Dual isotope cross-talk correction in Tc-99m/In-111 small-animal
SPECT

by

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Abstract

Dual-isotope imaging via energy discrimination is a major strength of SPECT but image quality is degraded by cross-talk interference. Direct application of clinically developed cross-talk correction techniques in small-animals is not ideal. Reduced subject size may allow adequate quantification with simpler cross-talk correction methods. This study evaluated four simple cross-talk correction methods for In-111/Tc-99m small-animal imaging: triple energy window (TEW), applied pre- and post-correction, convolution subtraction, and a vendor-supplied correction. Each method was evaluated using a three-syringe phantom filled with either Tc-99m, In-111, or a mixture of the two at five different ratios. The optimal method was tested on in-vivo rat images. A modified TEW applied in projection space visually provided the best removal of In-111 cross-talk and reduced it quantitatively by 96%. The mixed syringe ratios were recovered to within 5%. In-vivo testing provided inconclusive results indicating that a true dual-isotope study is still necessary.

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Glossary of Acronyms

ATP adenosine triphosphate.

CT computed tomography.

DEW dual energy window.

EC electron capture.

FBP filtered backprojection.

FDG fluoro-deoxy glucose.

FWHM full width at half maximum.

IT isomeric transition.

LEHR low energy high resolution.

MLEM maximum likelihood expectation maximization.

NaI(Tl) sodium iodide doped with thallium.

OSEM ordered subset expectation maximization.

PET positron emission tomography.

PMT photomultiplier tube.

PSF point spread function.

ROI region of interest.

SPECT single photon emission computed tomography.

TEW triple energy window.

Chapter 1

Introduction

1.1 Motivation

The two leading causes of death in Canada are cancer at 29.6% and heart disease at 21.3% [1]. Together these two account for just over 50% of the deaths in this country. These are staggering statistics and in an effort to improve them, a large portion of research is dedicated to studying these diseases and determining their causes, effects, treatments and prevention. One of the best methods of doing this is by studying these diseases in small animal models. By studying disease using small animal models, it is possible to probe the disease in an in-vivo situation that can be manipulated to express different genes, proteins or other risk factors [2].

There are many different methodologies that can be used to investigate these animals, however, one of the most powerful approaches is through imaging. Imaging has several advantages over conventional histology, the main one being that imaging is done in-vivo while histology is not. Imaging is therefore able to acquire information without disturbing the system. Another advantage is the statistical power of imaging. Since imaging is a non-invasive modality, in many situations, no additional control animals are required as each animal can act as its own control. The animal can be imaged pre-condition as the control and post-condition as the study. This reduces the effects of unpredictable variability that occurs between animals. The ability to use

smaller numbers of animals also reduces the costs associated with the study which is always an important consideration. Imaging also allows the animals to remain alive eliminating the need for their sacrifice to gain histological information and permitting serial imaging of the same animal. Lastly, imaging provides a visualization of the full three-dimensional volume of the animal instead of only a select sample.

1.2 Nuclear Medicine

In the field of nuclear medicine, radiation emitted by radioactively labelled pharmaceuticals is used to visualize the distribution of said pharmaceutical within the body. The tracer (pharmaceutical + radioactive isotope) is injected into the body and radiation that exits the body is detected by a camera forming projections (a sum of the activity distribution) which can be reconstructed into a 3D image of the isotope's distribution in the body. The distribution is related to the pharmaceutical to which the isotope is bound. The radioactive isotope is generally chemically bound to a compound involved in a certain metabolic process and therefore will accumulate in the body in places where this process is occurring. An example of this is fluoro-deoxy glucose (FDG) which is an analogue of glucose. It enters cells to be used as energy the same way glucose does. Once inside the cell, glucose is converted to adenosine triphosphate (ATP) by a multi-step process. Once FDG has undergone the first portion of this conversion process, it becomes trapped in the cell due to its differences from glucose. It then generates a signal that can be used for imaging [3].

There are two main types of nuclear medicine imaging: positron emission tomography (PET) and single photon emission computed tomography (SPECT). In both cases, gamma rays are detected. The two methods differ in how these gamma rays are created and the way in which they are detected. In SPECT, the gamma rays originate from the primary decay of an atom. In PET, the gamma ray arises from a secondary

interaction between an emitted positron and an electron causing annihilation. The energy of the positron and electron are converted into two annihilation photons that are emitted at 180° from each other and must be detected in coincidence in order to register as an event. This thesis focuses on small-animal imaging with SPECT.

Figure 1.1: A schematic of a SPECT camera and the data acquisition process. The detector of a SPECT camera is a NaI scintillation crystal fronted by a lead collimator, that rotates around a patient containing a distribution of radioactivity. As the detector rotates, data are acquired at a number of different positions (projections), three of which are indicated by the dashed components.

In SPECT imaging, the photons emitted by the radioactive isotope are detected by a crystal that spans a finite length covering only a portion of the camera bore. This detector is rotated around the patient at discrete angles, forming one projection of the activity distribution within the patient at each angle (see Figure 1.1). For each photon that is detected, information about its energy as well as its detected position are recorded. After all projections have been captured, a reconstruction algorithm is employed to determine the three-dimensional activity distribution.

1.2.1 Radioactive Decay

The phenomenon that allows us to do nuclear medicine imaging is radioactive decay. When an atom is unstable, it has extra energy that must be released. It does this by decaying to a more stable state and in the process, releases a particle or photon which carries away the extra energy [4]. Measuring these emitted particles is the basis of nuclear medicine imaging. The rate at which this decay occurs (or the activity) varies depending on the atom but is governed by the decay constant λ and is proportional to the number of decaying atoms present in the radioactive sample:

$$\frac{dN}{dt} = -\lambda N. \quad (1.1)$$

Integrating this relationship we find that the number of decaying atoms remaining (N) drops off exponentially with time where N_0 is the number of atoms present at time $t=0$.

$$N = N_0 e^{-\lambda t}. \quad (1.2)$$

Activity, which is the quantity dealt with in nuclear medicine, is a measure of the decay rate or number of atoms decaying per second therefore $A = \lambda N$. From this we can see that the activity of a sample will also decay exponentially with time (where A_0 is the activity at time $t = 0$).

$$A = A_0 e^{-\lambda t}. \quad (1.3)$$

This decay can take place by a number of different mechanisms including emission of an α particle, a β^- particle, a β^+ particle (also called a positron), or a photon via electron capture (EC) or isomeric transition (IT). In the case of the first two, these particles have low penetrating power and if they are emitted from inside the body, they are next to impossible to detect [4]. β^+ particles, like α particles, cannot penetrate

the body and be detected, however when they are emitted they annihilate with the body's electrons almost instantly and in doing so emit two photons of 511 keV that travel in opposite directions. Photons have much greater penetrating power than α and β particles and, therefore, exit the body permitting their detection. Measuring these photons in coincidence is the basis of PET imaging.

SPECT imaging uses unstable nuclei which emit photons directly either by electron capture (EC) or isomeric transition (IT). In electron capture, the nucleus of the atom captures an electron from one of its orbitals (usually those closest to the nucleus) and the proton transforms into a neutron. The vacancies left in the electron orbital shells by EC are usually filled by electrons transitioning down from higher orbital shells. The energy lost by these transitioning electrons is emitted as characteristic x-rays. These x-rays are usually of low energies (less than 25 keV) and do not exit the body [4]. An exception to this is Tl-201 which decays to mercury whose characteristic x-ray emissions are about 70 and 80 keV and can be used for imaging. This type of decay can also leave the daughter nucleus in an excited state which will decay via emission of a higher energy photon. These higher energy photons easily exit the body and can be detected. If the excited state of the daughter nucleus lasts for a long time, it is said to be in an isomeric state or a long-lived metastable state [4]. Isomeric states eventually also decay to a ground state and release their energy by emitting a photon (isomeric transition) which can be detected.

1.2.2 Tracers

The acquisition of nuclear medicine images requires the injection (or ingestion) of a radioactive tracer into the subject. A tracer can be a radioactive atom attached to a biological molecule or could also simply be a radioactive atom on its own [2]. An example of the latter is Tl-201. This tracer acts as an analogue of potassium in the body so it is taken up by the Na-K ATPase pump [3]. This tracer is taken up

preferentially by the heart and so serves as a marker of blood flow in heart tissues. Care is taken so that the active portion of the molecule is not disturbed by the addition of the radioisotope, an atom that is not normally found in a biological molecule. The behaviour of the tracer in the body is thus the same as the original molecule on which it is based [3]. In most cases, only very small concentrations (trace quantities) of the compound are used so as to measure the underlying process, but not disturb or alter it.

Another approach to tracers is to create an analogue that does not behave the same as the original molecule [3]. This is also done by labelling the molecule with a radioactive atom but in doing so, the chemical properties of the atom are altered. This is advantageous because it can create a situation where the tracer reacts only to a specific biological process instead of many. Alternatively the tracer can be trapped inside a specific type of target cell. This type of tracer is useful for isolating and targeting very specific processes. An example of this is Tc-99m tetrofosmin. This tracer relies on diffusion to enter the cell and once inside, the molecule becomes trapped by the mitochondria [3]. Some of the most common isotopes used in SPECT imaging are shown in Table 1.1.

Table 1.1: Common isotopes used in SPECT imaging adapted from Saha [5].

Isotope	Principal Decay Mode	Physical Half-life (h)	Gamma Energy (keV) (% Abundance)
Tc-99m	IT	6.0 hours	140 (90)
In-111	EC	2.8 days	172 (90), 245 (94)
Ga-67	EC	78 hours	93 (40), 184 (20), 300 (17), 393 (5)
I-123	EC	13.2 hours	159 (83)
I-131	β^-	8.0 days	284 (6), 364 (81), 637 (7)
Tl-201	EC	73 hours	167 (9.4), X-rays of 69-83 (93)

Isotopes Used

In this thesis, we investigated the combination of two isotopes: Tc-99m and In-111. These are valuable isotopes to study because they can be used in combination in

small-animal cardiac studies. Furthermore, both isotopes also have a range of other uses. Tc-99m is a metastable state of technetium which decays via the emission of a 140 keV photon and has a half-life of 6 hours. When attached to tetrofosmin, this tracer has a high affinity for uptake in the heart and as such is the isotope of choice for heart perfusion imaging. Tc-99m-tetrofosmin is also cleared quite quickly from the liver which is optimal when doing perfusion imaging due to the close proximity of the liver to the heart. In addition, Tc-99m has a short physical half-life so the patient is not radioactive for an extended period of time. After 24 hours, 93.75% of the original dose has physically decayed. The removal of the Tc-99m is accelerated even more by the biological half-life creating an even shorter effective half-life. After 48 hours, essentially all of the isotope has been eliminated from the body.

In contrast to Tc-99m, In-111 is a longer lived isotope with a half-life of 2.8 days, decaying via the emission of photons at 172 and 245 keV. One possible tracer labelled by In-111 is annexin which has a high affinity for cells that are undergoing apoptosis (programmed cell death). This can be used in combination with Tc-99m-tetrofosmin to show areas of irreparable damage to heart tissue. In addition to annexin, In-111 can also be used to label stem cells, giving the ability to image after treatments and determine where the stem cells congregate and track progress after the treatment. The long half-life of In-111 makes it ideal for this type of tracking as it remains in the body for weeks after injection allowing for multiple imaging sessions at different time points during a treatment.

1.3 Photon Interactions

The gamma rays that are produced by the decay of the tracer must pass through the patient, the collimator and interact with the detector scintillation crystal before they are registered in the detection electronics. As the photons pass through these

different materials they may interact. There are four types of interactions that may occur. The probability of each is determined by the energy of the photon as well as the type of material it is passing through [4]. The four interactions are Rayleigh scattering, Compton scattering, photoelectric absorption and pair production. Each will be discussed in further detail in the subsequent sections.

1.3.1 Rayleigh Scatter

In Rayleigh scattering, (also referred to as coherent scattering) the entire atom is excited by an incoming photon. The atom stabilizes by emitting a photon of the same incident energy but at a different angle. The scattering angle of the emitted photon decreases as it's energy increases [6]. This type of interaction occurs primarily at low energies and therefore is not a primary concern in SPECT imaging.

1.3.2 Compton Scatter

In contrast to Rayleigh scattering, in Compton scattering the photon interacts not with the whole atom but with one of the valence electrons. The photon transfers energy to the electron so that the electron is ejected from the atom, leaving the scattered photon with less energy (Figure 1.2) [6]. To satisfy the necessary conservation of energy rule, it can be written that:

$$E_i = E_f + E_{e-}. \quad (1.4)$$

Employing conservation of momentum, this can be rewritten to find the energy of the scattered photon in terms of the incident energy and the scattering angle of the photon.

$$E_f = \frac{E_i}{1 + \frac{E_i}{511\text{keV}}(1 - \cos(\theta))}. \quad (1.5)$$

On examination of Equation 1.5 one can deduce that as the incident energy increases, the distribution of scattered photons becomes more forward peaked. As well, as the scattering angle increases, more and more energy is transferred to the scattered electron [6].

Figure 1.2: Compton scattering of a photon. The electron acquires sufficient energy to escape the atom while the photon is scattered and leaves the atom in a different direction and with reduced energy.

