Hungary) and a 15 min PET scan was acquired. The PET system sensitivity was 4.67% for a 350-650 keV energy window and 4 ns 96 97 coincidence window (2). Subsequently, a CT scan was obtained for attenuation correction with a 55 kVp X-ray source, 600 ms exposure time in 180 projections over approximately 6 min. 98 PET images were reconstructed in Nucline v0.21 using Tera-Tomo 3D reconstruction with 4 99

Commented [JB1]: CT parameters for the PET

iterations, 6 subsets, 1-3 coincidence mode, voxel sized 0.4 mm (isotropic), energy window 100 101 400-600 keV with attenuation and scatter correction. Images were analysed in VivoQuant v.3.5, patch 2 software (Invicro LLC., Boston, USA). The activity was determined within a 102 103 cylindrical ROI slightly larger than the syringe. The same cylindrical ROI was used for each scan and translated or rotated to accommodate variations in the placement of each syringe. The 104 105 resulting activity from the PET/CT scan was compared to the decay-corrected activity measured in the dose calibrator. Quantitative assessment of image quality was assessed by 106 107 calculating the coefficient of variation for each image: a small spherical ROI was drawn over 108 the images and the standard deviation within the ROI was divided by the mean within that ROI (Supplementary figure 1). 109

110 111

#### 112 SPECT scanner phantoms

Plastic microcentrifuge tubes (1.5 mL) were filled with either <sup>99m</sup>Tc only (1 MBq), <sup>18</sup>F only (1 113 MBq), or mixtures of <sup>99m</sup>Tc (1, 10, 30, 50, 70 MBq) and <sup>18</sup>F (1 MBq) to achieve <sup>99m</sup>Tc:<sup>18</sup>F ratios 114 ranging from 1:1 to 70:1. Volumes were made up to 1 mL by addition of water. Radioactivity 115 in each tube was measured using a dose calibrator (Capintec, Ramsey, NJ, USA), calibrated to 116 the national standard. First the tube was filled with <sup>18</sup>F only and the activity measured. Then 117 the required activity of 99m/Tc was prepared in a second microcentrifuge tube, measured in the 118 dose calibrator, and transferred to the first tube. Residual 99mTc activity in the needle and 119 second tube was subtracted from the measured activity. Tubes were inverted several times and 120 121 vortexed for uniform distribution of radioactivity. Each tube was placed in the pre-calibrated nanoSPECT/CT Silver Upgrade (Mediso Ltd., Budapest, Hungary) and imaged with 122 acquisition time 15 min, frame time of 35 s using a 4-head scanner with  $4 \times 9$  (1 mm) pinhole 123 collimators in helical scanning mode, and CT images with a 55 kVp X-ray source, 1000 ms 124 exposure time in 180 projections over approximately 9 min. Images were reconstructed in a 125  $256 \times 256$  matrix, voxel size 0.3 mm (isotropic) using HiSPECT (ScivisGmbH) a 126 127 reconstruction software package, and images were fused using proprietary VivoQuant v.3.5, 128 patch 2 software (InVicro LLC., Boston, USA). The resulting activity from the SPECT/CT scan was compared to the decay-corrected activity measured in the dose calibrator. 129

130 131

#### 132 Compton scatter data correction

A method to correct for the effects of 511 keV scattered photons on SPECT image 133 134 quantification and quality was explored. A set of phantom experiments was performed on the nanoSPECT/CT as proof-of-concept. To obtain an <sup>18</sup>F scatter map, a microcentrifuge tube containing <sup>18</sup>F only (2 MBq, 1 mL) was placed in a 50 mL Falcon tube of water (to mimic 135 136 preclinical scatter conditions) and a 15 min SPECT scan was acquired, followed by a CT scan. 137 Next, a tube containing a mixture of <sup>99m</sup>Tc (50 MBq) and <sup>18</sup>F (2 MBq) was placed in a 50 mL 138 Falcon tube on the scanner and a 15 min scan was acquired, followed by a CT scan. Finally, a 139 tube containing <sup>99m</sup>Tc only (50 MBq) was placed on the scanner and a 15 min scan was 140 acquired. The scatter correction was applied by subtracting the <sup>18</sup>F-only SPECT scatter map 141 from the mixed-radionuclide SPECT scan, according to the programming code (Python 3) in 142 Supplementary figure 2a. Automatic scatter correction was applied by scaling the voxel values 143 in both the <sup>18</sup>F-only scan and the mixed-radionuclide scan using their "COUNTS Real World 144 145 Value Slope" as determined by the SPECT scanner calibration saved in the original dicom files. This allowed a matrix subtraction to be performed where each voxel value corresponded 146 147 directly with real world counts, as measured by the SPECT. The images were then converted back using the Value Slope for the original mixed-radionuclide image (Supplementary figure 148 2b). Both the original and scatter-corrected images were analysed in VivoQuant v.3.5, patch 2 149

