Dual SPECT imaging of $^{111}$In and $^{67}$Ga to simultaneously determine \textit{in vivo} the pharmacokinetics of different radiopharmaceuticals: a quantitative tool in pre-clinical research

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Keywords: \textit{In vivo} preclinical studies, dual-isotope imaging, Ga-67, In-111, Multi-pinhole SPECT

Abstract

Dual-isotope (DI) studies offer a number of advantages in pre-clinical imaging. These include: reducing study times when compared with sequential scans, reducing the number of animals required for any given study, and most importantly, producing images perfectly registered in space and time that provide simultaneous information about two distinct body functions. The ability of single photon emission computed tomography (SPECT) to measure and differentiate energies of the emitted photons makes it well suited for DI imaging. However, since scattered photons originating from one radioisotope may be detected in the energy window of the other and thus degrade image quality and quantitative accuracy, scatter and crosstalk corrections must be applied.

The decay characteristics of $^{111}$In and $^{67}$Ga, which are suitable for quantitative DI imaging for up to 2 weeks post-injection, led us to investigate the performance of simultaneous $^{111}$In/$^{67}$Ga SPECT imaging using a small-animal pre-clinical scanner. A series of phantom experiments were performed to investigate image quality and accuracy of activity quantification in $^{111}$In/$^{67}$Ga images acquired with three different collimators and reconstructed from different photopeak combinations. The triple energy window (TEW) method was used to correct for scatter and crosstalk. Based on these phantom studies, the optimal selection of collimator and energy window settings was determined. When using these optimal settings, submillimeter-size structures were distinguishable in the reconstructed images and quantification errors below 20% were achieved for both isotopes. The optimal parameters were subsequently applied to an \textit{in vivo} animal study. The determination of the distinct pharmacokinetic profiles of two polymer radiopharmaceuticals injected simultaneously, but by different administration routes (intravenously and intraperitoneally) into a single animal demonstrated the feasibility of simultaneous $^{111}$In/$^{67}$Ga SPECT.

1. Introduction

Unlike positron emission tomography (PET), which relies on coincidence detection of photons originating from positron annihilation and thus always having an energy of 511 keV for any radiotracer, single photon emission computed tomography (SPECT) is able to identify radiotracers labeled with different radionuclides based on the energies of their gamma emissions. Therefore, if two radiotracers are labeled with radionuclides emitting photons with different energies, they can be imaged simultaneously, and their distributions can be individually reconstructed. Dual-isotope (DI) studies have several advantages over sequential scanning. Not only acquisitions...
require less time compared to those performed sequentially, but DI images are also perfectly registered and provide information about different biodistributions occurring at the same time, thus corresponding to same physiological state. These characteristics may be particularly useful for the development of non-invasive tests to better understand the phenotype of a disease for diagnostic or treatment planning purposes (El Fakhri et al 2006, Hijnen et al 2012, Wolf et al 2016). Furthermore, DI imaging is a valuable tool to better understand the distribution and in vivo stability of multi-component drug delivery systems and nanomedicines (Fan et al 2017, Lamichhane et al 2017).

To date, dual-tracer imaging is only routinely used to stratify hyperparathyroid patients and prevent unnecessary surgery (Shcherbinin et al 2012a, Sommerauer et al 2015). However, it has also been used to evaluate the ratio of serotonin and dopamine transporters in depression (99mTc/123I) (Hsieh et al 2010), to accurately identify hepatic lesions (111In/99mTc) (Guo et al 2016), and to investigate myocardial perfusion and follow-up on the myocardial infarcts (201Tl/123I, 99mTc/123I) (Maskali et al 2005, Du et al 2014, D’estanque et al 2017).

In the last few decades, the extensive knowledge and rich experience in coordination chemistry facilitated the development of a wide variety of radiopharmaceuticals based on radiometals and the ability to target-specific ligands (peptides, small molecules and antibodies) (Anderson and Welch 1999, Wadas et al 2010, Ramogida and Orvig 2013, Price and Orvig 2014). Among these metals, 111In and 67Ga have shown excellent chemical and physical properties to design and develop SPECT imaging radiopharmaceuticals and are easily available at high specific activities (Wadas et al 2010).

Indium-111 has a physical half-life of 67.2 h, which is suitable for investigating tracer pharmacokinetics in vivo over a long time range, and emits photons with multiple energies (x-rays: 22–27 keV—82.8%, 171 keV—90.7%, and 245 keV—94.1%) (Blachot 2009) and is thus useful for SPECT diagnostic imaging (figures 1(a)–(c)). Gallium-67 has a half-life of 78.2 h and emits four groups of photons which can be used in SPECT imaging: 93 keV—38.8%, 185 keV—21.4%, 300 keV—16.6% and 393 keV—4.6% (Junde et al 2005) (figures 1(a)–(c)). The differences in the energies of photons emitted by 67Ga and 111In make them suitable for simultaneous imaging of these two radionuclides as most of the photopeaks do not overlap (figures 1(d)–(f)). Their similar long half-lives enable imaging of both radioisotopes over long time periods, up to approximately two weeks, which is especially favorable for imaging molecular entities with long biological half-lives, such as antibodies and nanomedicines. Moreover, the labeling procedures with both 67Ga and 111In are well established, simple to perform (often at room temperature) and yield high efficiency (regularly >95%) which may promote a rapid expansion of the use of these radionuclides in research. Consequently, the 111In/67Ga pair is a promising candidate for simultaneous DI SPECT imaging in pre-clinical studies.

However, one of the main challenges of dual-isotope imaging is the so-called ‘crosstalk effect’ between the radionuclides’ energy photopeaks. Crosstalk is defined as the detection of high energy photons (for instance, in this specific case (DI) originating from the radionuclide with higher energy emissions), which, upon scattering, fall in the energy window of the lower energy photopeak. The crosstalk effect is particularly important in DI imaging, as, if not corrected, it will result in imaging artifacts and quantification inaccuracies. In extreme situations, when the difference in energy of the two photons originating from the two radionuclides is small, the resulting photopeaks might partially overlap due to the finite energy resolution of the SPECT detectors. Several techniques have been proposed to address crosstalk related problems including the optimization of the radionuclide’s detection energy window selection, the development of sophisticated quantitative image reconstruction methods (De Jong et al 2002, Shcherbinin et al 2012b) and/or the use of neural networks (Bai et al 2007).