At the energies used in SPECT imaging, the majority of photons are scattered in the forward direction and retain a large portion of their initial energy. As a result, the majority of these scattered photons still have sufficient energy to travel through the patient and reach the detector. The detection of these scattered photons contaminates the primary (unscattered) photon signal and has undesirable effects on image quality.

Since this interaction depends on the presence of electrons, its probability increased in materials with a higher electron density [6]. Thus, the magnitude of scatter is dependent on the distribution of different tissues within the patient or object.

1.3.3 Photoelectric Absorption

Photoelectric absorption is similar to Compton scattering in that the photon interacts with the electrons of the atoms in the scattering material. In this interaction however,

the photon is fully absorbed by the electron, which is in turn ejected from the atom (Figure 1.3) [6]. The resulting energy of the ejected electron is the difference between the incident photon energy and the binding energy of the electron. The energy of the photon must be greater than the binding energy in order for this type of interaction to occur.

Figure 1.3: Photoelectric absorption of a photon. The photons energy is absorbed by the electron which then gains enough energy to escape the atom.

In photoelectric absorption the ejected electron is not necessarily a valence electron. As the energy of the photon increases, it will be able to eject electrons from inner shells which have higher binding energies. The electrons from the most inner shell will be preferentially ejected so long as the photon has sufficient energy to do so [6]. If an inner shell electron is ejected, it leaves a vacancy in that shell which needs to be filled. This vacancy is filled with an electron of lower binding energy. The vacancy of that electron is filled again with one of lower binding energy and so on, creating an electron cascade. This difference in binding energy between the two shells is released as characteristic x rays Auger electrons [6].

1.3.4 Pair Production

In the case of pair production, the incoming photon is now interacting with the nucleus of the scattering atom instead of the electrons [6]. The energy of the photon

is converted into an electron positron pair, hence the name. The threshold energy for this interaction is twice the rest mass of an electron (the sum of the electron and positron rest masses) or 1.022 MeV. Any energy the photon possesses above this threshold is converted to kinetic energy for the electron and positron [6]. Since the energies of the gamma rays emitted by most SPECT isotopes are well below 1 MeV, this type of interaction is not seen in SPECT imaging.

1.4 Anger Camera

The Anger Camera was first developed by Hal Anger in 1958 [7]. The traditional Anger camera consists of 5 components which each play a role in the detection of the photons (Figure 1.4). First, the photons meet the collimator which filters the photons based on their direction. Photons that pass through the collimator will then reach the scintillation crystal. When a photon interacts with the scintillation crystal, a pulse of light is emitted by the crystal. This pulse of light photons is amplified by a series of photomultiplier tubes (PMTs) and then sent through positioning electronics to determine the detection position [8]. This process is described in more detail in the following sections.

1.4.1 Collimator

The purpose of the collimator in the Anger camera is to limit the direction of photons that reach the scintillation crystal. By doing so, the ambiguity in the direction of the photon source is reduced making a clearer image and making it possible to reconstruct a 3D image. The collimator consists of a large slab of highly attenuating material (usually lead or tungsten) with a pattern of holes drilled into it. The configuration of the holes allows only certain photons to pass through while all others are attenuated in the material and do not reach the crystal. There are two main types of collimators:

Figure 1.4: The Anger camera used in SPECT imaging consists of five components: collimator, crystal, light guide, photomultiplier tubes and a computer. Photons are emitted from the radioactive source within the patient. Those photons not travelling perpendicular to the detector are blocked by the parallel hole collimator. Photons passing through the collimator interact with the crystal where they are converted to light photons which are directed towards the PMTs by the light guide. The PMTs convert the light photons to an electronic signal which is amplified and then sent to the computer for processing.

the parallel hole collimator and the pinhole collimator (shown in Figure 1.5). Each is discussed in more detail in the following two sections.

Parallel Hole Collimator

The parallel hole collimator is the most commonly used collimator in clinical imaging. It consists of thousands of tiny parallel holes drilled through the material. The parallel hole collimator allows only photons travelling normal to the detector to pass through. All other photons are absorbed in the septa (the walls between the holes) of the collimator [7]. By limiting the angle of the accepted photons, this design allows

Figure 1.5: Configuration of the two most common collimator types: A) parallel hole collimator and B) pinhole collimator. The parallel hole configuration contains thousands of small parallel holes which accept photons at only a small range of angles while the pinhole configuration contains only a single hole and accepts photons at a much wider range of angles. Here, h is the patient-to-collimator distance, d is the hole diameter, t is the septal wall thickness, l is the collimator length, f is the focal length of the collimator and θ is the acceptance angle of the collimator.

for improved resolution at larger patient-to-detector distances compared to pinhole collimators [9].

A popular version of the parallel hole collimator, called the low energy high resolution (LEHR) collimator, has been developed for low-energy applications. In this version, the holes are drilled close together so the septa are very thin and the thickness of the collimator is also decreased. These approach greatly improves the sensitivity for low energy applications without compromising the resolution [4].

When using a parallel hole collimator, the field of view of the camera as well as the sensitivity remain constant for all patient-to-collimator distances [6]. Although resolution decreases with distance from the collimator, this can be controlled using the ratio of hole diameter and collimator length [5]. In contrast, the resolution very close to the collimator is limited by the intrinsic resolution of the camera which is

typically 3-4 mm. Typical resolution using a LEHR is 7 mm at a distance of 10 cm from the collimator [7].

Pinhole Collimator

The pinhole collimator, as its name suggests, is comprised of a single circular hole drilled in the attenuating material. All photons that reach the crystal must pass through a single hole in the collimator, but may do so at a wide range of incident angles. This collimator acts as a magnifier with the amount of magnification depending on the ratio of the distance of the object from the pinhole (h) and the focal length of the pinhole (f) [10]. This type of collimator is ideal for imaging small organs or small animals as it can provide a large amount of magnification by placing the patient close to the collimator. With short patient-to-detector distances, this type of collimator is able to achieve improved resolution but at the cost of sensitivity [10]. With smaller patient to collimator distances, more photons will be attenuated by the collimator and will not reach the crystal. Pinhole collimators have limited use in the clinic but have been used extensively in small animal imaging. This will be discussed further in section 1.6.

1.4.2 Scintillation Crystal

The scintillation crystal is the part of the camera which actually detects the photons through absorption in the crystal. Depending on their energy and the composition of the scintillation crystal, the photons are generally absorbed by a photoelectric event, or by a cascade of Compton scattering events followed by a photoelectric event [11]. Compton scattered photons or characteristic x-rays released in photoelectric events may exit the crystal leaving only a portion of the energy deposited in the crystal. If a large amount of energy is lost, the photon event may be rejected as falling outside the permissible energy range. The typical crystal material is sodium iodide doped

with thallium (NaI(Tl)) with a thickness of 9.5 mm. The detection efficiency at this thickness is around 90% for 140 keV (Tc-99m) photons [5]

When the energy of the photons is deposited in the crystal, it creates electron-hole pairs. As these pairs recombine, they can emit light photons isotropically [12]. The number of electron-hole pairs, and consequently the number of visible light photons produced, is proportional to the deposited energy of the incident gamma ray. Thus, the magnitude of the signal generated by the crystal can be used to measure the energy of the incident gamma and thereby distinguish between gamma rays of different energies.

1.4.3 Photomultiplier Tubes (PMTs)

The detector crystal is coupled to a number of PMTs via a light guide. The purpose of the light guide is to ensure that the maximum number of light photons reach the PMTs. It does this by redirecting photons that would otherwise pass between the photomultiplier tubes [13]. When the light photons reach the PMTs, they are converted to an electronic signal by a photocathode. This electronic signal is very small so before any processing is done, the signal is amplified. This is done by the PMTs.

The photoelectrons created by the photocathode are sent through a series of dynodes with increasing electric potentials. The electric field created by the potential difference accelerates the electrons toward the dynodes and the impact of the electron on the dynode creates a shower of electrons. This shower of electrons is then accelerated towards the next dynode creating more showers (seen in Figure 1.6). This repeated creation of electron showers multiplies the number of electrons again and again [12]. This serves to amplify the signal to a level that is readable. The number of electrons after the multiplication process is proportional to the number at the beginning which is in turn proportional to the light photons converted. The number of light photons is proportional to the energy absorbed in the crystal, therefore the total

charge output from all PMTs is proportional to the energy of the absorbed gamma photon [12].

Figure 1.6: Signal amplification process that occurs within the photomultiplier tube. Light photons that are incident on the photocathode are converted to electrons which are then accelerated from dynode to dynode by increasing potential differences. The impact of the electrons on each dynode creates a shower of electrons which are accelerated and multiplied at subsequent dynodes.

1.4.4 Positioning Electronics

The PMTs are arranged in a hexagonally structured fashion to minimize the gaps between them. The isotropic emission of the light photons means that for a single gamma photon absorption, light photons will be absorbed in all PMTs but at different intensities with a larger portion reaching PMTs close to the absorption event and only a few reaching those farther away. This distribution of photons is used in determining the position of the initial absorption event by using linear combinations of the signals from each PMT [11].

In the original Anger camera, the resulting electronic signal from each PMT was sent through an analogue resistor network which weighted the different signals to determine the original detection position of the incident gamma ray [8]. Currently, the signal from each PMT is digitized. The signals are still weighted based on their positions within the PMT grid. The position of the detection event is then determined

through a positioning algorithm, which is commonly a maximum likelihood algorithm. Although this achieves a similar end point to that of the original analogue circuitry, it does so with much greater accuracy and flexibility.

1.5 Reconstruction

In SPECT imaging, the information that is collected by the detector is in the form of projections. Each element in a projection (or pixel) represents the sum of the activity in all locations along a line through the field of view [3]. This is depicted in Figure 1.7(a). Although projections can provide some useful information, if we want to know the activity concentration at a specific point (for instance in a tumour or heart infarct) is not easily seen in the projection data. To obtain this type of information it is necessary to reconstruct the projection data into a 3-dimensional representation of the activity distribution present within the field of view of the camera. There are several methods that have been developed to do this and they are described below.

1.5.1 Filtered Back Projection

One method of reconstruction is to take the projection values and back project them along the lines by which they were created (Figure 1.7). If the projections formed are $g(s_k, \theta_k)$ where s_k is the position along the detector and θ_k is the angular position of the detector, then the back projection image ($b(x, y)$) is found by

$$b(x, y) = \sum_{k=1}^p g(s_k, \theta_k) \delta\theta, \quad (1.6)$$

where p is the number of projection angles and $\delta\theta$ is the angular separation between projections [14]. Practically, this means finding the point s_k in each angular projection that makes a contribution to a specific point in b . The contributions from all $g(s_k, \theta_k)$ s are summed and then divided by p to find the value of $b(x, y)$. Since at different

angles, the pixels of the image contribute varying amounts, the sum of $g(s_k, \theta_k)$ must be weighted depending on the length of line that passes through it [14].

Figure 1.7: A visualization of the projection and backprojection operations. A) In the projection operation the activity (dark grey) present along each line through the patient (light grey) is summed at the detector to determine the projection pixel value. The activity distribution is given by $f(x,y)$, the projection angle by θ and the measured k th projection by $g(s_k, \theta_k)$ where s_k represents the transverse position on the projection. A graphical representation of the projection image is shown for multiple angles around the patient showing the shift in the location of the activity within the projection with angle. B) In the backprojection operation, the measured projections are smeared back across the field of view so that each point along the projection line contains the same amount of activity. This creates blurring in the image. Where the backprojected rays from different projections intersect, the values are summed.

It is important to note here that the operation of backprojection is not the inverse of projection and we do not get exactly the original activity distribution ($f(x,y)$) by doing this. Instead, we get a blurred version of $f(x,y)$. This is because the angular sampling in the image falls off with radial distance [14]. This can be seen in Figure 1.7 where the density of backprojected data is high near the centre but low near the edges. After back projection, the intensity of the image adds up well at the centre of the source, but is diluted as r increases causing $1/r$ blurring. The method developed to help with this blurring is filtered backprojection (FBP). Before back projecting, the

projections are filtered in the Fourier domain using a ramp filter (a filter where the weight at each frequency is the absolute value of the frequency itself) [14]. In the Fourier domain, low spatial frequency information is responsible for slowly changing pixel values in the spatial domain while high frequency information is responsible for rapidly changing pixel values. The ramp filter serves to suppress the low frequency portion of the data to eliminate the blurring caused by back projecting while giving a higher weighting to the high frequency information which increases the sharpness of edges in the back projected image. In the spatial domain this effectively deconvolves the image from the $1/r$ blurring kernel [14].

The drawback to this type of filtering is that although the edges are enhanced and the blurring is reduced, the noise in the image is also increased. Generally, object data is predominantly found in the lower frequencies [14]. By using the ramp filter, these frequencies are being suppressed while the high frequencies which are predominantly noise, are enhanced. The use of other types of low-pass filters have been implemented in an effort to restrict this noise enhancement (Butterworth, Hamming, Hann, etc..). These filters suppress the high frequency information associated with noise while leaving low frequency information unchanged [14]. Although this helps with the noise problem, it also degrades the spatial resolution of the image.