Commented [JB2]: Corrected kVp

software (Invicro LLC., Boston, USA). The activity in each image was determined using the
 same ROI, within a region slightly larger than the microcentrifuge tube. The activity quantified
 in both images was compared to the decay-corrected activity measured in the dose calibrator.

154 It is important to note that the radioactivity quantities used in these experiments reflect the 155 doses appropriate for our scanner system specifications; tested doses may need to be adjusted 156 for other systems.

#### 158 159 **Results**

160

157

161 Effect of <sup>99m</sup>Tc on PET scans

The effect of the presence of SPECT radionuclide <sup>99m</sup>Tc on <sup>18</sup>F PET scans was assessed by the use of phantoms. The PET scanner was calibrated prior to the start of the study following manufacturer procedures; <sup>18</sup>F-only phantoms measured in the dose calibrator and PET scanner showed good agreement (within 2 %).

In the presence of up to 100 MBq  $^{99m}$ Tc, 5 MBq  $^{18}$ F was accurately quantified by the PET scanner: an over-estimation of < 5% activity was observed in the presence of 50 and 100 MBq 166 167 <sup>99m</sup>Tc (Figure 1). At higher activities of <sup>99m</sup>Tc, PET scanner quantification became less accurate 168 and consistently under-estimated the amount of <sup>18</sup>F present. At 150 MBq of <sup>99m</sup>Tc, <sup>18</sup>F 169 quantification was underestimated by < 10% and became progressively worse with increasing 170 amounts of  $^{99m}$ Tc: at 350 MBg the scanner was underestimating activity of  $^{18}$ F by > 80% 171 172 (Figure 1). The live acquisition energy spectrum (Figure 2, Supplementary figure 3) showed that at these higher activities of <sup>99m</sup>Tc, photons at 140 keV (attributable to <sup>99m</sup>Tc decay) 173 overwhelmed the detection of 511 keV photons originating from <sup>18</sup>F decay suggesting that the 174 dead-time correction could not cope with the increased singles rate. Note that the true counts 175 decrease considerably when adding <sup>99m</sup>Tc to <sup>18</sup>F (Supplementary Table 1 -2). However, activity 176 quantification showed low errors up to 100 MBq added 99mTc (Figure 1) due to the intrinsic 177 dead-time correction of the scanner. Note also that both <sup>99m</sup>Tc only and water have similar 178 179 numbers of true counts which originate from the intrinsic radiation of the LYSO:Ce crystals.

The effect of <sup>99m</sup>Tc on PET image quality was examined (qualitatively) by observing image noise present with increasing <sup>99m</sup>Tc activity in the field-of-view. PET image quality was maintained in the presence of 5 MBq <sup>99m</sup>Tc when compared to its <sup>18</sup>F-only control (Figure 3). Addition of 50 MBq <sup>99m</sup>Tc caused image quality (in terms of signal-to-noise) to deteriorate noticeably, with images becoming more diffuse and lacking in definition. Image quality became progressively worse with increasing amounts of <sup>99m</sup>Tc (Figure 3). These qualitative observations were supported by quantitative analysis of the images: the coefficient of variation for each image increased with increasing amounts of <sup>99m</sup>Tc present (Figure 4).