The feasibility of simultaneous 111In and 67Ga 2D scintigraphic imaging was investigated in an early study by Wendt et al (2007). The authors developed a method to separate the images of the two isotopes based on principal component analysis and showed that their method performed qualitatively better than direct imaging of the 93 keV photopeak of 67Ga and the 245 keV photopeak of 111In. This method was subsequently tested on an animal study (Wong et al 2007), demonstrating that in vivo simultaneous scintigraphic imaging of 111In and 67Ga is feasible. Up to date, however, no systematic study investigating the feasibility of 111In and 67Ga imaging with quantitative SPECT has been reported.

Given the potential usefulness of the 111In and 67Ga radionuclide pair for quantitative pre-clinical dual-isotope imaging, the goal of this study was to investigate the imaging performance of simultaneous 111In/67Ga SPECT under different experimental conditions using a small animal scanner.

In this work, we determined an optimum dual imaging protocol for 111In and 67Ga by evaluating the image quality and accuracy of activity quantification using data acquired with three different collimators and several different energy window settings. Subsequently, to illustrate our conclusions, the resultant protocol was applied to an in vivo mouse study using simultaneous intravenous 67Ga and intraperitoneal 111In injection with the aim of determining pharmacokinetic profiles of both radiopharmaceuticals in a single animal and ultimately, demonstrating the feasibility of simultaneous preclinical 111In/67Ga SPECT imaging.
2. Materials and methods

2.1. VECT or/CT system, data acquisition and image reconstruction

All imaging studies were performed with the small-animal SPECT/PET/CT system VECT or/CT (MILabs, Netherlands). The system consists of three stationary NaI scintillation crystals arranged in a triangular configuration, an x-ray CT scanner and a series of interchangeable multi-pinhole collimators that allow the user to image both SPECT and PET radiotracers (Goorden et al 2012). Three different collimators were tested (table 1): (1) a general purpose (GP) collimator, suitable for imaging low-energy photons (van der Have et al 2009); (2) an ultra-high sensitivity (UHS) collimator (Ivashchenko et al 2015), designed for low activity studies, with a wall thickness of 30 mm, thus suitable for imaging medium-energy photons and; (3) the high-energy (HE) collimator, suitable for imaging high-energy emissions of both SPECT and PET radiotracers (van der Have et al 2016).

The SPECT data were acquired in list-mode where both the energy and the location of each detected photon were recorded. Following each SPECT acquisition, a CT scan was performed to visualize the phantom geometry or the animal’s anatomical features and create the transmission maps for use in attenuation correction. Prior to performing image reconstruction, the projection data were created from the list-mode data by selecting photons with energies corresponding to 111In and 67Ga photopeaks and scatter/background window settings. A preliminary visual analysis of the energy spectra of 111In and 67Ga photopeaks indicated that the photopeaks corresponding to 22–27 keV (x-rays) and 245 keV for 111In and the 93 keV and 300 keV photopeaks of 67Ga were the best candidates for simultaneous imaging of these radionuclides.

Figure 1. Normalized energy spectra of two point-sources of 111In and 67Ga acquired individually in air (a)–(c) and; the energy spectra of a point-source and a syringe containing a mixture of 111In + 67Ga (d)–(e). The energy spectra were measured with three collimators: general purpose, ultra-high sensitivity and high-energy collimator. Each energy spectrum was normalized by its corresponding maximum intensity peak.
radionuclides. Data from the 185 keV photopeak of $^{67}$Ga and the 171 keV photopeak of $^{111}$In were disregarded due to their considerable overlap. The 393 keV photopeak of $^{67}$Ga (not shown in figure 1) was also not considered due to its very low intensity.

The background and crosstalk under each photopeak window were estimated using the TEW method (Ogawa et al 1991). Since the accuracy of the scatter estimates depends on the selected photopeak width and the scatter window settings (Robinson et al 2016), we evaluated the performance of dual $^{111}$In and $^{67}$Ga SPECT by comparing images reconstructed individually from 16 different combinations of energy window settings (for each photopeak there were four different photopeak and scatter window settings). These 16 energy window settings are labelled as method $A_i$, $B_i$, $C_i$ and $D_i$ with the index $i = 1, 2, 3, 4$ corresponding to the $^{111}$In 22–27 keV x-rays, the $^{111}$In 256 keV photopeak, the $^{67}$Ga 93 keV photopeak, and the $^{67}$Ga 300 keV photopeak, respectively (table 2).

To account for the dependence of the NaI detector energy resolution on photon energy (energy resolution $\Delta E/E$ is proportional to $1/\sqrt{E}$ (Dorenbos et al 1995)), we performed window width re-scaling using the 140 keV photopeak window settings of $^{99m}$Tc as a reference. Using this approach, we adjusted the energy window setting for each photopeak of interest as follows:

$$W_X = W_{140} \times \sqrt{\frac{E_X}{E_{140}}}$$

Where: $W_X$ was the photopeak width (in %) for the photopeak energy $E_X$ (in keV), and $W_{140}$ was the reference photopeak width for 140 keV photons, the value of which was set at 20% (methods $A_i$ and $B_i$), 22.5% (method $C_i$) and 25% (method $D_i$). The scatter window widths were set at 10% for method $A_i$ (‘narrow’ scatter windows) and 20% (‘wide’ scatter windows) for methods $B_i$, $C_i$ and $D_i$.

All images were reconstructed using the pixel ordered subsets expectation maximization algorithm (P-OSEM) (Branderhorst et al 2010) with 16 subsets and 10 iterations and an isotropic 0.4 mm voxel grid. Subsequently, the reconstructed SPECT images were registered to the CT images, resampled to 0.16 × 0.16 × 0.16 mm³ voxels, and corrected for attenuation using the CT-based non-uniform Chang method (Wu et al 2011). The reconstruction of the images, the registration of SPECT to CT and the attenuation corrections were performed using the manufacturer’s software.