1.5.2 Iterative Methods

In iterative reconstruction methods we are looking at the same problem of finding a solution to f (the true activity distribution) from the measured data g where $g = Af$. The matrix A here is the matrix that defines the forward projection operation. The solution is found by creating an estimate at f , projecting it, and then comparing the estimate projections to the measured projections. The difference in the estimate and the measured projections is used to update the estimate and then the process is repeated until the two converge [14]. The difference between iterative algorithms

arises in how the estimate is updated and also in how much information is included in generating projections from the guess [14]. By using iterative methods, information to accurately model the physics of the camera can be included in the reconstruction process. The most popular iterative reconstruction method is the maximum likelihood expectation maximization method.

Maximum Likelihood Estimation Maximization

The maximum likelihood method takes into account the Poisson noise that exists in the projection data. When a radioactive isotope undergoes radioactive decay, it emits photons at an average rate which is measured by its activity (decays per second). In reality this rate is not constant and is governed by Poisson statistics [14]. If the number of photons emitted in a set period of time is measured many times, the average rate would be equal to the activity and the spread in the measurements would follow a Poisson distribution. Since when measuring projections we make only one measurement, we do not know at which point along the Poisson distribution this value would fall. The maximum likelihood expectation maximization (MLEM) algorithm estimates f by trying to find the value $\bar{f}$ that has the maximum probability of producing the measured projections [14]. The algorithm used in the MLEM method is as follows [14]:

$$\bar{f}_j^{(k+1)} = \frac{\bar{f}_j^{(k)}}{\sum_{i=1}^n a_{ij}} \sum_{i=1}^n \frac{g_i}{\sum_{j'=1}^m a_{ij'} \bar{f}_{j'}^{(k)}}. \quad (1.7)$$

In this equation, g_i is the i th pixel of a projection, $\bar{f}_j$ is the mean number of disintegrations in a pixel assuming a Poisson distributed variable and a_{ij} is the element in i th row and j th column of the matrix A . A more simplified way of looking at this

equation would be:

$$\text{image}^{(k+1)} = \text{image}^{(k)} \times \left(\text{normalized backprojection of } \frac{\text{measured projections}}{\text{projections of image}^{(k)}} \right). \quad (1.8)$$

This MLEM cycle is shown in Figure 1.8.

Figure 1.8: The steps of an iterations of the MLEM algorithm.

The initial estimate used in the MLEM algorithm is usually an image with a constant activity filling the entire field of view. This uniform image is projected to find the first projection estimate. The ratio of the measured and estimated projections is back projected and normalized and used to update the image to form the next estimate [14]. This process is repeated numerous times until the estimated projections match the measured projections with a good degree of accuracy. The MLEM algorithm is a robust reconstruction method but is usually slow to converge taking many iterations (usually 50-200) before a clinically acceptable image is achieved [14]. To increase the speed of convergence, the ordered subset expectation maximization (OSEM) method was developed.

Ordered Subset Expectation Maximization

The ordered subset expectation maximization (OSEM) method is a modification of the MLEM method. It uses the same algorithm to estimate and update the images. The difference is that it first splits the projections into groups or subsets. Each subset contains an equal number of projections that sample the rotation of the camera at equal intervals [14]. Each subset is used in turn to update the estimate of the object using the MLEM approach. One iteration of the OSEM method involves updating the estimated image by all subsets. The algorithm then rotates back to using the first subset. As the computation cost is roughly proportional to the number of projections used, by updating after each subset, the speed of the algorithm is increased by approximately the number of subsets used [14].

1.5.3 Attenuation Correction

As the photons pass through the patient, the photon beam is attenuated and the intensity of the beam is decreased due to the interactions discussed in section 1.3. The rate at which this decrease occurs is a function of the thickness of the material, x and the linear attenuation coefficient, μ [15]. The number of photons that reach the detector (and are included in the projection data) is less than would be expected in the absence of attenuation. Therefore, when the projections are reconstructed, the activity in the image is an under representation of the true distribution.

The amount of attenuation that occurs depends on the energy of the photon, with lower energy photons experiencing more attenuation than those with higher energies. For 140 keV photons (Tc-99m), the attenuation and consequently the error in the reconstructed activity distribution is on the order of 30% for a rat sized object while for In-111 photons it is slightly less (28%) [16]. Since we are trying to do quantitative imaging, having errors of this magnitude is unacceptable.

One method that has been developed to correct for losses due to attenuation uses

a computed tomography (CT) image acquired at the time of imaging. Since the pixel values of a CT image are related to the attenuation coefficient of the material, they can be used to generate an attenuation map of the patient. This attenuation map is used to model the attenuation in the MLEM algorithm in order to recover the true activity distribution. By doing attenuation correction, it is possible to achieve excellent quantitative accuracy with errors of less than 5% [16–18].

1.6 MicroSPECT Imaging

As discussed previously, small-animal imaging is a key element in the study of the inception, progression and treatment of many human diseases. Small-animal imaging provides a valuable tool in the assessment and study of disease with small-animal models. With the increasing availability of genetically engineered animal strains, we are able to follow the animals through the full progression of disease over their lifetime. By using molecular imaging, we are able to identify key differences between normal and diseased animals and translate these findings back to the clinic.

It is possible to use clinical SPECT cameras for small animal imaging, but this introduces a set of problems. One of the greatest challenges of small-animal imaging is that the resolution of a clinical camera is sufficient only for imaging human physiology. When imaging rats and mice, everything is on a much smaller scale and the resolution of clinical cameras is insufficient [19]. In addition, the use of clinical systems for animal research is complicated by accessibility in a busy clinical environment and by potential concern over the transfer of pathogens. Detection efficiency is also a problem with clinical systems as they are typically limited to one or two detector heads. In response to these issues, several systems have been designed that use multi-pinhole collimation combined with a clinical imaging system to generate high-quality small-animal SPECT images [20, 21].

The introduction of dedicated preclinical cameras was a leap forward in the world of small animal imaging. Not only did this eliminate many problems associated with disease transfer and camera accessibility but it also allowed for a camera specially developed for the needs of imaging small subjects. The most marked difference between the old clinical SPECT camera and the new preclinical cameras was the replacement of the parallel-hole collimator with a pinhole collimator [22]. This change made a remarkable improvement in the resolution of the system, making imaging of small structures of the rat or mouse much more practical. The introduction of dedicated preclinical SPECT cameras has improved many aspects of small animal imaging. Along with these improvements, this new class of camera also comes with its own set of challenges. The two largest obstacles to high-quality small-animal SPECT images are spatial resolution and detection efficiency.

1.6.1 Spatial Resolution

Spatial resolution refers to the resolution limit in the imaging plane. In pinhole imaging, which comprises the majority of small animal imaging, the spatial resolution is defined as

$$R_s = \sqrt{(d_e(1 - \frac{1}{M}))^2 + (\frac{R_i}{M})}, \quad (1.9)$$

where d_e is the effective diameter of the pinhole (taking into account penetration of the photons through the edges), M is the magnification of the image (one of the key advantages of pinhole collimation as discussed in subsection 1.4.1) which can also be defined as $\frac{f}{h}$ where f is the focal length of the pinhole and h is the source to pinhole distance (see Figure 1.5), and finally R_i is the intrinsic resolution of the detector [23]. The intrinsic resolution of the detector is determined by how well the crystal can pinpoint the location where the photon interacted. For NaI(Tl) crystals this is usually 2-3 mm [24]. The effective diameter of the pinhole is dependent on the attenuation

characteristics of the collimator material and can be defined as: $d_e = [d(d + \frac{2}{\mu} \tan(\frac{\theta}{2}))]$ [25]. One way to increase the spatial resolution is to move the subject closer to the pinhole, decreasing h and therefore increasing M . Another approach is to decrease the size of the pinhole. This has been done in the past with conventional Anger cameras. Ishizu *et al.* [26] were able to achieve a resolution of 1.65 mm using a pinhole diameter of 1.0 mm and focal length of 140 mm. Similarly, Jaszczak *et al.* [25] achieved a resolution of 1.5 mm using a pinhole diameter of 0.6 mm and a focal length of 153 mm.

The camera used in our experiments consisted of four detector heads. Each head had a collimator containing 9 pinholes each with a diameter of 2.5 mm. This combination can achieve a spatial resolution of ≤ 1.9 mm [27].

1.6.2 Detection Efficiency

Another challenge with microSPECT is the ability to acquire enough counts to get an image with appropriate statistics. This usually requires a large injected dose and/or long imaging times since the detection efficiency of the detector is limited. The detection efficiency is of course dependent on the geometry of the collimator, and for the pinhole collimators used in small animal SPECT, the detection efficiency is as follows.

$$G = \frac{d_e^2}{15h^2} N \epsilon. \quad (1.10)$$

Here, d_e is the effective diameter of the pinhole, N is the number of detectors, h is the source to pinhole distance (see Figure 1.5) and ϵ is the efficiency of the crystal [23]. Since the photon detection efficiency is directly proportional to the efficiency of the crystal, it is clear that we want to use the best crystal possible. The efficiency of a detector depends also on the thickness of the crystal. For NaI(Tl), which is common in SPECT detectors, a 9.5 mm crystal thickness yields an efficiency of 85-

90% for Tc-99m 140 keV photons, one of the most common SPECT isotopes [28]. The pinhole size has an even larger effect on the detection efficiency. Increasing the size of the pinhole lets more photons through, which increases the sensitivity. As discussed previously, increased hole diameter also degrades the spatial resolution of the image [23].

In small animal imaging, having high spatial resolution is imperative and therefore the system is usually configured to maximize the resolution rather than the sensitivity [29]. Although this does provide a loss in sensitivity, there are a few ways to combat this and minimize the loss. One way is to increase the number of detectors. Using multiple detector heads at different angles increases the number of photons that are detected at one time. An increase in sensitivity can also be used to provide the same amount of useful information with a shorter scan duration.

Another method used to increase detection efficiency is to employ multiple pinholes on the collimator [29]. Although some of the added information in this scenario is degraded as the resulting projections overlap, this still provides a significant increase in sensitivity. Our camera employs a combination of these two techniques. It consists of 4 detector heads placed at 90 degrees from each other and each detector head has a collimator with 9 multiplexed pinholes. It can achieve a sensitivity of > 2200 cps/Mbq [27] where cps refers to counts per second.

1.6.3 Energy Resolution

The energy resolution of the camera is mainly defined by the type of scintillation crystal used. As was mentioned during the discussion on the Anger camera in section 1.4, the energy of the incoming photon can be estimated based on the magnitude of the light output from the crystal. The magnitude of the light output for photons of a certain energy has some statistical variation, following a Gaussian distribution in energy with the peak energy being the energy of the photons. The width (full width

at half maximum (FWHM)) of this distribution is used to characterize the energy resolution of the crystal and is a property that varies with the material used [30].

The detector crystals in our camera are made of NaI(Tl), similar to that used in many clinical cameras, with a thickness of 6.4 mm. It has an energy resolution of 9.7% at 140 keV (Tc-99m) and 7.0% for 245 keV (In-111) [27].

1.7 Cross-talk

Small-animal cameras, like clinical systems, can distinguish photons based on their energies. When using different SPECT isotopes that emit gamma rays of different energies, it is possible to study multiple tracers and hence multiple metabolic processes simultaneously. The greatest challenge to multi- or dual-isotope imaging is the problem of cross-talk. The phenomenon of cross-talk in dual-isotope SPECT imaging refers to the presence of Compton scattered photons of one isotope in the energy window designated to measuring a different isotope [23]. Some of these scattered photons appear to the camera as if they have originated from another isotope since the camera discriminates solely by energy. These scattered photons cause an exaggeration of the isotope's signal leading to an over estimation of the activity present in the patient. In addition, the points of origin of the scattered photons will be falsely reconstructed since the reconstruction algorithm runs under the assumption that photons travel in straight lines.

In order to get an accurate activity distribution within the body, it is necessary to develop a method to correct for this cross-talk. Several methods have been developed for use in clinical SPECT imaging. In clinical SPECT the scatter fraction is quite large as the photons have to traverse up to 50 cm of tissue and bone before reaching the detector head. A collection of cross-talk correction (convolution subtraction, triple energy window, dual energy window and Monte Carlo methods) that have

been developed are discussed in this section. Many of these methods have been implemented for cross-talk correction in the clinic [31–40].