188 Effect of <sup>18</sup>F on SPECT scans

The effect of the presence of PET radionuclide <sup>18</sup>F on <sup>99m</sup>Tc SPECT scans was assessed by the use of phantoms. The SPECT scanner calibration was checked prior to the start of the study:
 <sup>99m</sup>Tc-only phantoms measured in the dose calibrator and SPECT scanner showed good agreement (within 5 %).

At equivalent activities of  $^{99m}$ Tc and  $^{18}$ F (1:1), quantification of  $^{99m}$ Tc was poor, with the scanner overestimating  $^{99m}$ Tc activity by > 150% (1 MBq vs. 2.75 MBq) (Figure 5). A 10-fold increase in the activity of  $^{99m}$ Tc relative to  $^{18}$ F dramatically improved scanner quantification

accuracy, reducing 99mTc activity overestimation to 10% (Figure 5). Further increases in the 196 quantity of <sup>99m</sup>Tc incrementally improved scanner quantification accuracy in the presence of 1 197 MBq <sup>18</sup>F. The adverse effects of <sup>18</sup>F on scanner quantification accuracy were mitigated 198 completely when <sup>99m</sup>Tc was in 70-fold excess compared to <sup>18</sup>F (Figure 5). SPECT image quality 199 was also affected by the presence of <sup>18</sup>F, with high levels of noise observed at <sup>99m</sup>Tc:<sup>18</sup>F ratios 200 of 1:1 and 10:1 (Figure 6). At ratios of 30:1 and above, noise levels observed qualitatively in 201 202 the images were significantly reduced and images became sharper (Figure 6). The acquisition 203 energy spectrum of an <sup>18</sup>F-only phantom on the SPECT scanner showed detection of a range of photon energies, including some in the 140  $\pm$ 10% (20% width) keV <sup>99m</sup>Tc energy window 204 (Figure 7). 205

#### 206 Compton scatter data correction

The SPECT scanner calibration was checked prior to the start of the study: <sup>99m</sup>Tc-only 207 phantoms measured in the dose calibrator and SPECT scanner showed good agreement (within 208 1 %): a <sup>99m</sup>Tc-only sample measured 50.8 MBq and 51.0 MBq in the dose calibrator and the 209 SPECT scanner, respectively. The mixed-radionuclide sample containing <sup>18</sup>F (2 MBq) plus 210 <sup>99m</sup>Tc (50.7 MBq, measured by dose calibrator) was quantified as 55.84 MBq on the SPECT 211 scanner in the  $140 \pm 10\%$  keV window, an over-estimate of 10 % resulting from the contribution 212 213 of Compton-scattered 511 keV photons to the 140 keV window. Upon subtraction of the <sup>18</sup>Fonly phantom counts from the mixed <sup>18</sup>F+<sup>99m</sup>Tc counts, the resulting "scatter-corrected" data 214 were quantified at 51.89 MBg, an over-estimation of only 2%. The coefficient of variation for 215 the original image and scatter-corrected image was 0.098 and 0.102, respectively. The resulting 216 image was visibly similar to the <sup>99m</sup>Tc-only control image, showing a substantial reduction in 217 218 noise and comparable activity compared to the mixed-radionuclide image (Figure 8). This could be implemented in practice as data correction: initially a SPECT scan of the <sup>18</sup>F present 219 is acquired before injection of <sup>99m</sup>Tc, to establish the <sup>18</sup>F down-scatter contribution; following 220 injection of <sup>99m</sup>Tc and acquisition of the SPECT scan, the <sup>18</sup>F down-scatter component is 221 subtracted to provide an accurate <sup>99m</sup>Tc uptake distribution. 222

#### 224 Discussion

223

225

Our specific motivation for this work originated from the need to compare directly two PET 226 227 tracers for the same biological target, both labelled with <sup>18</sup>F, to understand subtle differences 228 in behaviour between the two tracers in vivo. Since the two tracers were labelled with the same PET radionuclide, and hence had identical physical emission profiles, they could not be 229 compared simultaneously in the same animal. A consecutive imaging protocol (administration 230 and PET imaging of the first tracer, followed by administration and PET imaging of the second 231 232 tracer in the same animal) was possible, in theory. However, the need to allow the first tracer to decay sufficiently to prevent residual activity interfering with the second scan, combined 233 with limits on animal exposure to anaesthesia and recovery time (our specific animal licence 234 235 requires a minimum of 3 h between mouse recovery and being re-anaesthetised) means the 236 imposed delay between tracer administration could lead to significant physiological changes 237 (effects of anaesthesia, metabolism, tumour size etc.) in the animal between the two scans. Similarly, evaluating each tracer in a different animal introduces inherent variability between 238 mice, thus no longer maintaining a controlled environment for accurate tracer comparison. 239