The counts in the reconstructed images were converted into units of activity concentration by applying an experimental calibration factor for each isotope and each energy window setting. The calibration factors were determined following the procedure described by Wu et al (2010). In short, two point-sources of $^{111}$In (6.5 MBq) and $^{67}$Ga (6.5 MBq) were scanned individually for 5 min each and these data were reconstructed using the same parameters and corrections as those applied to the phantom studies. The calibration factors ($CF$) for each photopeak, energy-window setting, and collimator were determined using the following formula:

$$CF = \frac{A}{\sum_i R_i}$$

Where: $A$ is the activity of the point-source (MBq), $V$ is the volume of one voxel in the reconstructed image (ml) and $R_i$ is the normalized (according to the manufacturer’s defined procedure) count rate summed over all the voxel in the image. In total, 48 calibration factors were determined (for three collimators and 16 photopeaks) and their values are reported in the supplementary material.

### 2.2. Phantom experiments

Thirteen $^{111}$In/$^{67}$Ga SPECT/CT phantom experiments, divided into four studies, were performed. The objectives of these experiments were to evaluate the image quality and the activity quantification accuracy of simultaneously acquired $^{111}$In and $^{67}$Ga phantom data at varying activity concentration levels and phantom geometries. While the two first phantom studies (section 2.2.1–2.2.2) were performed under high activity concentration ratios, the third (section 2.2.4) comprised low activities of both radioisotopes in order to emulate typical conditions encountered in animal studies. For comparison, measurements of phantoms containing single $^{111}$In and $^{67}$Ga activities were performed to determine the reference image-quality and image quantification accuracy of these two radionuclides when imaged separately (section 2.2.3).

<table>
<thead>
<tr>
<th>Label</th>
<th>Material</th>
<th>Inner bore diameter [mm]</th>
<th>Wall thickness [mm]</th>
<th>Number of pinholes</th>
<th>Pinhole diameter [mm]</th>
<th>Pinhole opening angle [deg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP</td>
<td>Tungsten</td>
<td>44</td>
<td>18</td>
<td>75</td>
<td>0.6</td>
<td>30</td>
</tr>
<tr>
<td>UHS</td>
<td>Lead</td>
<td>46</td>
<td>30</td>
<td>54</td>
<td>2</td>
<td>21.2–25.1</td>
</tr>
<tr>
<td>HE</td>
<td>Tungsten</td>
<td>48</td>
<td>43</td>
<td>192</td>
<td>0.7</td>
<td>16–18</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of the general-purpose (GP), ultra-high sensitivity (UHS) and high-energy (HE) multi-pinhole collimators.
2.2.1. Image quality experiments

The quality of simultaneous $^{111}$In and $^{67}$Ga SPECT images was evaluated in terms of the smallest structure that could be resolved in the reconstructed images, and in terms of image-contrast. To this end, a micro-Jaszczak phantom, containing six sectors of rods with decreasing diameters, was filled with a uniform mix of equal amounts of $^{111}$In (2.35 MBq) and $^{67}$Ga (2.35 MBq). This phantom was scanned three times using the GP, UHS and HE collimators. The diameter of the rods ranged from 0.85 mm to 1.7 mm (figure 2(a)). The phantom was scanned for 4 h with the HE collimator, 5 h with the GP collimator, and 10 h with the UHS collimator, respectively. Different acquisition times were set to compensate for the decay of the isotopes as the scans were performed on different days. In total, 48 data sets acquired with the micro-Jaszczak phantom were reconstructed corresponding to the four energy window settings applied to the four investigated photopeaks of $^{111}$In and $^{67}$Ga for each of the three collimators ($4 \times 4 \times 3 = 48$) (table 2).

Intensity profiles through the 1.1 mm rods were computed on all reconstructed images of the micro-Jaszczak phantom as illustrated in figure 4. Additionally, the image-contrast ($C_d$) as a function of the hot-rod diameter ($d$) was quantified as follows: for each image, cylindrical VOIs were placed on the images of each hot-rod and on the space between these hot-rods. Each VOI had a diameter equal to 0.9 times the diameter of the rod and was 5 mm in height, expanding over a total of 30 axial slices in the reconstructed image. Contrast was calculated using equation (3) below, where $h_d$ represents the average voxel value of all the VOIs placed in the hot-rods and $b_d$ represents the average voxel value of all the VOIs placed in the space between the hot-rods.

$$C_d = \frac{h_d - b_d}{b_d}.$$  

(3)

Based on equation (3), an ideal imaging system would yield a contrast value of one for every rod diameter. The selected method to assess image quality, although not standard, is effective to quantify the resolvability of small hot regions, which is an important task in small-animal imaging (Walker et al 2014).

The effect of the selection of energy-window settings on the image contrast was assessed by calculating the average percent change in contrast ($\%\Delta C(X_i)$) with respect to $A_i$, obtained when another energy window setting $X_i$ was chosen:

$$\%\Delta C(X_i) = \frac{1}{6} \sum_d \frac{C_d(X_i) - C_d(A_i)}{C_d(A_i)} \times 100,$$

(4)

where $X_i$ corresponds to $B_i$, $C_i$, and $D_i$ (table 2). The quantity $C_d$ represents the image contrast for rod diameter $d$ (equation (3)). For any given energy window setting $X_i$, the percent change in contrast was averaged over the six rod diameters of the micro-Jaszczak phantom. Since configuration $A_i$ corresponded to the narrowest energy window settings, the quantity of equation (4) allowed us to investigate the change as a function of the increase in the energy window width.

Table 2. Energy window settings used for reconstruction of each of the photopeaks of $^{111}$In and $^{67}$Ga. The photopeak widths were determined according to equation (1).