1.7.1 Convolution Methods

Convolution methods provide an improvement on the manufacturer provided cross-talk correction by attempting to model the distribution of the scattered photons. This is done by applying a blurring kernel to the primary photons to simulate the broader spatial distribution of scattered photons [41, 42]. For the isotopes we are interested in, In-111 and Tc-99m, the measured data are represented by the following equations.

$$M_{Tc} = P_{Tc} + S_{In} = P_{Tc} + k_{In \rightarrow Tc} \cdot P_{In} * K_{g,In}. \quad (1.11)$$

$$M_{In} = P_{In} + S_{Tc} = P_{In} + k_{Tc \rightarrow In} \cdot P_{Tc} * K_{g,Tc}. \quad (1.12)$$

Here, M_j represents the total counts measured in photopeak j , P_j represents the primary counts present in photopeak j , S_j represents the scattered counts present in photopeak j , $k_{i \rightarrow j}$ is a scaling factor indicating the fraction of photons from photopeak i that are scattered and measured in photopeak j , and $K_{g,j}$ is a two-dimensional Gaussian blurring kernel that is spatially symmetric and is representative of the spatial distribution of scattered photons from photopeak j . Since the process of Compton scattering causes photons to lose energy, the S_{Tc} in the second equation is essentially zero and it reduces to $M_{In} \approx P_{In}$. As well, with a 10% energy resolution, the 140 keV and 172 keV peaks are sufficiently separated that there is no significant direct overlap of the primary photopeaks. The first equation then simplifies to:

$$M_{Tc} = P_{Tc} + k_{In \rightarrow Tc} \cdot M_{In} * K_{g,In}. \quad (1.13)$$

From this equation, we can solve for P_{Tc} to find the primary photons only.

$$P_{Tc} = M_{Tc} - k_{In \rightarrow Tc} \cdot M_{In} * K_{g,In}. \quad (1.14)$$

The advantage of this method is the fact that already acquired energy windows can be used without the need for additional information. It is also relatively simple to implement and it is not computationally expensive compared to other scatter modelling methods. The main drawback of this method is that the formulation of the scatter estimate is still fairly simplistic and does not incorporate the differences in scatter that arise in different materials. This approach also assumes that the scatter kernel is spatially invariant. This is reasonably accurate in the middle of homogeneous media, but breaks down both in inhomogeneous media (such as the thorax) and near the edges of objects.

1.7.2 Triple Energy Window Method

The triple energy window (TEW) method has been implemented by Ichihara *et al.* [43], Feng *et al.* [42] and Zhu *et al.* [44]. The TEW method is a geometric scatter correction method which employs two small energy windows above and below the primary energy window to measure the scattered photons [43, 45]. The energy spectrum and measured energy windows of the two isotopes can be seen in Figure 1.9. The counts in the two scatter windows are averaged to find the scatter counts within the primary window and then are subtracted from the total. To illustrate this process more clearly, let's define the parameters: C_p represents counts from primary photons in the primary window, C_s represents counts from scattered photons in the primary window and C_t represents total counts in the primary window. The counts measured

are a combination of the primary and scattered photons so therefore

$$C_p = C_t - C_s. \quad (1.15)$$

Now if we define the widths of the energy windows as w_{s1} being the width of the lower energy scatter window, w_{s2} being the width of the higher energy scatter window and w_p being the width of the primary photopeak window, we can define the scattered counts as [43]

$$C_s = \left(\frac{C_{s1}}{w_{s1}} + \frac{C_{s2}}{w_{s2}} \right) \frac{w_p}{2}. \quad (1.16)$$

These scattered counts are subtracted from the total measured counts on a pixel by pixel basis across the image to form the corrected image.

Figure 1.9: An energy spectrum of a dual-isotope study with the energy windows required for TEW correction shown. The energy distribution of the primary (unscattered) Tc-99m photons is represented by the dashed curve, the primary In-111 photons are represented by the dotted curve and the scattered In-111 photons are represented by the solid curve. The energy windows needed for a TEW correction are shown as shaded regions on the figure.

The main advantage of this method is that it estimates the scatter on a pixel by

pixel basis from a measured scatter estimate. By doing this, there is no underlying assumption about the spatial distribution of the scattered photons. This could potentially provide a much more accurate estimate of the cross-talk contamination. The most obvious drawback of this method is that it requires the measurement of two additional energy windows which, on some systems that do not have list-mode capabilities (the ability to measure photons of all energies simultaneously), can require extra acquisition time. An additional drawback to this method is that choosing the position of the scatter windows is a delicate process. They must be placed far enough away so that they contain only scatter photons and no primary photons but close enough that they can accurately represent the scatter in the location of the photopeak. When the two photopeaks are close in energy, as was the case for this study, this problem is amplified due to the potential for overlap of the two photopeaks.

1.7.3 Dual Energy Window Method

The dual energy window method is similar to the triple energy window method in that it uses a secondary window to estimate the scatter in the primary window. The dual energy window method on the other hand uses only one secondary window that is placed at an energy lower than that of the primary window and has a larger energy width. The counts measured in this secondary window when scaled by the appropriate scatter fraction are used as the estimate of scatter in the primary window [46]. This method is most useful when there are no higher energy photons that can scatter into the photopeak. In this situation, the upper scatter window of the TEW method would contain no counts and therefore the TEW method collapses down to the dual energy window (DEW) method. In our study, there is a significant amount of scatter from In-111 gamma rays into the Tc-99m photopeak and the approach is therefore not accurate.

The advantage of this method is its simplicity, however, the distribution of scat-

Figure 1.10: Energy spectrum of a dual-isotope study with the energy windows required for a dual energy window (DEW) based correction shown. The energy distribution of the primary (unscattered) Tc-99m photons is represented by the dashed curve, the primary In-111 photons are represented by the dotted curve and the scattered In-111 photons are represented by the solid curve. The energy windows needed for a DEW correction are shown as shaded regions on the figure.

ter is highly dependent on the scattering angle of the photon as well as the number of scattering events experienced by the photon [47]. This is a problem because the scatter inside the primary window is generated by photons which have not lost a significant amount of energy (small scattering angles) and consists primarily of photons which have only undergone a single scattering event. The scatter in the lower, wider secondary window however, can contain photons with much wider scattering angles and many more interactions in order to get them to this lower energy. Because of these additional interactions, the spatial distribution of these multiply scattered photons is different than singly scattered photons.

1.7.4 Monte Carlo Methods

As mentioned, the limitation of many of the scatter correction techniques was their inability to accurately model the spatial distribution of scattered photons. The methods that have been the most successful at doing this are those that employ Monte Carlo simulations or accurate computer modelling to determine the scatter estimates. When combined with a transmission scan like a CT that unveils the distribution of scattering structures within the patient, Monte Carlo simulations are able to model the scattering process very accurately on a patient by patient basis [47].

The main issue with the use of Monte Carlo techniques is their complexity. In order to achieve accurate estimates, the model must incorporate all interactions and the computing time for this is immense. More recently, with the improvement of these methods and the introduction of accelerated Monte Carlo techniques, these methods have become more practical for clinical applications [47–51]. The reconstruction times required for these methods have been substantially reduced and they are receiving wider acceptance in the SPECT imaging community.

1.7.5 Cross-talk Correction in Small Animal SPECT

Cross-talk correction in small animal SPECT is not as well studied as it is in clinical SPECT. There is potential that the techniques that have been developed for clinical SPECT will also work in small animal SPECT although there are some fundamental differences between clinical and small animal SPECT that must be considered.

First, because of the nature of what we are trying to achieve by doing small animal imaging, the demands of the images are a bit different. In clinical imaging, the purpose of the study is usually to make a diagnosis. Thus, the physician is examining the image for the presence or absence of activity in a certain structure. To do this, the physician needs an image with some quantitative accuracy in order for the activity distribution to be correct, but more importantly the physician requires

an image with superior qualitative elements (clear boundaries, uniform distribution, etc.). In small animal imaging, it is already known what condition the animal has since it was induced intentionally. What we want to know instead is how the condition is changing, and to get these results the images must be much more quantitatively accurate. Therefore, we require a cross-talk correction method that can achieve this.

The next difference is the camera. The pinhole collimator used on small animal systems is different than the parallel hole collimator used on most clinical systems. As a result, the distribution of photons scattering in the collimator is different, and more importantly, the number of photons that scatter in the collimator before detection is low [47]. Since this type of scatter is less prominent in small animal imaging, this simplifies the necessary correction. In addition, septal penetration by high energy gamma emissions or contaminants is less frequent with pinhole collimators than with parallel hole collimators [47], potentially changing the nature of the cross-talk contamination.

Another key difference is the size of the patient. In small animal imaging, the subject is a rat or mouse that is 3-10 cm in diameter and 20-300 g instead of a human who is typically 25-45 cm in diameter and 70-150 kg. This is a decrease in size by several orders of magnitude and as a result, there is much less scattering material for the photons to pass through before exiting the body. This decreases scattering substantially and therefore the scatter fraction measured in small animal imaging is much smaller than in typical clinical imaging.

As a result of the reduced scatter component, it is entirely possible that the simpler cross-talk correction techniques may be sufficient to solve this problem. There has been some work done to investigate the amount of scatter present in small animal SPECT imaging [17, 18, 52] and energy window based methods were deemed acceptable. Little work, however, has been done to investigate the problem of cross-talk correction in dual-isotope small-animal imaging. It is our expectation that because

of the factors mentioned above, these simple window based cross-talk corrections will be sufficient for this type of correction as well.

1.7.6 Manufacturer Method

Bioscan Inc. (Washington, DC), the manufacturer of the image analysis software Invivoscope provides a very simple method for cross-talk removal. This method is a simplified version of the convolution subtraction technique discussed previously in subsection 1.7.1, omitting the convolution with a blurring kernel. The basis of the correction is the assumption that the number of unwanted scatter photons present in the photopeak windows is simply a fraction of the primary photons detected in the photopeak window of the isotope from which the scattered photons originated.

$$M_{Tc} = P_{Tc} + S_{In} = P_{Tc} + k_{In \rightarrow Tc} P_{In}. \quad (1.17)$$

$$M_{In} = P_{In} + S_{Tc} = P_{In} + k_{Tc \rightarrow In} P_{Tc}. \quad (1.18)$$

Here, M represents the counts measured in the photopeak window, P represents the primary counts measured, S represents the scattered counts measured and k represents the scatter fraction. To find the number of primary counts measured in the photopeak window, or in other words the true activity present in the image, we must simply solve the system of linear equations for P_{Tc} and P_{In} , and this is what Invivoscope does with user specified scatter fractions.

The advantage of this method is that it is very simple to solve and implement. Moreover, it requires no additional energy windows to be measured. The limitation of this method is in determining the scatter fractions. Since the scatter that occurs during an imaging session is partially dependant on what is being imaged, different materials will produce different scatter fractions. It is therefore difficult to assign a single value to all cases. In addition to the scatter fraction difficulties, this method

also does not incorporate any modelling of the scatter distribution which is not the same as the primary distribution. This method is perhaps more suited for removal of cross-talk from two isotopes that are very close in energy, for example Tc-99m (140 keV) and I-123 (159 keV), where the cross-talk is primarily due to insufficient separation of the primary photopeaks and not due to scattered photons.

1.8 Objective

The goal of this research was to determine the cross-talk correction technique for use in Tc-99m/In-111 small-animal imaging that achieved the most quantitatively accurate results. Our hope was that due to reduced collimator scatter when using a pinhole collimator along with the decrease in patient size, a simple cross-talk correction method would provide us with sufficiently accurate images. Cardiac repeatability studies have shown that variability in animals over time and also between different animals can introduce an error in quantitative accuracy of 3-7% [53]. This level of error is unavoidable and can therefore serve as a reference for reasonable and achievable level of quantitative accuracy. We therefore hypothesize that we can achieve a similar level of accuracy and that simple cross-talk correction methods will be able to achieve quantitative accuracy with an error of 5% or better. A description of the studies we performed to evaluate this hypothesis is presented in chapter 2. In chapter 3, we discuss some of the limitations of our method as well as some of the possible future directions for this work.

1.9 Contribution

During this study, I was assisted by Julia Lockwood, Imaging Technologist at the University of Ottawa Heart Institute with phantom preparation during phases 1 and 2. All of the camera operation and data acquisition was performed by myself. During

phase 3, Julia assisted with the animal handling and injection. Camera operation and animal monitoring was a combined effort between Julia and myself. After the data was acquired, I implemented all of the scatter correction methods used in this thesis off-line in the MATLAB programming environment: the manufacturers correction, convolution subtraction and triple energy window (in both projection and image space). I also developed and then implemented the modified triple energy window approach that was used in phase 2 and phase 3 of this work. To perform attenuation correction on the In-111 data sets, I measured the conversion factors used to transform the measured CT values into appropriate attenuation coefficients. The reconstruction software used during phases 2 and 3 for CT and SPECT reconstruction were written by Jared Strydhorst.

I have presented this work twice in the form of a poster:

1. R. Timmins and R. G. Wells, "Cross-talk correction in dual isotope $^{111}\text{In}/^{99\text{m}}\text{Tc}$ small animal SPECT imaging," in *Imaging Network of Ontario 10th Symposium Conference Record*, pp. 165, 2012.
2. R. Timmins and R. G. Wells, "Cross-talk correction in dual isotope $^{111}\text{In}/^{99\text{m}}\text{Tc}$ small animal cardiac SPECT imaging," in *Society of Nuclear Medicine Annual Meeting Supplement 1*, pp. 574p-575p, 2012.

I have also presented this work in the form of an oral presentation:

1. R. Timmins and R. G. Wells, "Cross-talk correction in dual isotope $^{111}\text{In}/^{99\text{m}}\text{Tc}$ small animal cardiac SPECT imaging," in *The University of Ottawa Heart Institute 5th Annual Molecular Function and Imaging Symposium Record*, pp. 22, 2012.