Our solution was to adopt a paired-control approach, using a single SPECT (<sup>99m</sup>Tc) tracer (for the same biological target) in conjunction with each PET tracer, where each tracer can be imaged in the presence of the other because <sup>18</sup>F and <sup>99m</sup>Tc possess different emission profiles. Thus the need to understand the impact of each radionuclide on the scanning of the other presented itself, and mixed-radionuclide <sup>18</sup>F/<sup>99m</sup>Tc phantom experiments were carried out to determine the crossover effects of each radionuclide on the scans. Of course, dual radionuclide
PET/SPECT imaging is not only relevant to our niche example; it is of value more generally,
to enable evaluation of different, but related, biological systems at (almost) the same time using
different tracers labelled with different radionuclides.

Firstly we examined the effect of 99mTc on 18F PET scans. Dead-time effects are observed on 249 the PET scanner when there is too much of any radionuclide. A previous Noise Equivalent 250 Counting (NEC) study performed on our PET scanner showed count-rate peaks at 430 kcps at 251 36 MBq and 130 kcps at 27 MBq for <sup>18</sup>F in mouse and rat phantoms, respectively (2). The 252 injected activity for a mouse in our PET scanner is typically 2-10 MBq; 5 MBq <sup>18</sup>F was used 253 in this phantom experiment, which is well below the NEC peaks, and therefore quantification 254 255 is not affected by dead-time when this quantity of <sup>18</sup>F alone is used. However, when combined with 99mTc, we must assess the contribution of 99mTc photons emitted to scanner dead-time, and 256 hence the effects on PET image quality and quantification. We see that when combined with 257 lower amounts of <sup>99m</sup>Tc (<100 MBq), <sup>18</sup>F quantification is unaffected, but 140 keV photon contribution from higher amounts of <sup>99m</sup>Tc (>150 MBq) prevents coincident 511 keV PET 258 259 photons being recorded, causing the scanner to significantly underestimate <sup>18</sup>F activity. In 260 practice, a typical <sup>99m</sup>Tc SPECT scan requires only 10-40 MBq injection. These results enable 261 us to assess the feasibility of a hypothetical imaging protocol as follows: (i) administration of 262 SPECT tracer (99mTc, 40 MBq) (ii) SPECT image acquisition (1 h) (iii) administration of PET 263 tracer (<sup>18</sup>F, 5 MBq) (iv) PET image acquisition (1 h), in the presence of <sup>99m</sup>Tc. Our phantom 264 studies show that at these levels of activity, <sup>18</sup>F PET quantification is not affected by the 265 presence of <sup>99m</sup>Tc. However, even at levels of <sup>99m</sup>Tc where quantification accuracy is 266 maintained, PET image quality does appear to be compromised. In the presence of 50 MBq 267 <sup>99m</sup>Tc, images becomes a little more diffuse with reduced definition. While this slight decrease 268 in image quality is unlikely to be problematic for the majority of imaging scenarios, it could 269 270 make identification and visualisation of very small biological structures (e.g. tumour metastases) more difficult. 271

Next we examined the effect of <sup>18</sup>F on <sup>99m</sup>Tc SPECT scans. In the presence of <sup>18</sup>F, <sup>99m</sup>Tc SPECT
 image quantification and quality were affected by Compton scatter, leading to an over estimation of <sup>99m</sup>Tc and increase in noise. However, when <sup>99m</sup>Tc activity was 70-fold higher
 than <sup>18</sup>F this over-estimation could be mitigated completely and image quality preserved.