<table>
<thead>
<tr>
<th>Isotope: photopeak center</th>
<th>Method</th>
<th>Photopeak width (%)</th>
<th>Photopeak width (keV)</th>
<th>Scatter width (%)</th>
<th>Scatter width (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{111}$In: 22 keV</td>
<td>$A_1$</td>
<td>50.4</td>
<td>11.1</td>
<td>10</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>$B_1$</td>
<td>50.4</td>
<td>11.1</td>
<td>20</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>$C_1$</td>
<td>56.7</td>
<td>12.5</td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>$D_1$</td>
<td>63.1</td>
<td>13.9</td>
<td>20</td>
<td>2.8</td>
</tr>
<tr>
<td>$^{111}$In: 245 keV</td>
<td>$A_2$</td>
<td>15.2</td>
<td>37.2</td>
<td>10</td>
<td>3.7</td>
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<tr>
<td></td>
<td>$B_2$</td>
<td>15.2</td>
<td>37.2</td>
<td>20</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>$C_2$</td>
<td>17.1</td>
<td>42.0</td>
<td>20</td>
<td>8.4</td>
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<td></td>
<td>$D_2$</td>
<td>19</td>
<td>46.6</td>
<td>20</td>
<td>9.3</td>
</tr>
<tr>
<td>$^{67}$Ga: 93 keV</td>
<td>$A_3$</td>
<td>24.7</td>
<td>23.0</td>
<td>10</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>$B_3$</td>
<td>24.7</td>
<td>23.0</td>
<td>20</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>$C_3$</td>
<td>27.8</td>
<td>25.6</td>
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</tr>
<tr>
<td></td>
<td>$D_3$</td>
<td>30.8</td>
<td>28.6</td>
<td>20</td>
<td>5.7</td>
</tr>
<tr>
<td>$^{67}$Ga: 300 keV</td>
<td>$A_4$</td>
<td>13.7</td>
<td>41.1</td>
<td>10</td>
<td>4.1</td>
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<tr>
<td></td>
<td>$B_4$</td>
<td>13.7</td>
<td>41.1</td>
<td>20</td>
<td>8.2</td>
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<td>$D_4$</td>
<td>17.1</td>
<td>51.3</td>
<td>20</td>
<td>10.3</td>
</tr>
</tbody>
</table>
2.2.2. Image Quantification Experiments

2.2.2.1. Quantification accuracy of simultaneous $^{111}$In and $^{67}$Ga SPECT as a function of the radioisotopes’ concentration ratio

A multi-source phantom experiment was designed to evaluate the quantification accuracy of simultaneous $^{111}$In and $^{67}$Ga SPECT for different ratios of $^{111}$In to $^{67}$Ga activity concentration. The multi-source phantom consisted of a strip of 6 0.2 ml polymerase chain reaction (PCR) tubes placed inside a 20 ml plastic syringe filled with water (figure 2(b)). Each compartment of the phantom was filled with a 0.07 ml solution of $^{111}$In and $^{67}$Ga activities. The activity concentration of $^{67}$Ga in each compartment was kept constant at 8.8 MBq ml$^{-1}$ whereas the activity concentrations of $^{111}$In ranged from 46.2 MBq ml$^{-1}$ in the first compartment to 2.1 MBq ml$^{-1}$ in the sixth compartment. As a result, the ratios of $^{111}$In-to-$^{67}$Ga activity concentrations in the six compartments were 5.2, 3.4, 1.9, 1.0, 0.5 and 0.2, respectively. Similar to the image-quality experiments, the phantom was scanned with each of the three collimators (1 h acquisitions). The scans FOVs were adjusted to focus on the multi-source’s regions filled with activity, as shown in figure 2(b). The projection data were reconstructed using the four energy window settings for each $^{111}$In and $^{67}$Ga photopeak. As a result, 48 reconstructed images of the multi-source phantom were obtained.

The $^{111}$In and $^{67}$Ga activity concentrations of the multi-source phantom were estimated by placing cylindrical VOIs (diameter = 3 mm, height = 3 mm) on each compartment on each of the 48 images. Such VOIs were chosen to avoid the influence of the partial volume effect on the determination of the activity concentration. The estimated activity concentrations were then compared to their true values by calculating the percent error:

$$\% \text{Error} = \frac{C_{\text{SPECT}} - C_{\text{DC}}}{C_{\text{DC}}} \times 100.$$  

Where: $C_{\text{SPECT}}$ and $C_{\text{DC}}$ represent the activity concentrations measured in the SPECT images and in the dose-calibrator (Atomlab 500, Biodex Medical System, NY, USA), respectively.

2.2.3. Image performance of single $^{111}$In and $^{67}$Ga SPECT

Two additional experiments were performed to determine the image-quality of separate $^{111}$In and $^{67}$Ga imaging, used as reference when evaluating simultaneous DI imaging. In a first experiment, the micro-Jaszczak phantom was filled with 0.5 MBq of $^{111}$In and scanned for 15 h using the UHS collimator. In a second experiment, that same phantom was filled with 9.3 MBq of $^{67}$Ga and scanned for 1 h using the HE collimator. The UHS and HE collimators were selected for this investigation as these collimators are most suitable to stop the medium and high-energy emissions of $^{111}$In and $^{67}$Ga, respectively. Acquisition times were adjusted to collect a number of counts comparable to those of the dual-isotope studies.

Furthermore, two 0.2 ml PCR-tube compartments filled with 0.07 ml of $^{111}$In (14.2 MBq ml$^{-1}$) and $^{67}$Ga (18.6 MBq ml$^{-1}$) solutions, respectively, were scanned to determine the reference quantification accuracy of single $^{111}$In and $^{67}$Ga SPECT. The $^{111}$In and $^{67}$Ga sources were scanned for 10 min each using the UHS and HE collimators, respectively. The photopeak data in the single-isotope experiments were reconstructed using window settings $A_i$ (table 2) as these settings yielded the best image performance in DI experiments (see section 3.2).