Chapter 2

Cross-talk correction in Tc-99m/In-111 dual-isotope imaging for small-animal SPECT

2.1 Introduction

This thesis examines two classes of cross-talk correction methods that have been developed for clinical SPECT applications: 1) convolution based correction methods and 2) energy based methods. Within the convolution class, the methods we tested were 1a) convolution subtraction [41, 42] (see subsection 1.7.2) and 1b) the manufacturer-provided method (see subsection 1.7.6). For the energy-window based methods, we tested: triple energy window method [42–44] (see subsection 1.7.1) as implemented both in 2a) projection space (pre-reconstruction) and 2b) image space (post-reconstruction). We have focused on testing applications for cardiac imaging though the results are generally applicable to other small-animal imaging settings. The testing of these methods was completed in three distinct phases and as such, this thesis will outline the three phases in the order in which they were completed. The

three phases of this research are as follows:

1. comparison of the different correction techniques
2. testing the best method over a range of cross-talk concentrations
3. evaluating the performance of the best method in-vivo

The methodology and results of each phase will be discussed individually followed by a discussion of all phases at the end.

2.2 Phase 1: Method Comparison

2.2.1 Purpose

The purpose of this phase was to compare a set of four cross-talk correction methods and determine which was the most accurate quantitatively as well as qualitatively.

2.2.2 Methodology

Cross-talk Correction Techniques

For this study, we tested four cross-talk correction techniques. These were: the technique provided by the manufacturer (described in subsection 1.7.6), the TEW method (described in subsection 1.7.2) and the convolution subtraction method (described in subsection 1.7.1). The TEW method was implemented in both projection space and in image space as two separate techniques. These four methods were chosen because they are simple to implement and not computationally intensive. As discussed in subsection 1.7.5, this simplicity is hypothesized to be sufficient for the smaller amount of scatter that is present in small animal SPECT imaging. The implementation of each algorithm is shown in Figure 2.1, Figure 2.2, Figure 2.3 and Figure 2.4.

Figure 2.1: A block diagram showing the implementation of the manufacturer's correction method. In this diagram, everything before the Recon operation is projection data while everything after is image data. Here, P_j represents the primary photons from isotope j , S_j represents the scattered photons from isotope j , I_x represents the reconstructed image formed using projections of x , $\tilde{I}_x$ represents the estimate of the image of x type of photons and $k_{i \rightarrow j}$ is the fraction of photons from isotope i measured in the window of isotope j . The reconstruction algorithm used in the Recon operation is described in the reconstruction portion of this section. The solution operation in this method is done on a pixel by pixel basis.

Figure 2.2: A block diagram showing the implementation of the convolution subtraction method. In this diagram, everything before the Recon operation is projection data while everything after is image data. Here, P_j represents the primary photons from isotope j , S_j represents the scattered photons from isotope j , I_x represents the reconstructed image formed using projections of x , $\tilde{I}_x$ represents the estimate of the image of x type of photons, K_j represents the Gaussian blurring kernel used to approximate the spatial distribution of scattered photons from energy j keV and $k_{i \rightarrow j}$ is the fraction of photons from isotope i measured in the window of isotope j . The reconstruction algorithm used in the Recon operation is described in the reconstruction portion of this section. The scatter image estimate operation is applied to 2D slices on a pixel by pixel basis.

Figure 2.3: A block diagram showing the implementation of the triple energy window method in image space. In this diagram, everything before the Recon operation is projection data while everything after is image data. Here, P represents the primary Tc-99m photons, S represents the scattered In-111 photons, I_x represents the reconstructed image formed using projections of x photons, $\tilde{I}_x$ represents the estimate of the image of x type of photons, w_k represents the width of energy window k in keV. The reconstruction algorithm used in the Recon operation is described in the reconstruction portion of this section. The scatter image estimate operation is applied on a pixel by pixel basis.

Figure 2.4: A block diagram showing the implementation of the triple energy window method in projection space. In this diagram, everything before the Recon operation is projection data while everything after is image data. Here, P represents the primary Tc-99m photons, S represents the scattered In-111 photons, I_x represents the reconstructed image formed using projections of x photons, $\hat{P}$ represents the estimate of the projections due to primary Tc-99m photons, w_k represents the width of energy window k in keV. The reconstruction algorithm used in the Recon operation is described in the reconstruction portion of this section. The scatter image estimate operation is applied on a pixel by pixel basis.

Phantom

The phantom constructed for this study consisted of three 10 ml plastic syringes (Becton Dickinson #309604). The first syringe was filled with Tc-99m-pertechnetate solution, the second with In-111-chloride in aqueous solution and the third a mixture of Tc-99m and In-111. Each syringe was diluted with water as needed to produce a 2 ml volume of activity the desired activity. The plunger of the syringe was compressed to hold this volume only. The activities in each syringe can be found in Table 2.1.

Table 2.1: Activities in syringe phantoms used in phase 1. Errors are the reading errors associated with the dose calibrator used to make the measurements.

Isotope	Tc-99m syringe	In-111 syringe	Mixed syringe
Tc-99m activity (MBq)	29.34 ± 0.07	-	6.55 ± 0.07
In-111 activity (MBq)	-	23.35 ± 0.07	25.94 ± 0.07

The syringes were placed in a pyramid configuration on the camera bed with the two single-isotope syringes forming the base of the pyramid and the dual-isotope syringe sitting on top (Figure 2.5).

Figure 2.5: This is a cross-sectional view of the pyramid configuration of the phantom. The two single-isotope syringes were placed directly on the camera bed and formed the base of the pyramid while the mixed-isotope syringe was placed on top as indicated. The activities found in each syringe can be found in Table 2.1

Image Acquisition

All imaging for this study was done on the Bioscan nanoSPECT/CT in-vivo preclinical imaging system (Washington, DC) seen in Figure 2.6.

Figure 2.6: Photo of the Bioscan NanoSPECT/CT camera

The system consists of four detector heads, 90 degrees apart, to completely surround the field of view. Each detector head has a set of interchangeable collimators. The collimator we used had 9 multiplexed pinholes of 2.5 mm diameter on each head and is referred to as Apt2 by the camera system. Projections were acquired for 3 different primary energy windows and 2 scatter windows to be used in the TEW correction. The centroid energy and widths of each energy window are shown in Table 2.2. The widths of the primary peaks are narrower than what is normally used in single-isotope studies due to the proximity of the Tc-99m peak at 140 keV and the lower In-111 peak at 172 keV. The energy windows we used are similar to those that were optimized by Zhu *et al.* [44].

Table 2.2: Energy windows used for all imaging.

Energy Window	Low Scatter	Tc-99m Primary Photopeak	High Scatter	In-111 Primary Photopeaks	
Centroid Energy (width)	126 keV ($\pm 2.5\%$)	140 keV ($\pm 7.5\%$)	156 keV ($\pm 2.5\%$)	172 keV ($\pm 8\%$)	245 keV ($\pm 9\%$)

A scout scan was done to assign a field of view that contained the activity. A field of view of 19.2 mm was chosen where the edges of the field were placed at the edges of the activity contained within the syringe. Projections of each energy window (Table 2.2) was then acquired via a helical SPECT scan consisting of 48 projections of 150 seconds each for a total scan time of 30 minutes. This amounts to 12 projections formed by each of the four detector heads for a total of 48 projections. The projections contained approximately 6.7×10^7 counts in the Tc-99m photopeak window and 9.5×10^7 counts in the In-111 photopeak window.

Reconstruction

Reconstruction of the images was done using the vendor provided OSEM iterative method on the 'High' setting. This reconstruction method includes a 3D Gaussian filter (FWHM=0.63 mm) applied between iterations. The reconstructed voxel size was $0.6 \times 0.6 \times 0.6 \text{ mm}^3$. The reconstruction was done using 24 iterations where the first 8 iterations contained 6 subsets, the next 8 iterations contained 4 subsets, and last 8 iterations contained 3 subsets [53].

The three different cross-talk correction techniques were applied to the primary photopeak image of Tc-99m in image space for all of the three techniques and also in projection space for the TEW method (refer to Figures 2.1-2.4).

Data Analysis

After reconstruction, there were four corrected Tc-99m photopeak images. To determine the correction method that achieved the best results, the techniques were

evaluated on their ability to remove the In-111 scatter from the Tc-99m window image in the area of the In-111 syringe. This was evaluated by quantifying the amount and distribution of residual scatter via a plot of the standard deviation of pixels versus the average pixel value. The best quantitative method was the method that had the smallest standard deviation paired with an average pixel value close to zero.

A region of interest (ROI) was created around the In-111 syringe phantom. The ROI was created by setting a threshold so that pixels with more than 30% of the maximum value were included in the ROI and those with less were not. This was done in three dimensions so that the ROI contained the full volume of activity.

Figure 2.7: Shown is a cross-sectional view through the three syringes similar to the view shown in Figure 2.5. The image corresponds to a combined image of the Tc-99m and In-111 photopeak energy windows. The number of counts recorded is shown in a false colour scale. The ROI used for measurements is represented by the white outline around the In-111 syringe. Selection of the ROI was done using a threshold of 30% of the maximum pixel value within the In-111 syringe. Selection of the ROI was done on each slice containing In-111 activity to create a 3-dimensional volume representing the syringe.

The ROI was defined on the In-111 photopeak image since that is where the syringe could be fully visualized. Once defined, the ROI was superimposed on the Tc-99m photopeak image for measurements. The total scatter counts present in this

region was measured in the uncorrected image and in each of the corrected images. The amount of residual scatter after reconstruction was expressed as a percentage of the scatter present in the uncorrected image for each correction method. In addition, the standard deviation of pixel values within the ROI was measured to determine the variability of the correction method. A lower standard deviation would indicate a more complete removal of the scatter.

2.2.3 Results

The measured residual scatter for each method is shown in Table 2.3. It was found that although each method was able to reduce the In-111 scatter by a significant amount (see Table 2.3), the TEW method was superior. After using the convolution subtraction method to remove the cross-talk compared to the TEW methods, approximately three times more scatter remained. When comparing the two TEW correction methods (projection space and image space), there was little difference in the amount of scatter they removed, projection space implementation leaving $4\pm4\%$ and image space implementation leaving $5\pm6\%$.

Table 2.3: Measured residual In-111 scatter in the Tc-99m image of the Tc-99m syringe after correction. Residual scatter is measured as a percentage of the scatter present before correction.

Method	Manufacturer Correction	Convolution Subtraction	TEW (image space)	TEW (projection space)
% Residual Scatter	-32 ± 9	14 ± 5	5 ± 6	4 ± 4
Average Pixel Value	$(-6\pm1)\times10^4$	$(2.1\pm0.3)\times10^4$	$(0.79\pm0.08)\times10^4$	$(0.76\pm0.07)\times10^4$

The measurements of total scatter present before and after correction were done using a threshold value to determine the ROI. Variation of this threshold does effect the number of pixels captured and therefore the amount of cross-talk. Increasing the threshold and including less pixels in the ROI increased the apparent percent removal of scatter while decreasing the threshold did the opposite. Due to the proximity of

the other syringes, the threshold of 30% was used to avoid including pixels from the wrong syringe.

Since only one imaging session was performed, the error in the scatter removal was estimated by examining the variation of removal between pixels and between different slices in the image. As well, the effect of the threshold on scatter removal was also examined. This was done by determining the standard deviation of the 100 pixels with the highest intensity. This serves as an estimate of how changing the maximum pixel value would affect the ROI definition. The standard deviation was found to be 4% of the average so the threshold was then shifted by $\pm 4\%$ to determine the effect this had on the measured scatter removal. These errors were combined to get the total error on each measurement.

To better quantify the scatter removal, the average pixel value and standard deviation within the In-111 syringe were found for each of the corrected Tc-99m photopeak images. When the standard deviation of each method was plotted against the average (Figure 2.8), there was larger separation between the methods. An ideal method would have an average of zero with a low standard deviation.

This plot shows that the manufacturer correction provides little improvement over the uncorrected data. It also shows that the TEW correction performed in projection space should provide the best correction of the scatter. A paired t-test with the two TEW methods was performed to determine if the two methods provided statistically different results. The results of the paired t-test were $p < 0.001$ which indicates that the two methods are indeed different, with TEW performed in projection space resulting in the most quantitatively accurate correction.

The images were also evaluated qualitatively to determine the distribution of the residual scatter within the image. Upon visual examination of the scatter distribution within each image, it was clear that the TEW method in projection space was the best method in this respect as well. A transverse slice through the phantom for each

Figure 2.8: Plot of the standard deviation versus average pixel value within the ROI for each correction method. The correction method that is closest to (0,0) provides the most quantitatively accurate correction. Error bars indicate the standard error on the average and on the standard deviation. The correction methods are indicated in the legend.

correction method is shown in Figure 2.9. It is clear by looking at the images that the TEW in projection space has much less residual scatter than the other methods visually as well. When we look at the convolution subtraction method (Figure 2.9d) and the TEW method in image space (Figure 2.9e) there is much more structure to the residual scatter and it is located with high intensities in specific locations. Comparing this to the TEW in projection space image (Figure 2.9f) the distribution of scatter is dispersed throughout the entire region of the syringe and is more uniform.

