RESEARCH ARTICLE

GATE Validation of Standard Dual Energy Corrections in Small Animal SPECT-CT

Sanghyeb Lee1,2, Jens Gregor1, Stephen J. Kennel2,3, Dustin R. Osborne2*, Jonathan Wall2,3

1 Department of Electrical Engineering and Computer Science, University of Tennessee, Knoxville, TN 37996 USA, 2 Department of Radiology, Graduate School of Medicine, University of Tennessee, Knoxville, TN 37920 USA, 3 Department of Medicine, Graduate School of Medicine, University of Tennessee, Knoxville, TN 37920 USA

* dosborne@utmck.edu

Abstract

This paper addresses 123I and 125I dual isotope SPECT imaging, which can be challenging because of spectrum overlap in the low energy spectrums of these isotopes. We first quantify the contribution of low-energy photons from each isotope using GATE-based Monte Carlo simulations for the MOBY mouse phantom. We then describe and analyze a simple, but effective method that uses the ratio of detected low and high energy 123I activity to separate the mixed low energy 123I and 125I activities. Performance is compared with correction methods used in conventional tissue biodistribution techniques. The results indicate that the spectrum overlap effects can be significantly reduced, if not entirely eliminated, when attenuation and scatter is either absent or corrected for using standard methods. In particular, we show that relative activity levels of the two isotopes can be accurately estimated for a wide range of organs and provide quantitative validation that standard methods for spectrum overlap correction provide reasonable estimates for reasonable corrections in small-animal SPECT/CT imaging.

Introduction

Dual isotope SPECT imaging using radiiodide is a useful technique for performing preclinical comparative effectiveness studies of biological agents, such as antibodies and peptides in individual animals. This technique significantly enhances comparison of reagents in vivo without interference from biological variability in the animal model. We have previously used dual-energy SPECT imaging of amyloid-reactive biological agents, 125I-labeled serum amyloid P component (SAP) and 99mTc-labeled peptide, in a murine model of systemic visceral amyloidosis [1]. This is a very powerful technique for quantitatively comparing two biological radiotracers in an individual animal; however, to date we have used 99mTc and radiiodide for this purpose, which involves two different methods of radiolabeling. Ideally, in comparative effectiveness studies both biological radiotracers should be labeled using the same technique, i.e., oxidative radiiodination of tyrosine side chain moieties [2]. This capability would allow quantitative
comparison of novel amyloid-reactive peptides and the development of next generation re-
agents with improved binding properties [3, 4].

Dual energy imaging techniques are well established, e.g., $^{99m}$Tc-labeled perfusion agent and $^{123}$I-labeled neurotransmitter agents have been used in simultaneous acquisition for SPECT brain imaging [5]. $^{99m}$Tc and $^{201}$Tl are likewise used in rest and stress myocardial perfu-
sion SPECT imaging [6]. The method for correcting spectrum overlap effects between these ra-
dionuclide pairs is relatively straightforward due to the energy gap in the emitted $\gamma$-photons. In contrast, the separation of radiiodine isotopes is more complicated because, although the pri-
mary $\gamma$-ray spectra of $^{123}$I and $^{125}$I are distinct and well-separated, the low energy spectra are
identical with attenuation and scatter effects exacerbating the problem further. We show, and
quantitatively validate with Monte Carlo techniques, that spectrum stripping methods used in
gamma counters for separating the contributions from each isotope provides a sufficient esti-
mation of the degree of spectrum overlap and can confidently be applied accurately to SPECT/
CT imaging [7]. We further show that this simple method is quite robust with little deviation
in the correction regardless of object size or relative activity concentrations with regard to typi-
cal mouse sizes and shapes.

Our work is centered around GATE-based Monte Carlo simulations for the MOBY mouse
phantom [8, 9]. We use statistical system modeling to quantify the amount of spectrum overlap
between $^{123}$I and $^{125}$I in the presence and absence of scatter and attenuation for different organs
as well as different mouse sizes. Performance of the separation method is compared with con-
ventional gamma counter tissue biodistribution measurements of two amyloid-reactive biological
radiotracers in a mouse model of systemic, reactive amyloidosis [10].