2.2.4. $^{111}$In/$^{67}$Ga imaging performance under challenging imaging conditions: low activity concentration and small structures

2.2.4.1. Accuracy of simultaneous $^{111}$In and $^{67}$Ga SPECT imaging for extended sources with low activity concentrations

This experiment was designed to investigate the quantification accuracy of simultaneous $^{111}$In and $^{67}$Ga SPECT for an extended object of a size similar to that of a mouse. To this end, a 20 ml plastic syringe was filled with an 8 ml aqueous solution of $^{111}$In and $^{67}$Ga activities. The activity concentration of both $^{111}$In and $^{67}$Ga was 0.95 MBq ml$^{-1}$. Simulating a typical animal study, the phantom was scanned for 30 min using the GP, UHS and HE collimators. The configuration of this phantom allowed us to investigate the quantification accuracy in a situation of low radioactivity concentration distributed over a relatively large volume, resulting in a low number...
of counts per projection. In addition, the scatter distribution of the uniform extended source was different from that of the individual multi-sources and from that of the calibration sources (point-sources in air).

The activity concentration of the syringe phantom was measured by drawing a large cylindrical VOI at the center of the reconstructed image (radius = 5 mm, height = 30 mm) and calculating the mean voxel value. The obtained activity concentration was compared to the true value (measured with the dose-calibrator) by computing the percent error between these two measurements. To illustrate the variability in the voxel values within each VOI, the standard deviation of the percent errors across all voxels in the VOI was also determined.

2.2.4.2. Image quality and quantification accuracy of small structures under low-count conditions
To evaluate the effect of less favorable experimental conditions on the quality and quantification accuracy of dual-isotope $^{111}$In/$^{67}$Ga SPECT, the data obtained after scanning both the micro-Jaszczak and the multi-source phantoms using the HE collimator were reconstructed using only 10% of the total recorded counts. The data acquired with the HE collimator were selected for this investigation since it was the one showing the best performance based on the phantom experiments (sections 2.2.1 and 2.2.2). Furthermore, we quantified the activity concentration by segmenting each compartment of the multi-source phantom with a cylindrical VOI (diameter = 4.25 mm, height = 7.5 mm) that covered the entire source (rather than a small VOI within the source boundaries). This segmentation method might reflect better the one applied during animal studies where often the entire organ or tumor is delineated.

2.3. In Vivo Imaging
The animal study was performed in accordance with the canadian council on animal care (CCAC) using the protocol approved by the Animal Care Committee (ACC) of the University of British Columbia (A16-0150). Under isoflurane, two 3-month-old healthy female CD1 mice (Charles River Laboratories, Montreal, QC, Canada) were administered $^{67}$Ga-labeled 500kDa hyperbranched polycerol (HPG) (Saatchi et al 2012a) intravenously (IV) via the tail vein (7.2 MBq and 6.0 MBq, respectively). Immediately afterwards, the animals were administered the same polymer labeled with $^{111}$In (Saatchi et al 2012b) into the peritoneal cavity (9.6 MBq and 8.3 MBq, respectively). The animals were immediately placed in the scanner and 20 min whole-body scans were acquired at approximately 0, 1, 4, 5, 6 and 24 h post-injection. Throughout the entire scanning procedure, mice were kept under isoflurane anesthesia and body temperature was maintained constant using a heated imaging bed.

Animal studies were performed using the HE collimator (study 1) and UHS collimator (study 2). The animal projection data were reconstructed using method $A_2$ applied to the 245 keV of $^{111}$In and method $A_3$ applied to the 93 keV photopeak of $^{67}$Ga. These energy window settings yielded the optimum, and most robust quantification results based on the phantom experiments (see section 3.2).

To obtain the pharmacokinetic profiles of the two radiopharmaceuticals, Standardized Uptake Values (SUV-mean) were determined for a spherical volume of interest (3 mm in diameter) placed on the image of the heart. Intravenously administered $^{67}$Ga-HPG remains largely within the blood pool (Saatchi et al 2012a) whereas $^{111}$In-HPG is slowly absorbed via the peritoneal lymphatics into the blood, where it will continue to circulate with a half-life similar to that of $^{67}$Ga-HPG (Mactier et al 1987, Saatchi et al 2012b). Therefore, the $^{111}$In and $^{67}$Ga signals were expected to converge in the heart (representative of the blood) after several hours.

3. Results

3.1. Image quality experiments
Figures 3 and 4 show trans-axial slices of the micro-Jaszczak phantom reconstructed with the energy window methods $A_i$ applied to the photopeaks of $^{111}$In and $^{67}$Ga for the single and dual-isotope experiment, respectively. Visually, the single-isotope images were comparable to those of the dual-isotope study. The smallest rod size distinguishable in the dual-isotope data acquired with the GP and HE collimators was 0.85 mm, whereas in images reconstructed from UHS data it was 1.1 mm (93 keV peak of $^{67}$Ga). Images reconstructed with method $B_i, C_i$ and $D_i$ were visually indistinguishable from those shown in figure 4 (data not shown).

Figure 5 shows normalized voxel intensities along a line crossing the 1.1 mm rods of the reconstructed images of figure 4.

The contrast as a function of the rod diameter evaluated from the micro-Jaszczak phantom images reconstructed with method $A_1$ (table 2) is depicted in figure 6. In dual-isotope images, the best contrast was obtained in images reconstructed from the 93 keV photopeak of $^{67}$Ga. Both the GP and HE collimators yielded similar contrast values ranging from 0.75 to 0.90 for this photopeak, only slowly decreasing for smaller rods, whereas the contrast in images acquired with UHS rapidly decreased for rods below 1.3 mm in size. The image contrast of single and dual-isotope acquisitions of $^{67}$Ga photopeaks was comparable.
Figure 3. Transaxial slices from SPECT baseline images of the $^{111}$In (top) and $^{67}$Ga (bottom) micro-Jaszczak phantoms acquired with the ultra-high sensitivity ($^{111}$In) and high-energy ($^{67}$Ga) collimators. The displayed images were obtained by adding ten axial slices. For reference, the rod dimensions (in mm) are reported in the first image.