In practice, a good 1 h dynamic PET scan can be achieved with 3 MBq <sup>18</sup>F alone and, taking 276 into account decay ( $t_{1/2} = 109.8$  min), no more than 2 MBq of <sup>18</sup>F tracer would be residual in 277 the animal after the 1 h scan. Our results enable assessment of the feasibility of an alternative 278 279 hypothetical imaging protocol, as follows: (i) administration of PET tracer (<sup>18</sup>F, 3 MBq) (ii) PET image acquisition (1 h) (iii) administration of SPECT tracer (<sup>99m</sup>Tc, > 140 MBq, to 280 maintain 70:1 <sup>99m</sup>Tc:<sup>18</sup>F ratio) (iv) SPECT image acquisition (1 h) in the presence of <sup>18</sup>F. Our 281 phantom studies show that at these radionuclide activity ratios, <sup>99m</sup>Tc SPECT image 282 quantification and quality is not affected by the presence of <sup>18</sup>F. However, although we can 283 control this radionuclide activity ratio at the time of SPECT tracer injection, we cannot know 284 285 if this 70:1 ratio is maintained in vivo - the biodistribution of both PET and SPECT tracers would need to be similar for this to be true. This, plus the need for otherwise unnecessarily 286 287 high levels of radioactivity, makes this protocol an inferior option.

From a biological perspective, it would be preferable to co-administer both tracers at the same time, but based on our findings, there is no combination of imaging sequence and tracer quantity (without waiting several hours for radionuclide decay) that would allow the two radionuclides to be injected *simultaneously* and each scanned in the presence of the other, without having a major effect on image quality and quantification. Furthermore, images of each tracer at the same time after injection could not be obtained with simultaneous injection.

We also explored simple computational methods for correcting for the effects of Compton 294 scatter on the SPECT scanner. One approach involves using the <sup>18</sup>F-only PET scan to identify 295 sources of <sup>18</sup>F, and then subtracting this from the mixed-radionuclide SPECT scan. This process 296 297 becomes quite complex because we are attempting to combine data collected from two different and essentially unrelated pieces of equipment; differences between the PET and SPECT 298 scanners must be accounted for and this requires additional calibration steps to be done for 299 300 each set of measurements obtained on the scanners. An additional complication of this method 301 is the need to maintain animal position between the two scanners so that images can be accurately co-registered. Even with the most careful transfer between scanners (the animal bed 302 is compatible with both scanners and could be transferred without removing the mouse), a 303 304 registration step would be required using the CT scans of the mice to obtain a "best case" match for the scatter map. While it would be possible to produce a warp field for the mouse shift in 305 position, this quickly becomes an overly-complex solution. A more practical approach might 306 therefore be achieved using the SPECT scanner directly to obtain an <sup>18</sup>F scatter map, prior to 307 injection of <sup>99m</sup>Tc. This method would involve incorporating an additional SPECT scan into 308 the scanning protocol and makes the assumption that scatter present in the <sup>18</sup>F-only scatter map 309 is comparable to that obtained when the SPECT tracer is also present (i.e. no change in 310 biodistribution between SPECT scans). The scatter map must also be corrected for radionuclide 311 312 decay between scans. Our proof-of-concept phantom experiment demonstrated that this scattercorrection method could be used easily and successfully to remove noise caused by Compton 313 314 scattering of <sup>18</sup>F photons. The proposed preclinical imaging protocol would then be as follows: (i) administration of PET tracer (<sup>18</sup>F) (ii) PET image acquisition (iii) SPECT image acquisition 315 (to obtain <sup>18</sup>F scatter map) (iv) administration of SPECT tracer (<sup>99m</sup>Tc) (v) SPECT image 316 acquisition (in the presence of <sup>18</sup>F) (vi) scatter map image subtraction. This approach builds 317 the scatter map acquisition into the scanning protocol, and would therefore be applicable to any 318 319 PET/SPECT radionuclide combination, any radionuclide activity, and any scanner model, without requiring calibration for each experiment, making this method an attractive option for 320 dual radionuclide PET/SPECT imaging. 321