**Results and Discussion**

**Conventional gamma counter methods**

Determination of the relative amounts of mixed isotopes in a sample was achieved using a
gamma counter by first measuring the contribution of each radionuclide separately in the ener-
gy windows to be measured and applying spectrum stripping methods [7]. This method is rou-
tinely employed and has been used to determine the spectrum overlap from histological data
[2, 5]. This technique provided a single correction factor that is typically used for the entire his-
tology by subtraction of a percentage of counts from the low energy $^{125}$I window that are de-
derived from $^{123}$I. For this study, the percentage determined from counting individual source
standards in the gamma counter was 43%. The $^{125}$I data from gamma counting of samples of
liver, spleen, kidneys, and heart were corrected for spectral overlap (spillover) by subtracting
43% of the counts measured in the $^{123}$I (high energy) window.

**Quantitative Analysis of spectrum overlap**

Under ideal imaging conditions with no scatter, attenuation, or energy blurring effects, the
simulated ratio $e_{123}$ was 0.49. When energy blurring was simulated, the ratio $e_{123}$ decreased
to 0.42. This expected result occurred because the energy blurring effect is stronger in the low
energy window than in the high-energy window leading to truncation loss. When attenuation
and scatter effects were included in the simulation, we observed that ratio $e_{123}$ increased (Fig
1a). This occurs as a result of high-energy photons scattering into the low energy window. In-
terestingly, most of the scattering events were found to have taken place in the detector hous-
ing. In other words, the water sphere phantoms gave rise to a relatively small amount of
scatter. Using the MOBY phantom under ideal imaging circumstances, the $e_{123}$ ratio was
found to be 0.49. However, when attenuation and scattering was introduced, ratio $e_{123}$
dropped to 0.40. The total count in the high-energy window decreased by 20% because many
of the high-energy photons were scattered (Fig 1b). In contrast, the total count in the low-energy window increased 22% as a result of the scattered high-energy photons being detected in the low-energy window. When scatter and attenuation corrections were both applied, an e123 ratio of 0.53 is obtained (Fig 1b). Visual comparison of simulated versus real data (Fig 2) show similar trends with each indicating less counts in the 123I high energy window and slight reduction in counts when the spectrum stripping correction methods were applied to the low energy window.

**Spectrum overlap estimation and correction**

Amyloidosis in the AA mouse is most prevalent in the spleen, kidneys, liver, and heart; therefore we focused our analyses on these organs. Ratios of e123 were calculated for each of these organs in different sized MOBY phantoms (Table 1). Notably, the low-to-high energy ratio increased slightly (5–10%) as the size of the mouse phantom was increased. The activity detected in the high energy window was associated solely with 123I γ-photons, whereas the activity detected in the low energy window was a mix of 123I and 125I both γ-photons and x-ray photons. High and low energy 123I events are were statistically correlated, thus the low energy 123I component could be accurately estimated from the high energy counts. Separation of the mixed 123I and 125I activity signals detected in the low energy window could then be accurately achieved.

Simulations were carried out for different scanner and radiotracer concentration configurations to verify that the spectrum overlap effects can be suppressed using the simple ratio based correction method. The MOBY phantom was 4 cm wide and 6 cm long. The kidney was used as the source of emissions. For both the MGP and MWB collimators, the relative estimate error increased dramatically when scatter and attenuation medium were included in the simulation and predictably, decreased when appropriate corrections were applied (Table 2). When equal quantities (μCi) of 123I and 125I were simulated, the relative estimation error was 10% under ideal imaging conditions. When the amount of 125I was two-fold greater than 123I, the relative estimation error decreased because the spectrum overlap was reduced. Conversely, when the amount of 123I was present at two-fold the amount of 125I, overlap played a more dominant role due to the relative increase in 123I-derived x-ray photons and the scattered 123I high-energy γ-photons being detected in the low-energy window (Table 2).
Materials and Methods