Figure 2.9: Each image shows the same transverse slice through the three syringe phantom. The top row shows the uncorrected images in the Tc-99m energy window A and the In-111 energy window (B). The bottom row shows the Tc-99m energy window images after correction. Syringe I is the mixed isotope syringe, II is the In-111 syringe and III is the Tc-99m syringe. The images show the best In-111 scatter removal occurs with TEW correction in projection space with the most even distribution of residual scatter. All images are shown in log scale to better visualize the residual scatter.

2.3 Phase 2: Cross-talk ratios

2.3.1 Purpose

The purpose of this phase was to determine whether the best correction method as determined in phase 1 (TEW in projection space) could achieve a constant accuracy that is independent of the cross-talk magnitude present.

2.3.2 Methodology

Cross-talk Correction Technique

The cross-talk correction technique used in this phase was the TEW correction applied in projection space but with a modification. On examination of the energy spectrum of the two isotopes, it was observed that although the Tc-99m photopeak (140 keV) and the lower energy In-111 photopeak (172 keV) were separated enough that two photopeaks did not overlap, they did overlap with the scatter windows. This is due to the energy resolution of the NaI(Tl) detector which is 10%. As a result, primary photons from both photopeaks were being detected in the scatter windows and being considered as scatter for the purposes of the TEW correction (see Figure 1.9). This misrepresentation of the scatter was causing an over-estimation of the cross-talk in the Tc-99m photopeak and therefore an over-correction of the image was performed.

To determine the magnitude of this over-estimation, the proportion of primary photons being detected in each scatter window needed to be determined. This was done by measuring the energy spectrum of the two isotopes independently. Since the camera cannot measure the spectrum directly, it was determined by measuring the number of photons in energy windows of 5 keV width from 115-160 keV for Tc-99m and from 145-205 keV for In-111. Once the spectrum was sampled, it was fit with a linear + Gaussian function where the Gaussian represents the photopeak distribution and the linear function is used to fit the background and self-scatter within the source.

The fits are shown in Figure 2.10. From the Gaussian fit, the proportion of primary photons measured in the scatter window was found by integrating over the ranges of the energy windows.

Figure 2.10: Plots of the measured spectrum and linear + Gaussian fit (solid line) for each photopeak. The dashed lines indicate the location of the scatter windows. These fits were used to determine the number of primary photons measured in the scatter windows for use in the modified TEW method.

Once these values were known, the primary counts from both isotopes were subtracted from the scatter windows prior to the application of the TEW cross-talk correction. The combination of these two steps, will be referred to as the modified TEW method. A block diagram showing how the modified TEW method was implemented can be found in Figure 2.11.

Phantom

The phantom used in phase 2 was the same as in phase 1 (see Figure 2.5), however, instead of one Tc-99m + In-111 mixed syringe, five were created of varying Tc-99m:In-111 activity ratios. The Tc-99m:In-111 ratios used were approximately: 4.5:1, 3:1, 1:1, 1:4, 1:8. The activities can be found in Table 2.4.

Figure 2.11: A block diagram showing the implementation of the modified triple energy window method. In this diagram, everything before the Recon operation is projection data while everything after is image data. Here, P_j represents the primary photons of isotope j , S_j represents the scattered photons of isotope j , $k_{i \rightarrow j}$ represents the fraction of primary photons from isotope i that are measured in window j , I_x represents the reconstructed image formed using projections of x photons, $\tilde{P}$ represents the estimate of the projections due to primary Tc-99m photons, w_k represents the width of energy window k in keV. The reconstruction algorithm used in the Recon operation is described in the reconstruction portion of this section. The scatter image estimate operation is applied on a pixel by pixel basis.

Table 2.4: The activities found in the syringe phantoms used in phase 2. Errors are the reading errors associated with the scale of the dose calibrator used to make the measurements.

Syringe	Tc-99m activity (MBq)	In-111 activity (MBq)
Tc-99m only	18.20 $\pm$ 0.07	-
In-111 only	-	22.83 $\pm$ 0.07
4.5:1	5.59 $\pm$ 0.07	31.78 $\pm$ 0.07
3:1	7.40 $\pm$ 0.07	25.90 $\pm$ 0.07
1:1	18.50 $\pm$ 0.07	17.80 $\pm$ 0.07
1:4	24.90 $\pm$ 0.07	7.73 $\pm$ 0.07
1:8	33.00 $\pm$ 0.07	5.66 $\pm$ 0.07

Image Acquisition

Five sets of images were acquired, one set for each mixed isotope syringe. In each case, a pilot CT scan was done to assign the field of view. A field of view of approximately 25 cm was assigned so that the full volume of solution was contained and the edges of the field were placed just outside the activity contained within the syringe. A CT image was then acquired using a tube voltage of 45 kVp. The image was acquired in 360 projections each of 1500 ms. A helical SPECT scan was then performed with 48 projections of 150 seconds each for a total scan time of 30 minutes. The axial extent of both the CT and SPECT scans was that which was defined in the pilot scan.

Reconstruction

Reconstruction was done off-line using the OSEM iterative method developed by Strydom and Wells [54]. This method includes point spread function (PSF) modelling created via ray tracing. The ray tracing was done for each of the nine multiplexed pinholes. During the reconstruction process, forward projection and back projection were done separately through each pinhole then summed over all pinholes. A total of 32 iterations were performed: the first 8 with 6 subsets, the second 8 with 4 subsets, the third 8 with 3 subsets and the last 8 with 1 subset. This method was used to be able to include a CT-based attenuation correction as part of the reconstruction algo-

rithm. Post-reconstruction filtering was done using a Gaussian filter with $\sigma = 0.6$. The reconstructed voxel size was $0.6 \times 0.6 \times 0.6 \text{ mm}^3$.

Attenuation Correction

Attenuation correction was performed on all images during reconstruction. In order to perform the attenuation correction, it was necessary to acquire an attenuation map for each isotope. This was done by obtaining a CT image for each phantom and from this, CT values were converted to attenuation coefficients. The attenuation coefficient is dependent on the material the photon is passing through and also on the energy of the photon. Therefore, the conversion had to be done separately for each isotope.

Figure 2.12: Experimental set-up used to perform attenuation measurements for an In-111 point source. The pinhole collimators were removed from all four detector heads and replaced with detector shields on three sides. A parallel collimator was placed in front of the top detector. The source was placed on the bottom detector shield surrounded by a platform to support the attenuating cylinders, which were placed between the source and detector on top of the platform. Measurements with and without the attenuating cylinder in place were used to estimate the broad beam attenuation coefficient of the material.

The conversion from CT value to attenuation coefficient follows a bilinear relationship [55]. The slopes of the bilinear curve used for Tc-99m were taken from Strydhorst

and Wells [56] while the slopes for In-111 were measured through experiment. The experimental method of doing this was also taken from Strydhorst and Wells [56]. To make these measurements, a point source was created using a capillary tube of 1 mm internal diameter which was filled with a 2 mm long solution of In-111-chloride. The point source was placed on the camera bed and surrounded by a stack of microscope slides to create a platform. A cylinder with a density designed to simulate a specific tissue or organ within the body was also placed in the camera between the source and the detector head to attenuate the beam of photons (see Figure 2.12). The pinhole collimator was replaced with a parallel hole collimator in an effort to narrow the beam of photons hitting the detector. The total number of counts in the single detector over 100 seconds was measured and then measured again with the attenuating cylinder removed. A CT was also acquired to determine the CT unit of the material. This was done for twelve different attenuating cylinders of varying densities (a complete list can be found in Table 2.5). Using the ratio of the counts measured with the attenuating cylinder in place to those measured without, the attenuation coefficient of each material was calculated according to Equation 2.1, where μ_m is the attenuation coefficient of the material, N_w and N_{wo} are the number of counts detected with and without the attenuating material present and x is the length of the attenuating cylinder.

$$\mu_m = -\frac{\ln\left(\frac{N_w}{N_{wo}}\right) - \mu_{air}x}{x}. \quad (2.1)$$

Unlike Tc-99m, In-111 has two emission energies at 172 and 245 keV and the attenuation coefficients for the two energies will not be the same. Instead of having to correct each peak separately, in an effort to simplify the attenuation correction process for In-111, the projections from the two peaks were summed and assigned an

effective attenuation coefficient based on their detection ratio such that:

$$\phi_{172keV} + \phi_{245keV} = (\phi_{0,172keV} + \phi_{0,245keV}) \exp^{-\mu_{eff}x}, \quad (2.2)$$

where ϕ is the photon flux, μ is the attenuation coefficient and x is the distance travelled in the material. If we solve for the effective attenuation coefficient, we get the following equation [57]:

$$\mu_{eff} = \frac{\ln(\exp(-\mu_{172keV}x) + \alpha \exp(-\mu_{245keV}x)) - \ln(1 + \alpha)}{-x}. \quad (2.3)$$

Here, α is the ratio of detection of the two energies. This factor incorporates both the emission probability of the two energies as well as the detection efficiency of each energy in the detector. The coefficient α was measured as $\alpha = \frac{\phi_{245keV}}{\phi_{172keV}}$ where the values of ϕ were measured as the number of counts from an In-111 point source found in each energy window. This method was tested by Seo [57] and it was found that this method could accurately recover the true activity concentration, and did a better job than using attenuation coefficients from one energy peak only. Using the 172 keV peak only, Seo [57] found their method overestimated the activity by 4% and using only the 245 keV peak, the activity was underestimated by 3%.

Once the effective attenuation coefficients were found for all cylinders, the values were plotted against CT number and fit with a bilinear curve. This curve was used to convert values from the CT images to attenuation coefficients to create the attenuation map for In-111 during reconstruction.

Data Analysis

The effectiveness of the modified TEW cross-talk correction technique was evaluated by measuring the activity of the mixed isotope syringe in the corrected Tc-99m-window images and comparing it to the known Tc-99m activity of that syringe

Table 2.5: Composition of the attenuating cylinders used to sample the CT-to-attenuation-coefficient curve for In-111

Material	Density (g/cm ³)
Lung LN-300	0.28
Lung LN-450	0.4
Adipose	0.9
Breast	0.96
Solid Water	0.99
Brain	1.05
Liver	1.07
Inner Bone	1.09
Bone B-200	1.11
CB2-30%	1.28
CB2-50%	1.46
Cortical Bone	1.69

(obtained from the dose calibrator measurement). This was done by creating a ROI around the syringe in the same way as in phase 1, then measuring the total activity present within the ROI. The ROI was created by setting a threshold value of 30% so that all pixels with a value greater than 30% of the maximum were included in the region.

2.3.3 Results

The CT value to attenuation coefficient conversion produced a bilinear curve as expected, and is shown in Figure 2.13. When the measured attenuation coefficient of water was compared to the accepted value of 0.135 cm^{-1} [58] for photons of 208 keV (the effective energy of In-111), it was found that the measured value underestimated the attenuation coefficient by 0.0394 cm^{-1} . The slope of the first portion of the curve was adjusted to pass both through the origin and the accepted value for water. For the second portion of the curve, the slope was kept the same as that which was measured but the intercept was changed so this line would also pass through the point for water. The uncertainty on each CT measurement was found using the variation

in reconstructed CT pixel value within the cylinder. The uncertainty in the measurement of attenuation coefficient was determined from the Poisson variation due to the photon counting of each acquisition. Since each was a high count acquisition, these errors were small.

Figure 2.13: Plot showing the bilinear curve measured from experiment for use in converting reconstructed CT values to attenuation coefficients. Conversion was used to create attenuation maps for attenuation correction during reconstruction. The values used to perform the conversion were adjusted from the measured fit (solid line) to better match the known attenuation coefficient of water. The shifted values are represented by the dashed line.

Measuring the Tc-99m concentration present in the mixed syringe from uncorrected images provides a clear indication that cross-talk correction is indeed necessary. The largest error at the highest In-111 concentration was greater than 80% while even the smallest error at the lowest In-111 concentration was over 10% (see Figure 2.14). There was also a clear dependence on the concentration of In-111 present.

Cross-talk correction using the direct application of TEW method provided substantial reduction in cross-talk and was able to reduce the error from the true value

by approximately a third of the error with no correction (Figure 2.14). However, this method over-corrected the images and at high In-111 concentrations, the error was still 30% and not sufficient for good quantitative imaging. The dependence of the error on the In-111 concentration was also still clearly present.

Figure 2.14: Plot of the error in the activities measured from the Tc-99m images compared to the known true activities measured from the dose calibrator. The dashed lines show the $\pm 5\%$ region desired for quantitative small-animal imaging. After modification of the TEW method, the errors in the measured values are reduced to within this range.

After making the modification to the TEW method and applying the modified correction to the Tc-99m images, the error from the true Tc-99m concentration was further reduced. After the modified correction, the error had stabilized and remained constant across the five activity ratios tested. With this correction method, the Tc-99m activity can be measured with an accuracy of $4 \pm 1\%$ over all In-111 concentrations. Errors in the measured activity concentrations were found using the same method as was used in phase 1 of this work.