Figure 4. Transaxial slices from the SPECT images of the $^{111}$In + $^{67}$Ga micro-Jaszczak phantom acquired with the general purpose, ultra-high sensitivity and high-energy collimators. The projection data were reconstructed using energy windows settings $A_i$ applied to the $^{111}$In and $^{67}$Ga photopeaks. For each collimator and photopeak, the displayed images were obtained by adding ten axial slices. For reference, the rod dimensions (in mm) are reported in the first image. The red dashed line illustrates the trajectory of the line profile of figure 5.
Dual-isotope images of $^{111}$In exhibited higher contrast in data reconstructed with the 22–27 keV x-ray peak than in that of the 245 keV photopeak for the GP and UHS collimators. For the HE collimator, however, the 245 keV photopeak of $^{111}$In yielded better contrast than the low energy peaks. The contrast of the single-isotope 22–27 keV and 245 keV peak images was approximately 33% higher than that of the dual-isotope ones for the tested UHS collimator.

Figure 7 shows the relative change of contrast in the micro-Jaszczak phantom images reconstructed with energy window settings $A_i$, $B_i$, and $D_i$ with respect to settings $A_i$ applied to the $^{111}$In and $^{67}$Ga photopeaks. For comparison, the image-contrast of individual acquisitions of $^{111}$In and $^{67}$Ga, namely ‘Single’, are also included.

3.2. Image quantification experiments

The quantification errors of the activity concentrations estimated from the multi-source phantom images (for each collimator and all energy window settings) are plotted in figure 8 ($^{111}$In photopeaks) and figure 9 ($^{67}$Ga photopeaks). For $^{111}$In, the images reconstructed with the 22–27 keV x-ray peak resulted in low quantification errors (<10%) for the GP and HE collimator at $^{111}$In-to-$^{67}$Ga ratios $\geq 1$, and images reconstructed with the 245 keV photopeak yielded the best quantification for the UHS collimator at all investigated ratios. The selection of the energy-window setting had low impact on quantification errors of $^{111}$In images, especially for the 22–27 keV x-ray peak. Overall, the quantification errors of dual-isotope $^{111}$In photopeaks were comparable to those of the single-isotope acquisition.

For $^{67}$Ga images (figure 9), the best quantification was obtained from images reconstructed with the 93 keV photopeak for all the collimators. The images reconstructed from the high-energy peak of $^{67}$Ga resulted in large underestimations of the activity concentration, especially for $^{111}$In-to-$^{67}$Ga ratios $>1$. Furthermore, these errors were substantially affected by the selection of energy window settings (especially for GP and UHS), and were minimized when data were reconstructed with window settings $A_4$. In the single-isotope case, the quantification errors of both 300 keV and the 93 keV peak were less than 5%.
3.3. $^{111}$In/$^{67}$Ga imaging performance under challenging conditions: low activity concentration and small structures

Figure 10 shows the sagittal images (reconstructed with energy window settings $A_i$) of the uniform syringe phantom acquired with the GP, the UHS and the HE collimator using each of the $^{111}$In and $^{67}$Ga photopeaks. The images exhibit very high noise, especially those acquired with the GP and the HE collimators. Please note that no smoothing filter was applied to these images. The average activity concentration along an axis perpendicular to the syringe’s axis is plotted and compared to the true activity concentration. The average quantification errors within each syringe VOI and their variability across voxels are summarized in table 3.

Figure 11 shows the image quality and quantification accuracy of the DI phantom experiments (sections 2.2.1 and 2.2.2.1) under low-count conditions. Overall, we observed an average 7% decrease of the image-contrast in...
both 67Ga and 111In images with respect to the high-count case. The quantification accuracy was generally worse for low-count studies, although errors were still within ±20% except for 111In at very low In-to-Ga ratios.

### 3.4. In Vivo Imaging

Figure 12 shows the biodistribution of 111In-HPG and 67Ga-HPG in the mice from the simultaneously acquired 111In/67Ga SPECT/CT images during the first 24 h after injection. The temporal behaviors of the two radiotracers in the heart are presented in figure 13. The data show instant blood distribution and maximum heart activity of 67Ga-HPG after its intravenous injection, followed by mono-exponential clearance with a measured effective half-life of 22.1 and 30.6 h for study 1 and 2, respectively. 111In-HPG was absorbed from the peritoneal cavity into the circulatory system, reaching a maximum blood concentration at approximately 5.5 h post-injection for

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**Table 3.** Quantification errors (%) ± standard deviation across voxels of the activity concentration measured in the syringe phantom for each collimator and photopeak (reconstructed with energy window settings A_i).

<table>
<thead>
<tr>
<th>Isotope: photopeak</th>
<th>General purpose</th>
<th>Ultra-high sensitivity</th>
<th>High-energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>111In: 22–27 keV</td>
<td>−9 ± 30</td>
<td>−10 ± 30</td>
<td>−17 ± 99</td>
</tr>
<tr>
<td>111In: 245 keV</td>
<td>−19 ± 19</td>
<td>−8 ± 22</td>
<td>−31 ± 73</td>
</tr>
<tr>
<td>67Ga: 93 keV</td>
<td>−29 ± 17</td>
<td>−32 ± 17</td>
<td>−36 ± 70</td>
</tr>
<tr>
<td>67Ga: 300 keV</td>
<td>−12 ± 43</td>
<td>−18 ± 40</td>
<td>−45 ± 86</td>
</tr>
</tbody>
</table>

---

**Figure 10.** Sagittal SPECT images of the uniform syringe phantom acquired with the general purpose (first column), the ultra-high sensitivity (second column) and the high-energy collimator (third column). The line profiles across the images are shown for each reconstructed photopeak (fourth column). Note: to reduce statistical fluctuations, each line profile was calculated as the average value within a ‘strip’ of 200 voxels (dashed box) perpendicular to the line profile, as illustrated in the figure.

**Figure 11.** Contrast (a) and quantification errors (b) of 111In/67Ga images reconstructed from 10% of the total counts compared to the values obtained in images reconstructed from all (100%) counts; data were acquired with the high-energy collimator. The 111In and 67Ga images were reconstructed using the 245 keV and the 93 keV photopeak data, respectively.
both mice and then decreasing slowly thereafter with an effective half-life of 38.2 and 30.3 h for study 1 and 2, respectively.