GATE Model and Data Processing

Spectrum overlap between $^{123}$I and $^{125}$I was quantitatively assessed by running GATE simulations for a selected set of imaging conditions. We used a validated GATE model of the Siemens Inveon multi-modality imaging platform (Fig 3a), which features a dual-head SPECT system with interchangeable collimators [11]. This model is available for download at the following hyperlink: GATE Model Inveon Download.
Two tungsten collimators were used in the simulations. Collimator shape and material characteristics were modeled according to the manufacturers specifications in order to accurately account for resolution and other relevant data acquisition effects. The mouse whole-body collimator (MWB) has a five-pinhole configuration with 1.0 mm pinholes that supports whole body imaging of mice (Fig 3b). The mouse general purpose (MGP) collimator is a single pinhole, high-resolution imaging collimator with a 1.0 mm pinhole.

Simulated mouse data were generated using the MOBY phantom software (Duke University, Durham, NC) which features realistic organ shapes while maintaining the flexibility to model anatomical variations (Fig 3c) [9]. Liver, spleen, kidneys, and lung organs were simulated using the software and the resulting map of attenuation coefficients were converted to voxelized phantoms for use as the material in the realistic GATE model. 125I and 123I concentrations for each of the simulated organs were determined from real mouse data and the appropriate concentration distributed uniformly throughout each of the simulated organs for use as the source distribution input for the GATE model. Parameters used for the MOBY phantom are included as supporting information, S1 Data.

A 45 minute SPECT acquisition was simulated with the two detectors located respectively at 90 and 270 degrees at the start of simulation. Sixty projections were acquired with projection data acquired every 6 degrees over a 360-degree gantry rotation. For the MGP collimator, a 25 mm radius of rotation was used. For the MWB collimator, a 30 mm radius of rotation was used.
The Inveon Acquisition Workplace (IAW) software version 1.5 (Siemens Medical Solutions USA, Inc., Knoxville, TN) was used to acquire and reconstruct tomographic images from the simulated phantom projection data and real animal images. Using this software, CT-based attenuation correction and dual-energy window scatter correction \cite{12} was applied to data from both the high and the low energy windows. Data were also reconstructed without attenuation and scatter correction to determine its impact on calculated ratios.

Each iodine isotope was modeled using their three primary energy peaks. All other energy peaks were omitted. For $^{123}$I, the energy peaks used were 528 keV (1.1%), 159 keV (48.4%), 31 keV (9.3%), and 27.5 keV (41.1%). For $^{125}$I, the energy peaks used were 35.5 keV (4.5%), 31 keV (17.6%), and 27.5 keV (77.9%). One detector head was configured to have an energy window of 148170 keV with an energy blurring of 11% while the other detector head was set to have an energy window of 2545 keV with an energy blurring of 28%. Fig 4 shows the two spectra as detected with the MGP collimator.

**Spectrum Overlap Estimation and Correction**

Data were separated into high and low energy windows represented as HI and LO, respectively. Each window contains all the counts acquired within that window for a given isotope where we use the terminology HI123 and LO123 referring the spectrum based $^{123}$I activities, and LO125 for the low energy $^{125}$I activity. Under ideal circumstances, where attenuation, scatter, and effects such as detector induced energy blurring can be ignored, it is well-known that the low-to-high energy $^{123}$I ratio given by:

$$e_{123} = \frac{LO_{123}}{HI_{123}}$$ \hspace{1cm} (1)

can be used to estimate the $^{125}$I activity in the low energy window using the formula:

$$(LO_{125} = LO - e_{123}HI)$$ \hspace{1cm} (2)