When we looked at the In-111 only syringe to assess the cross-talk removal in the Tc-99m image in that region, we saw a consistent removal of the cross-talk (Figure 2.15). This is expected since the concentration in this syringe was the same in all images (with the exception of minor differences due to radioactive decay). The residual scatter in the corrected Tc-99m images was $12\pm 2\%$ of the scatter present before correction (Figure 2.15).

Figure 2.15: Plot showing the variation in cross-talk measured in the In-111 syringe over a range of In-111/Tc-99m activity ratios for the modified TEW corrected Tc-99m energy window images. The solid line shows the average of the data and the dashed line shows the standard deviation. The values show a consistent reduction in scatter with only minimal variation.

After measuring the quantitative accuracy of the In-111 attenuation corrected images, we found that there was an error of $-3\pm 1\%$ on measurements of the In-111 only syringe and $-6\pm 1\%$ on measurements of the mixed syringe. Errors were found using the same method as in phase 1.

2.4 Phase 3: Feasibility in-vivo

2.4.1 Purpose

The purpose of this phase was to determine if the modified TEW method could achieve a similar accuracy in an in-vivo setting compared to that achieved in phantoms.

2.4.2 Methodology

Cross-talk Correction Techniques

The cross-talk correction technique used was the modified TEW method. It was applied to all Tc-99m photopeak images prior to reconstruction according to Figure 2.11.

Animals

Ethical approval for this study was acquired from the University of Ottawa's Animal Care Committee (protocol HI-286). In this phase, the correction method was tested via imaging of Sprague-Dawley rats. Once the rats arrived at the Heart Institute, they were allowed to acclimatize to their new environment for one week before any imaging was done. In order to isolate the two isotopes and have an accurate measurement of the true activities present, only one isotope, Tc-99m, was injected. Tc-99m was injected in the form of Tc-99m-tetrofosmin, an established clinical myocardial perfusion tracer. This tracer is known to concentrate in the heart with a high target to background ratio. The injection of Tc-99m was kept at 81 ± 4 MBq for all rats.

The other isotope, In-111, was placed in a 5 ml plastic syringe (Becton Dickinson #309646) and placed on the chest of the rat during imaging. The syringe was filled with In-111-chloride in aqueous solution and diluted with water to produce a volume 2 ml containing the desired activity. The plunger of the syringe was compressed to hold the 2 ml volume only. Three syringes of In-111 were created, each containing a

different concentration of activity. The activity concentrations were: 0.8695, 2.4975 and 6.8080 MBq/ml. The syringe was then placed on the rats chest in the left anterior oblique position to minimized its distance from the rat's heart. The positioning was checked with a pilot CT scan and adjusted if necessary. In doing this, our hypothesis was that the In-111 syringe would be close enough to the heart that some of its photons would scatter in the heart and be detected in the Tc-99m photopeak window in a location where Tc-99m was also present.

Image Acquisition

In addition to the physical decay of the isotope, the body naturally clears the compound from the body, referred to as biological decay. It was necessary then to determine a model for the rate at which Tc-99m-tetrofosmin is cleared from the rat heart. This was done by imaging a single rat at 20 minute intervals for a total of 3.5 hours. The rat was injected with a dose of 80 MBq and placed on the camera bed. Once in the camera, a pilot CT scan was performed to set a field of view that contained the rat's heart. The field of view was set so as to bracket the heart and a small amount of tissue above and below, approximately from the top of the liver to the level of the aortic sinus. Once the field of view was set, images were acquired consecutively over the 3.5 hour period. Each image consisted 96 projections of 50 seconds each for a total scan time of 20 minutes.

During the main imaging protocol to test the modified TEW cross-talk correction method, each of the six rats was injected with 81 ± 4 MBq of Tc-99m-tetrofosmin 30 minutes before the start of the SPECT imaging protocol. During these 30 minutes, the Tc-99m-tetrofosmin was allowed to distribute throughout the body. During this time, the rat was positioned on the camera bed and a pilot scan was performed to set a field of view which contained the rat heart. The field of view used was 35 cm long and as before, the field of view was set so as to bracket the heart and a small

amount of tissue above and below, approximately from the top of the liver to the level of the aortic sinus.. In addition, a CT image of the rat was acquired for attenuation correction. CT images were performed with a tube voltage of 45 kVp and contained 360 projections of 1500 ms.

Thirty minutes after injection, a series of helical SPECT scans were performed on each rat. The rats were imaged a total of 8 times. Each scan contained 96 projections of 50 seconds for a total scan time of 20 minutes. The first scan was of the rat only (no In-111 syringe in place). This image was used to determine the true initial concentration of Tc-99m present in the heart without the presence of In-111 scatter. The second to seventh scans were done with an In-111 syringe present on the rat's chest. The rats were imaged twice with each In-111 syringe, once to acquire the Tc-99m photopeak windows as well as the scatter windows and then a second time to acquire the two In-111 photopeak windows. The final scan was again with only the rat (no In-111) to get a final estimate of the true Tc-99m activity concentration in the heart.

The rats were anaesthetized with Isoflurane at 1-2% prior to injection and remained anaesthetized throughout the entire imaging procedure. The temperature of the scanner bed was monitored throughout the procedure and was maintained at 37 degrees Celsius. This was done because the rats are very sensitive to hypothermia during anaesthesia. The heart rate and breathing rate of the rats were also monitored throughout the procedure to make sure they remained at an acceptable and healthy level. The mass, average heart rate and average respiration rate for each rat is shown in Table 2.6

Reconstruction

The reconstruction method used on the rat images was the same as described in phase 2 of this thesis. Briefly, projection data were corrected with the modified TEW

Table 2.6: Mass, average heart rate and average respiration rate measured over the imaging session for each rat. The uncertainty in the mass is the error due to the measurement scale while the error in heart rate and respiration rate is due to variation over the imaging procedure.

Rat	Mass (g)	Heart Rate (beats per minute)	Respiration Rate (breaths per minute)
1	255.9 $\pm$ 0.2	360 $\pm$ 15	56 $\pm$ 7
2	257.0 $\pm$ 0.2	386 $\pm$ 11	62 $\pm$ 4
3	266.3 $\pm$ 0.2	352 $\pm$ 17	46 $\pm$ 7
4	259.0 $\pm$ 0.2	343 $\pm$ 17	57 $\pm$ 4
5	364.1 $\pm$ 0.2	374 $\pm$ 8	59 $\pm$ 7
6	367.1 $\pm$ 0.2	329 $\pm$ 17	51 $\pm$ 2

approach and then an OSEM reconstruction was performed off-line with attenuation correction [54].

Data Analysis

After reconstruction of the images, the first step in analysing them was to determine the model which would be used to estimate the clearance of Tc-99m-tetrofosmin from the heart of the rat. To determine the model used for estimation, the serial study of a rat with only Tc-99m-tetrofosmin was used. Two model fits were tested on the serial study data to determine which provided the most accurate representation of the heart clearance. Since the length of the serial study matched the approximate length of the imaging procedure on one rat, we can use the fitted model to estimate the activity in the heart at each imaging time point during the study. To determine the activity in the heart for each image of the serial study, an ROI of 30% threshold was placed around the heart and the total activity within the region was measured. This was measured for each of the eight acquired images.

Each measured heart activity were corrected for isotope decay and then plotted against time. The data was fit with a linear and mono-exponential model to determine which provided the best fit. During the correction study, an estimate of the true activity concentration in the heart was determined by interpolating a linear clearance

of Tc-99m tetrofosmin between the first and last image acquired where no In-111 was present in the field of view. An ROI was selected around the rat's heart in each of the two images and the total activity present in the region was measured. From these two activities, the true Tc-99m activities in the hearts of the other three Tc-99m images were estimated.

For the three corrected Tc-99m images wherein In-111 was also present during imaging, an ROI was selected around the rat's heart and the total activity present in the region was measured. This measured activity was then compared to the estimate of the true activity. The measured activity was normalized by the true activity estimate for easier comparison across all rats.

2.4.3 Results

The rate of clearance of Tc-99m tetrofosmin in the heart for a single rat was found by plotting the measured heart activity every 20 minutes for 3.5 hours (Figure 2.16). There is a clear decline in the heart's Tc-99m concentration over time so this needs to be accounted for when estimating the true Tc-99m concentration. The data were fit with a linear and exponential decay curve to determine the best model. The linear model provided a slightly better fit to the data with an R^2 value of 0.9633 compared to 0.9590 for the exponential fit. The errors in the normalized activity values were determined with a method similar to that used in phase 1 of this work.

The linear fit had a slope of -0.0393 (-0.04615, -0.03244) h^{-1} . The uncertainty on this fit was found by using the slope, to calculate the activity then also using the confidence boundaries of the slope to calculate the activity. The variation in the activities calculated from the confidence boundaries from those calculated using the slope of best fit was $\pm 2\%$. For all activities estimated using linear interpolation, we have assumed they carry this 2% error.

The heart's Tc-99m activity was measured from the Tc-99m images where no

Figure 2.16: Plot showing the modelled fit for the biological clearance of Tc-99m-tetrofosmin from the rat heart with time. Both an exponential model and a linear model were tested with the linear model providing a better fit in this time frame. This model was used to estimate the heart activity in the presence of In-111.

cross-talk correction had been applied. Comparing these measured values to the estimates of the true activity found using the tetrofosmin clearance model (Figure 2.17), the maximum error found was 3% at the lowest In-111 concentration. This is already an accuracy superior to what we hypothesized we could achieve and what we were able to achieve in phase 2. This indicates that there was only minimal, if any, In-111 cross-talk being measured in the heart. Comparing the activity concentrations in the corrected images with the estimates of truth, there was a slight increase in the error. On examination of the errors in each measurement, we see that in almost all cases, the error bars cross 1, where a normalized activity value of 1 would mean a perfect measurement. Errors in these measurements were found using the same method as in phase 1 to determine the effect of the threshold and the pixel variation. These two errors were combined with the error of the tetrofosmin clearance model to find

Figure 2.17: Plot of measured Tc-99m activity normalized to the true activity. The plot shows the error in the measured activity due to residual In-111 scatter in the region. The solid lines represent Tc-99m measurements in the rat heart before and after correction. The dashed lines represent the In-111 activity in the location of the In-111 syringe in the Tc-99m image.

the total error in each measurement. The errors were found for each image of each rat and then a weighted average and standard deviation was found for each syringe concentration.

2.5 Discussion

The simplest method to implement and the one already integrated into the manufacturer's software package for cross-talk correction is much too simplistic to give accurate quantitative results. It does not consider the spatial distribution of Compton scatter in any way and this is apparent in the resulting images. By only scaling the primary counts with the scatter fraction, and due to the Gibbs artefact causing

rings to appear in the image, the resulting image is under-corrected near the centre of the syringe where the scatter distribution is the most dense and over-corrects at the edges of the syringe (see Figure 2.18). Although this may average out to a bias difference of zero over the whole area of the syringe, the magnitude of the over- and under-correction is so large on a pixel by pixel basis that it is not a feasible method for quantitative imaging.

Figure 2.18: Plot of image pixel value as a function of r where r is the radial distance from the centre of the In-111 syringe. Pixel values were measured in the Tc-99m photopeak image after correction with the manufacturer provided method. Profiles were measured at 5 degree increments around 360 degrees. Each line represents a single profile.

The convolution subtraction method improves on some of the issues present in the manufacturer's correction. Instead of using only a scatter fraction scaling of the primary photons, it adds a blurring kernel to model the distribution of scattered photons around a source. This helps with the over- and under-correcting issues but still assumes a radially symmetric scatter distribution. This would be a correct as-

sumption in an ideal case where the scattering medium consists of a uniform infinite material. In in-vivo imaging however, the scattering medium is an animal which consists of distinct structures of different materials which are bounded and are in no way homogeneous. If it is unable to removing the scatter in a simple phantom of a single scattering material (Figure 2.9), it would likely show even worse results when used in animal imaging.

The TEW method eliminates scatter distribution problems by assuming no spatial relationship between the primary In-111 distribution and the scatter distribution. Instead it estimates the scatter at each position independent of the In-111 concentration. As a result, we see the best results with this method, both qualitatively and quantitatively.

We observed that there was a statistically significant difference between the two implementations of the TEW method, applied in projection and image space, where the implementation in projection space produced a more accurate and uniform correction of the scatter. Both methods provided similar average pixel value within the corrected In-111 syringe, but they were statistically different due to their standard deviation in those pixel values. Part of the explanation for this may lie in the underlying mechanisms of the OSEM iterative method. When we apply the TEW correction in projection space we are changing the noise properties of the data. After subtraction there is no longer a Poisson noise relationship. This is a problem because the OSEM algorithm is based on there being a Poisson noise relationship. Changing this relationship would then cause errors in the reconstruction process. Since the reconstruction algorithm is non-linear, we know that superposition and subtraction do not hold in image space either. Because of this, it is unclear how well the TEW method would hold there. The most accurate approach to alleviate these problems might be to incorporate the scatter estimate into the reconstruction algorithm however we did not explore this option.

After testing the TEW correction method in a situation where both isotopes are present (the mixed syringe of the phantom), it is evident that the increasing presence of In-111 contributes to an increased error in the accuracy of the measured Tc-99m. The increase in In-111 means not only an increase of scatter but also an increase in primary photons being detected in the primary window. There is a clear need for modification of the TEW method to achieve better quantitative accuracy, especially in these high In-111 concentration scenarios.