4. Discussion

4.1. Image quality

Figure 4 shows that, despite the complex energy spectra of $^{111}\text{In}$ and $^{67}\text{Ga}$, DI studies using simultaneous acquisition of these two radioisotopes allow us to distinguish structures as small as 0.85 mm (for GP and HE) and 1.1 mm (for UHS), consistent with what was observed when performing scans with the individual radioisotopes (figure 3). Low image contrast was clearly visible when images were reconstructed from data acquired in the 245 keV for $^{111}\text{In}$ and 300 keV for $^{67}\text{Ga}$ photopeak with the GP and UHS collimators (see figures 5 and 6). Such degradation of image quality was caused primarily by the collimator and pinhole edge penetration of these high energy emissions.

Interestingly, the 22–27 keV $^{111}\text{In}$ images acquired with HE collimator exhibited lower contrast than those reconstructed using high-energy photopeaks despite the fact that the 22–27 keV x-ray peak of $^{111}\text{In}$ is resting on a relatively low background (figure 1(f)). This effect could be due to the fact that the collimator-detector response built-in into the system matrix of the HE collimator is optimized for very high-energy photons.

The analysis of figure 7 indicates that the increase in photopeak width has only a small effect on the image contrast in DI studies. In particular, we observed a decrease of contrast no greater than 5%, on average, for methods $B$, $C$, and $D$ with respect to method $A$. The observed trend was expected because the larger width of the

![Figure 12](image-url)
photopeak window allows for a larger amount of scattered and crosstalk photons to be accepted. Such increase of scatter, however, might not be fully compensated by the TEW scatter correction.

Overall, in terms of image quality, the best performance was obtained for projection data acquired with the HE collimator and reconstructed using window settings $A_i$ applied to the 93 keV of $^{67}$Ga ($A_3$) and the 245 keV of $^{111}$In ($A_2$).

### 4.2. Image quantification

The investigation of the ‘multi-source’ phantom allowed us to explore the quantification accuracy of simultaneous $^{111}$In and $^{67}$Ga pre-clinical SPECT when imaging different ratios of $^{111}$In to $^{67}$Ga. Such analysis is important as the concentration of each radionuclide in a specific target region is unknown a priori and could vary depending on the tracers being investigated.

Our study has shown that quantification accuracies of images reconstructed using the 22–27 keV x-ray peak and the 245 keV photopeak of $^{111}$In remained within $\pm 20\%$ for $^{111}$In-to-$^{67}$Ga ratios $\geq 0.5$ and for all the investigated collimators (figure 8).

Interestingly, the quantification performance of $^{111}$In was not substantially affected by the selection of energy window settings except for the 245 keV photopeak of $^{111}$In at low $^{111}$In-to-$^{67}$Ga ratios ($\leq 1$). Under such conditions, the crosstalk and contamination from the 300 keV peak of $^{67}$Ga into the upper scatter window of the 245 keV of $^{111}$In becomes important, affecting the scatter estimate under the 245 keV photopeak.

As expected, imaging $^{111}$In at low $^{111}$In-to-$^{67}$Ga ratios (below 1) is challenging because $^{67}$Ga emissions dominate the signal in the measured data. Nevertheless, the quantification errors of the 245 keV photopeak were within $\pm 20\%$, and these errors were less sensitive to the $^{111}$In-to-$^{67}$Ga ratio than those of the 22–27 keV x-ray peak. Therefore, we recommend using the 245 keV photopeak to quantify the activity of $^{111}$In in dual $^{111}$In and $^{67}$Ga SPECT studies.

The results presented in figure 9 show that quantitative measurements of $^{67}$Ga in simultaneous $^{111}$In/$^{67}$Ga SPECT could only be achieved when the data were reconstructed using the 93 keV photopeak (errors within $\pm 20\%$ for $^{111}$In-to-$^{67}$Ga ratios $\leq 5$). It is evident by looking at the energy spectra of figure 1(d)–(f) that quantifying the 300 keV photopeak is very difficult mostly due to its low intensity and the proximity of the 245 keV peak of $^{111}$In which has high intensity (relative to the 300 keV peak), even at low $^{111}$In-to-$^{67}$Ga ratios. In fact, when this photopeak was measured individually (single-isotope case), the quantification accuracy remained within 5%.

### 4.3. DI $^{111}$In/$^{67}$Ga imaging performance under challenging imaging conditions

The results presented in figure 10 and table 3 are important for evaluation of the ability of the pre-clinical system to quantify low activity concentrations of $^{111}$In and $^{67}$Ga uniformly distributed over extended sources, resulting in low density of counts. Indeed, the reconstructed images of the uniform syringe exhibit large non-uniformities, except for the data acquired with the UHS collimator. Please note that no smoothing filter was applied to these images. It is worth noting that the calibration factors applied to our phantom studies were derived from individual, crosstalk-free images of $^{111}$In and $^{67}$Ga point-sources scanned in air. The large differences in scattering conditions between the calibration sources and the syringe phantom (see figures 1(a)–(c) versus (d) and (e)),
combined with the inaccuracies of the TEW scatter correction, might explain the large quantification errors reported in table 3. The overall negative bias (which corresponds to an underestimation of activity concentration in images) suggests that, in these conditions, the TEW method might be over-estimating the fraction of scatter photons under each photopeak.

Figure 11 shows that quantifying small structures under low-count DI studies for a wide range of In-to-Ga ratios is still feasible as most of the errors are within ±20%.

4.4. In Vivo Study

The animal study, with an administration of the isotopes into anatomically separate sites, provided an example of the scanner’s ability to provide separated in vivo activity distributions of 67Ga and 111In from simultaneously acquired data. As shown in figure 12, intravenously administered 67Ga-HPG was only found within the blood pool while intraperitoneally administered 111In-HPG was only dispersed through the abdominal region at the earliest imaging point.