The ability to obtain accurate parallel scans with PET and SPECT tracers would allow the 322 limits of molecular imaging to be extended and would be useful for comparison of different 323 324 radiotracers or to image multiple related molecular processes simultaneously to obtain a deeper understanding of interlinked processes. Examples could include temporospatial mapping of 325 anti-cancer drug delivery (e.g. via liposomal formulation) and response in relation to the 326 disease site (6), and in advanced cell-based therapies where the trafficking of cells to disease 327 328 sites has to be quantified alongside mapping of the disease and response to therapy. Similarly, SPECT imaging of the biodistribution of therapeutic radionuclides that emit both imageable 329 gamma photons (e.g. <sup>177</sup>Lu, <sup>67</sup>Cu, <sup>188</sup>Re) could be performed alongside PET imaging (e.g. with 330 [<sup>18</sup>F]FDG or other tracer) of metabolic response to therapy. It would also enable imaging of 331 multiple molecular characteristics of disease in an animal to obtain metabolic and gene 332 expression profiles or characterise diseases such as cancer where heterogeneity is to be 333 334 expected.

#### 336 Conclusion

335

Sequential PET and SPECT data acquisition whilst both positron- and gamma-emitters are present is feasible under certain conditions without substantial influence on image quantification accuracy. Suitable combinations of injection sequence and imaging sequence can be devised to match the biological requirements of a particular experiment, using existing commercial small animal PET and SPECT scanners, with only minor or insignificant effects of each radionuclide on the scan of the other. However simultaneous tracer injection is not feasible under any combination of imaging sequence and tracer quantity, and does not allow images of each tracer to be obtained at the same time after injection.

345 346

## 347 Conflict of interest

348 The authors declare that the research was conducted in the absence of any commercial or

349 financial relationships that could be construed as a potential conflict of interest.

## 350 Author contributions

LL and PB contributed conception of the study; LL, PB, JEB, JKB, AR, MF, HO, JJ, KS, JB

- and CP contributed design of the study; JEB, JKB, AR, MF, JK and CP contributed phantom
- data acquisition; JKB, JK, JEB and KS contributed image analysis; HO and JJ contributed code
- development; JEB wrote the first draft of the manuscript; LL, PB, JEB, KS, JK contributed to
- manuscript revision. All authors read and approved the submitted version.

#### 356 Funding

357 This work was supported by the Wellcome EPSRC Centre for Medical Engineering at KCL

- 358 [grant number WT 203148/Z/16/Z], the KCL/UCL Comprehensive Cancer Imaging Centre
- funded by CRUK and EPSRC in association with the MRC and DoH (England), the EPSRC

360 Centre for Doctoral Training in Medical Imaging at King's College London and Imperial

361 College London, Wellcome Multi User Equipment Grant "A multiuser radioanalytical facility

362 for molecular imaging and radionuclide therapy research" and the National Institute for Health

363 Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation

Trust and KCL [grant number IS-BRC-1215-20006]. PET and SPECT scanning equipment at KCL was funded by an equipment grant from the Wellcome Trust under grant number WT

084052/Z/07/Z. The views expressed are those of the authors and not necessarily those of the

367 NHS, the NIHR or the Department of Health.

#### 368 Acknowledgements

#### 369 The authors would like to thank the King's College London & Guy's and St Thomas' PET

370 Centre for the supply of <sup>18</sup>F, and Guy's and St Thomas' NHS Trust Radiopharmacy for the

371 supply of <sup>99m</sup>Tc.

### 372 Supplementary Material

373 One file

#### 374

### 375 References

1. Chapman SE, Diener JM, Sasser TA, Correcher C, Gonzalez AJ, Avermaete TV, et al.

- 377 Dual tracer imaging of SPECT and PET probes in living mice using a sequential protocol.
- 378 Am J Nucl Med Mol Imaging. 2012;2(4):405-14.

2. Szanda I, Mackewn J, Patay G, Major P, Sunassee K, Mullen GE, et al. National

Electrical Manufacturers Association NU-4 performance evaluation of the PET component of
 the NanoPET/CT preclinical PET/CT scanner. J Nucl Med. 2011;52(11):1741-7.