To investigate how each physical and detector interaction influences the low energy photon spectrum overlap, GATE simulations were carried out for the following scenarios: (1) ideal imaging situation (assumption of no attenuation or scatter), (2) energy-blurring was considered, and (3) energy-blurring, attenuation, and scatter effects were all taken into account and the source was located inside different sized water spheres with radius varying from 0.5 cm to 3.0 cm.
cm. Each simulation was designed to represent 200 μCi $^{123}$I in a uniform sphere (radius = 3.91 mm) with the activity distributed over a 250 mL volume. The effect of different activity concentration ratios of $^{123}$I to $^{125}$I were also examined in our simulations with this uniform spherical phantom. The ratios simulated were 1:1, 1:2, and 2:1 for ideal conditions (no attenuation or scatter), attenuation and scatter simulated but not corrected, and attenuation and scatter simulated but corrected for in reconstruction.

GATE simulations were run to estimate generic organ based $e_{123}$ ratios for different sized mice with length varying from 4cm to 8cm. The $e_{123}$ ratios were pre-computed for different organs under various circumstances using the MOBY phantom output in the GATE simulation. These pre-computed $e_{123}$ ratios were then used to estimate the amount of spectrum overlap effects in the low energy window of real mouse data using Eq (2). The accuracy of the correction was captured by the difference between the estimated and the actual $^{125}$I activity measured relative to the latter. We refer to this as the relative estimation error given by:

$$r_{^{125}} = \frac{\text{Actual}_{^{125}} - \text{Estimate}_{^{125}}}{\text{Actual}_{^{125}}}$$

where Actual denotes the actual number of I photons detected and Estimate denotes the estimated number computed using Eq (2).

Each simulation was repeated five times to acquire a mean value for each result. Standard deviations were calculated for each series of simulations and compared. The coefficient of variation (COV) was calculated for series of simulations. COVs of 20% and 10% were deemed acceptable for individual and entire population averages, respectively.

**Animal Studies**

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Tennessee. Animals for this study are housed in our IACUC approved dedicated animal facility.

To compare our models with standard methods, we acquired SPECT and CT scans of three mice with systemic AA amyloidosis using an Inveon trimodal (PET/SPECT/CT) imaging system. Each mouse was injected with 145 μCi of $^{125}$I-protein 1 and 45 μCi of $^{123}$I-protein 2. After the appropriate uptake time (2 h after injection of the second protein), the mice were euthanized by isoflurane overdose and prepared for SPECT/CT imaging. Sixty projections were acquired at 6-degree intervals over 360-degrees. A full energy window from 0-300 keV was acquired and subsequently histogrammed into three energy windows for both detector heads. For detector 1, with voltage and gain set to acquire $^{123}$I emission data, we used 127–143keV, 143-175keV, and 175-191keV windows. For detector 2, with voltage and gain set to acquire $^{125}$I emission data, we used 15-25keV, 25-45keV and 45-55keV windows. SPECT and CT scans were used to estimate $e_{123}$ ratios using Eq (1).

After image acquisition, the mice were sacrificed, necropsied, organs harvested with a small piece placed in a tared vial, and counted in a Wizard 3 1480 gamma counter (Perkin Elmer, Waltham, MA). The amount of $^{123}$I and $^{125}$I radioactivity in each sample was measured. Spectrum overlap corrections for this technique were applied by using pure samples of both isotope and calculating the low-energy component of the $^{123}$I. These data were also used to calculate the $e_{123}$ ratios using these standard methods. Because of differences between output of the imaging system and gamma counter, data acquired from both systems were corrected to provide units of percent injected dose per gram (％ID/g) decay corrected to the animal injection time.
Supporting Information

S1 Data. Data parameters file for MOBY phantom generation.
(ZIP)

S1 ARRIVE Checklist. Completed ARRIVE checklist.
(PDF)

Author Contributions

Conceived and designed the experiments: SL DO JG SK JW. Performed the experiments: SL DO SK. Analyzed the data: SL DO JG. Contributed reagents/materials/analysis tools: JW DO SK. Wrote the paper: SL DO JW JG SK.

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