This study may be considered as a proof-of-concept that pharmacokinetic assessment of the radiotracers is possible (figure 13). Given the high molecular weight of 111In-HPG, the polymer was absorbed via peritoneal lymphatic into the circulatory system (Mactier et al. 1987). The radiotracer reached peak plasma levels faster than other polysaccharides, such as the dialysis agent icodextrin, when administered into the peritoneal cavity (5.5 h versus 12.7 h) (Mactier et al. 1987, Moberly et al. 2002). The elimination plasma half-life of 111In-HPG at approximately 34 h (average of study 1 and 2) was comparable to previous studies with the polymer (Saatchi et al. 2012b). However, the plasma biological half-life of 67Ga-HPG was found to be shorter at 26 h (on average), in contrast to previous studies that reported a 50.7 h for this parameter; it is difficult to ascertain why the 67Ga-labeled dendrimer was cleared quicker given the n = 2 sample size used in this study (Saatchi et al. 2012a). However the main goal of this study was to demonstrate imaging feasibility rather than exploring the underlying biology, for which more studies need to be performed.

It is worth noting that although study 1 and study 2 were performed with different collimators, the effective half-lives and measured SUV values of the two animals were comparable. Such observation suggests that although the UHS collimator is not the optimal choice according to the phantom studies, it stills provides interpretable images; however a study including a large animal sample size would be required to fully compare the imaging performance obtained with the two collimators in live animal imaging.

4.5. Recommendations and Limitations of the Study

Overall, we recommend using the HE collimator for preclinical DI 111In and 67Ga imaging due to the following reasons: (1) such collimator minimizes the penetration of high-energy photons emitted by 111In and 67Ga, which degrades the image contrast and spatial resolution and; (2) it yields quantitatively reasonably accurate images of 111In (245 keV peak) and 67Ga (93 keV peak) with errors typically within ±20% for a wide range of 111In-to-67Ga ratios.

If this collimator is not available, then the medium-energy collimator (such as the UHS) might be suitable. Despite its limited ability to differentiate very small structures (<1.1 mm in size) in the reconstructed images (figure 4), the UHS collimator yielded quantification errors of 111In and 67Ga activities within ±20% for all the investigated 111In-to-67Ga ratios. The UHS collimator offered the best quantitative performance under very low activity concentration conditions (table 3). This is particularly important for small animal studies, where often acquisition times or injected activities are low.

Lastly, we would not recommend using a low-energy GP collimator for DI 111In/67Ga due to the large amount of collimator penetration from high-energy photons emitted by these two isotopes.

It is important to mention some of the limitations of our study. Firstly, the image-quality of simultaneous 111In/67Ga acquisitions was only investigated using the micro-Jaszczak phantom containing equal activity concentrations of both radionuclides. Similarly, the quantification accuracy was investigated for a limited number of In-to-Ga ratios. Nevertheless, the trend of figures 8 and 9 allows us to extrapolate quantification errors for In-to-Ga ratios that were not directly measured in our experiments.

Finally, it is worth noting that the TEW method is a simple approach to estimate the amount of scatter, background and crosstalk under the photopeak. Other, more sophisticated methods tailored to compensate for crosstalk and scatter in dual-isotope SPECT have been proposed for 99mTc/123I (Du and Frey 2009, Sommerauer et al. 2015), 99mTc/201Tl (De Jong et al. 2002) and 99mTc/111In (Du et al. 2007). These could potentially improve the performance of simultaneous 111In/67Ga imaging compared to the TEW approach. Nonetheless, we decided to apply the TEW method because (a) the number of scattered photons in small-animal studies is low and, (b) it is available in most pre-clinical and clinical reconstruction software.

Although the experiments were carried out using the VECTor scanner, the main results and conclusions can be extended to other pre-clinical SPECT imaging systems with appropriate collimators, as long as these systems employ NaI scintillator detectors and allow for CT-based attenuation and TEW based scatter corrections. These
results, however, might not be fully applicable to clinical systems as the amount of scatter in human studies is substantially higher than that of small animals and therefore the effectiveness of the aforementioned method to remove crosstalk might be low. This challenge could be addressed by developing alternative modified TEW methods which have shown improvement of the quantification accuracy in $^{111}$In/$^{99m}$Tc dual tracers in small-animal SPECT compared to the standard TEW (Prior et al. 2016). On the other hand, the combination of $^{111}$In and $^{67}$Ga in clinical studies might not be desired due to the potentially high radiation delivered to patients as both isotopes have long half-lives. Therefore, other isotope combinations, such as $^{111}$In and $^{99m}$Tc, might be more suitable for humans studies (Zhu et al. 2007, Heiba et al. 2017).

5. Conclusion

Herein, we developed and optimized a quantitative $^{111}$In/$^{67}$Ga dual-isotope SPECT imaging protocol to be used in pre-clinical imaging research. Medium or high-energy collimators allow to minimize collimator penetration, downscatter and crosstalk. Different energy windows settings were tested in order to find the optimal settings for imaging $^{111}$In and $^{67}$Ga simultaneously. Our findings demonstrated that activity quantification errors below 20% were obtained when reconstructing photopeak windows centered at 245 keV ($^{111}$In) and 93 keV ($^{67}$Ga) over a wide range of $^{111}$In/$^{67}$Ga activity ratios. The results of animal studies obtained for two different collimators suggested that concomitant injections of both radiotracers did not affect their individual biological half-life and biodistribution.

Acknowledgments

Funding for this research is acknowledged from the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery Grant 312284-10 to U Häfeli, NSERC Discovery Grant 240670-13 to V Sossi), the Canada Foundation for Innovation (project no. 25413), and the Lundbeck Foundation, Copenhagen, Denmark. This research was also in part supported by the Novo Nordisk Foundation (Grand Challenge Programme: NNF16OC0021948; Natural Sciences and Engineering Research Council of Canada: 240670-13, 312284-10). We are grateful for the support of technicians and veterinarians at the Centre for Comparative Medicine, University of British Columbia, Canada (http://invivomaging.ca).

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