- 382 3. Sandler MP, Videlefsky S, Delbeke D, Patton JA, Meyerowitz C, Martin WH, et al.
- Evaluation of Myocardial-Ischemia Using a Rest Metabolism Stress Perfusion Protocol with
- F-18 Deoxyglucose Technetium-99m Midi and Dual-Isotope Simultaneous-Acquisition
- Single-Photon Emission Computed-Tomography. J Am Coll Cardiol. 1995;26(4):870-8.
- 386 4. Ichihara T, Ogawa K, Motomura N, Kubo A, Hashimoto S. Compton Scatter
- Compensation Using the Triple-Energy Window Method for Single-Isotope and Dual-Isotope
   Spect. J Nucl Med. 1993;34(12):2216-21.
- 389 5. Wu J, Ma TY, Liu H, Xia Y, Chen S, Wang S, et al. Feasibility Studies of
- 390 Simultaneous PET and SPECT Dual-tracer Imaging with a Stationary Multi-pinhole
- 391 Collimator Inserted to Animal PET Detector. Ieee Nucl Sci Conf R. 2012:2788-91.

392 6. Man F, Lim L, Volpe A, Gabizon A, Shmeeda H, Draper B, et al. In Vivo PET

393 Tracking of (89)Zr-Labeled Vgamma9Vdelta2 T Cells to Mouse Xenograft Breast Tumors

Activated with Liposomal Alendronate. Mol Ther. 2019;27(1):219-29.

395

396

402

407

411

415

419

397 Figure captions

Figure 1. Effect of different amounts of <sup>99m</sup>Tc on the accuracy of PET scanner quantification of 5 MBq <sup>18</sup>F. The effect on scanner quantification was assessed by comparing the amount of <sup>18</sup>F measured by the dose calibrator to that measured by the PET scanner; n=3, mean ± SD.
 Grey box inset provides zoom of 0-150 MBq region.

**Figure 2.** Live acquisition energy spectrum obtained during a PET scan of a mixed radionuclide phantom containing 5 MBq <sup>18</sup>F and 250 MBq <sup>99m</sup>Tc. The yellow peak at 140 keV corresponds to the energy of <sup>99m</sup>Tc  $\gamma$  photons, which reduces detection of <sup>18</sup>F coincident photons at 511 keV.

**Figure 3.** PET-CT MIPs of mixed-radionuclide  ${}^{18}\text{F} + {}^{99\text{m}}\text{Tc}$  phantoms. Each syringe contains having  ${}^{18}\text{F}$  (5 MBq) mixed with increasing amounts of  ${}^{99\text{m}}\text{Tc}$  (0-350 MBq). An  ${}^{18}\text{F}$  only (5 MBq) control is included for comparison.

Figure 4. Effect of increasing amounts of <sup>99m</sup>Tc on the PET image quality of 5 MBq <sup>18</sup>F,
quantified by calculating the coefficient of variation within an ROI for each image (SD/mean);
n=3.

Figure 5. Effect of <sup>18</sup>F (1 MBq) on the accuracy of SPECT scanner quantification of increasing amounts of <sup>99m</sup>Tc. The effect on scanner quantification was assessed by comparing the amount of <sup>99m</sup>Tc measured by the dose calibrator to that measured by the SPECT scanner.

Figure 6. SPECT-CT MIPs of <sup>99m</sup>Tc only and mixed-radionuclide <sup>99m</sup>Tc + <sup>18</sup>F phantoms. Each tube contained either <sup>99m</sup>Tc only (1, 10, 30, 50, 70 MBq) or <sup>99m</sup>Tc (1, 10, 30, 50, 70 MBq)
 mixed with <sup>18</sup>F (1 MBq). All images scaled to the same threshold and intensity.

Figure 7. Live acquisition energy spectrum obtained during a SPECT scan of an <sup>18</sup>F-only
 phantom (1 MBq). A range of energies is evident, including energies in the 140.5 keV ±10%
 (i.e. 20% width) <sup>99m</sup>Tc energy window (red).

**Figure 8**. Proof-of-concept for SPECT scatter data correction method. The <sup>18</sup>F-only scatter map image was subtracted from the <sup>99m</sup>Tc + <sup>18</sup>F mixed-radionuclide image to achieve the final scatter-corrected image. The <sup>99m</sup>Tc-only image is included for comparison.