Removing the primary photons was done using a Gaussian estimated fit to the primary photopeak distribution and setting the primary photons in the scatter window as a fraction of those measured in the primary photopeak window. This is a good approximation when the photons measured in the photopeak window are dominated by true primary counts so that the scattered photons skew the estimate only minimally. As a result, the modified correction worked well in areas where both isotopes were present (the mixed syringe).

The problem with this method of estimating the primary contribution to the scatter window arises in areas where there is no Tc-99m present. In these areas, the counts measured in the primary Tc-99m window are entirely scatter. In addition, with no Tc-99m present there is no need to remove primary Tc-99m counts from the scatter window. Unfortunately, the code cannot distinguish this situation so it still attempts to carry out the subtraction. Since the photopeak window has only scatter counts, any estimate based on these counts will actually be removing scatter counts from the scatter window as well, and therefore underestimate and under correct the scatter in that pixel. This effect is seen in Figure 2.15 for the phantom experiments and also in Figure 2.17 for the rat experiments.

One way to compensate for this issue might be to take an iterative approach to the modified TEW correction. After applying the correction once, the new estimate of primary Tc-99m photons in the photopeak window could be compared to the value

before correction and a convergence criterion set. This way, if the change between the values is large, which would be the case when only scatter is present, the correction would be repeated. An end criterion could also be set so that when the difference between estimates falls below the convergence criterion then we can conclude that a reasonable scatter estimate has been achieved. Testing this approach would be one of the next steps in validating this method.

A limitation of our method is that we have only validated in one phantom configuration. This phantom contained only two types of scattering media (the water and the plastic of the syringe) which have similar scattering properties to biological tissue but lack its complexity. To better validate this correction method it would have been useful to test its accuracy in a number of different phantom configurations, some of which could model the complexity of rat body.

When determining the parameters of the bilinear curve used to convert CT images to attenuation maps, we noted that all of the measured attenuation coefficient values were lower than expected based on the accepted values found in Hubbell and Seltzer [58]. This is due to the fact that our values were measured using a broad beam configuration and not a narrow beam. The values needed for attenuation correction however, are narrow beam values. We have adjusted the slopes and intercepts of our lines in an effort to better match the narrow beam values. For the portion of the curve that converts materials less dense than water, we know that this shift is reasonable because the attenuation coefficient for water are well defined.

The shifts performed on the portion of the line used to convert materials more dense than water such as bone are not as easily justified although we know the lines must intersect at water. Although the accuracy of conversion for large CT values is unclear, the amount of material present in a rat that would have CT values of this magnitude is very small and as a result would have only minor effects on the accuracy of the attenuation correction. Based on this knowledge, we felt this is a

justifiable approach. One way to test the validity of this shift would be to perform a reconstruction with the original conversion curves and another with the shifted curves. Comparing these two images would also help in quantifying the error in the shifted method. This is a crucial next step in validating the In-111 attenuation correction.

After using this attenuation map conversion model, the resultant images had a measurement error of approximately 5%. This is of the same order of magnitude as the accuracy achieved for the Tc-99m measurements showing that we were able to achieve a consistent accuracy across all images. Although determining the narrow beam conversion coefficients may be able to provide additional accuracy, we have demonstrated that a simple shift of the broad beam values provides a good approximation.

The purpose of phase 3 of this project was to have a dual isotope study where the true concentration of each isotope was known in order to determine the accuracy of the correction method. In order to do this in a true dual isotope study, the animals used must be sacrificed, dissected and organs decayed and placed in a well counter. The signal from the well counter also suffers from cross-talk. Thus, a cross-talk correction method would need to be employed here as well and this method would first need to be validated. In an effort to avoid this, we attempted to simulate a dual-isotope experiment without actually injecting both isotopes. Unfortunately, the syringe could not be placed close enough to the heart (without surgical implantation) and as a result the number of In-111 photons that scattered into the heart of the rat was almost negligible. With only minimal In-111 scatter in the heart, the accuracy of the measured uncorrected Tc-99m activity is quite high.

The errors that we do see are small, ranging from -3% to +3% but with errors of a similar magnitude. The measured values after correction were slightly larger in magnitude (-3% to -7% differences) but were also accompanied with larger errors which are all larger than the values themselves. Since the error associated with each

measurement makes the measurement indistinguishable from a measurement with perfect accuracy, it is very difficult to draw any conclusions about this cross-talk correction's performance ability in-vivo. In order to differentiate quantitative value of images before and after cross-talk correction, it is necessary for a larger proportion of cross-talk to be present in the regions of Tc-99m-tetrofosmin uptake. This confirms the need to do a true dual-isotope in-vivo study.

Although we were not able to make conclusions about our cross-talk correction method's accuracy in an in-vivo environment, we were able to verify one of its limitations. When measuring the residual scatter present in the location of the In-111 syringe after correction, we saw the same problem of incomplete removal identified in phase 2. At the highest In-111 syringe concentration, we see 14% residual scatter. This is comparable to the 12% that was measured in phase 2 when using a similar activity concentration. Although we are still unsure of our method's accuracy in-vivo, we are seeing some results consistent with those found in the phantom experiments. This indicates that with further exploration this method might have the potential to become a quantitatively accurate method of correcting cross-talk.

2.6 Conclusions

After careful examination of four different cross-talk correction techniques for use in In-111/Tc-99m dual isotope small animal imaging, we determined that the best method for removing the In-111 cross-talk in the Tc-99m photopeak window was a modified Triple Energy Window method. This method provided an level of accuracy acceptable for quantitative small animal imaging when tested in a simple phantom configuration. When tested in an in-vivo study, however, the experimental design failed to provide an acceptable configuration to accurately test the method. The limited success of this portion of the study made it clear that a true dual-isotope

study would be necessary to determine the usefulness of this cross-talk correction method in small animal imaging.

Chapter 3

Conclusions and Future Work

3.1 Conclusions

In this thesis, the development and testing of a modified triple energy window method of cross-talk correction in dual-isotope imaging was described. This technique was able to achieve an accuracy of $4\pm 1\%$ compared to the true activity concentrations in phantom experiments. It was also able to remove a substantial amount of In-111 scatter when no Tc-99m was present. Further modifications to the method, however, are required to achieve better accuracy in these areas. When tested in-vivo, the modified TEW method was able to remove the shadow of the In-111 syringe in the Tc-99m image but the amount of cross-talk measured in the heart from In-111 was too small to provide a clear measure of the accuracy of the correction in-vivo.

The accuracy we have demonstrated, $4\pm 1\%$, is a vast improvement on the accuracy achieved without cross-talk correction. To gain a better understanding of how good this accuracy is, it is important to compare the error caused by cross-talk to the other sources of error present in small-animal imaging. These additional sources of error also place limits on the quantitative accuracy that can be achieved in small-animal imaging. Chen *et al.* [16] showed that the effects of attenuation introduced

a -30.6% error while the effects of self-scatter introduced an error of 9.1% in a rat sized phantom using Tc-99m. The use of attenuation and scatter correction methods has reduced these numbers drastically. Vanhove *et al.* [18] were able to achieve a quantitative accuracy of $-9.5 \pm 10.4\%$ in rats by implementing both an attenuation correction and a TEW scatter correction. Although this was a single isotope study, the correction techniques used were similar to ours so it is a useful comparison study. The errors we measured in both our phantom study and our rat study are less than this value and are therefore at an appropriate level for quantitatively accurate small animal imaging.

All of our measurements were done using a total activity measurement within a volume of interest. The volumes of interests used in this study were on the order of 2 cm^3 . The spatial resolution of our system is 1.6 mm. At this size, there is a large reduction in the partial volume effects and these are not expected to cause additional error in the measurements. When measuring smaller volumes, this can become a concern. Partial volume errors become more and more magnified with smaller and smaller structures and for structures as small as 2 mm in diameter, the error can be as much as 50% [52]. When doing small animal imaging we are working with much smaller structures than in human imaging and it is here where partial volume effects become more of a concern. These effects must be considered in further validation of this method.

Using the modified TEW method to remove In-111 cross-talk from Tc-99m images would provide quantitatively accurate images to study the relationship between tissue death and heart function in the context of heart disease. It could also provide researchers with the ability to study other processes using different molecules labelled with these isotopes. The accuracy of the method, however, may be limited to this combination of isotopes. If different isotopes are used, the energy distribution of the emitted photons will change and the validity of this method, especially with the im-

plemented modification, may no longer be valid. Our method relies on the energy distributions of these specific isotopes. For example, if I-123 was used instead of In-111, the emission energies would be 140 and 159 keV. These two photopeaks are even closer than Tc-99m and In-111. Primary contributions would not only be a problem in the scatter window but in the primary photopeak windows as well. With these additional considerations, perhaps a TEW method would not be the best candidate for a quantitatively accurate cross-talk correction method.

We have demonstrated that our modified TEW method works in certain situations, but it has its limitations. As mentioned, it achieves good accuracy in areas where both isotopes are present. Without further testing of this additional modification to the method, the use of the modified TEW cross-talk correction method is limited to situations where there is substantial overlap in the distribution of isotopes in the animal. However, it is this situation where correction is most critical. The modified TEW correction approach that was developed in this work provides an excellent first step in generating quantitatively accurate small-animal dual-isotope results in the presence of cross-talk, but further studies are needed to extend and improve upon this technique.

3.2 Future Work

As we have indicated, our modified TEW method leads to an under-correction in areas where only one of the two isotopes is present. We have hypothesized that this is an artefact of the modification implemented to remove the primary photons from the scatter windows and have suggested applying the correction iteratively as a potential solution. This would minimize the removal of true scattered photons from the scatter estimate and could result in a more accurate cross-talk removal. This implementation of this iterative approach is the next step of this work. In addition, the variation

found in the scatter estimate before and after TEW implementation needs to be examined with and without the iterative approach. This would serve to determine an appropriate criterion for stopping the iterations.

As a result of our experimental set-up, we were not able to effectively examine the accuracy of our modified TEW method in an in-vivo environment. This validation is key to the acceptance of this method and therefore this must be revisited. The shortcomings of our design drive us towards doing a true dual-isotope imaging study with both isotopes injected into the animal. This would provide true overlap of the activity distributions and allow for a larger presence of In-111 contamination. Since acquiring accurate measurements for this type of study would also require a cross-talk correction method for the well counter, a first approach could be to determine if a different pseudo-dual-isotope design could be successful. This can be done by examining the In-111 scatter distribution by placing an In-111 source inside a water phantom the size of a rat to determine how large the spread of the distribution is and if we can reasonably expect any number of scattered photons to be present anywhere outside the heart.

Another aspect for future investigation is the effect this method has on partial volume effects. This can be done by creating mixed isotope sources of decreasing diameters but containing identical activity concentrations. In the absence of partial volume effects we would expect that activity measurements on these images would produce identical errors regardless of size. This will not be the case however and these measurements would allow us to determine our methods sensitivity to these effects.

References

- [1] Statistics Canada, *Leading causes of death in Canada*. Ottawa, ON. 2008. Catalogue #: 84-215-XWE.
- [2] S. R. Cherry, "In vivo molecular and genomic imaging: new challenges for imaging physics," *Physics in Medicine and Biology*, vol. 49, pp. R13–R48, 2004.
- [3] R. Weissleder, B. Ross, A. Rehemtulla, and S. Gambhir, *Molecular Imaging: Principles and Practice*. PMPH USA Ltd Series, Shelton, CT: People's Medical Publishing House, 2010.
- [4] S. Cherry, J. Sorenson, and M. Phelps, *Physics in Nuclear Medicine*. Philadelphia, PA: Elsevier Health Sciences, 2012.
- [5] G. B. Saha, *Physics And Radiobiology of Nuclear Medicine*. New York, NY: Springer, 2006.
- [6] J. T. Bushberg, *The Essential Physics of Medical Imaging*. Philadelphia, PA: Lippincott Williams & Wilkins, 2002.
- [7] M. N. Wernick and J. N. Aarsvold, *Emission Tomography: The Fundamentals of PET and SPECT*. San Diego, CA: Elsevier Academic Press, 2004.
- [8] H. O. Anger, "Scintillation camera," *Review of Scientific Instruments*, vol. 29, pp. 27–33, 1958.

- [9] H. O. Anger, "Sensitivity, resolution, and linearity of the scintillation camera," *Nuclear Science*, vol. 13, pp. 380–392, 1966.
- [10] J. Prekeges, *Nuclear Medicine Instrumentation*. Sudbury, MA: Jones and Bartlett Publishers, 2010.
- [11] T. E. Peterson and L. R. Furenlid, "SPECT detectors: the Anger Camera and beyond," *Physics in Medicine and Biology*, vol. 56, pp. R145–R182, 2011.
- [12] S. Ahmed, *Physics and Engineering of Radiation Detection*. London, UK: Academic Press, 2007.
- [13] W. Semmler and M. Schwaiger, *Molecular Imaging I. Handbook of Experimental Pharmacology*, Berlin, Germany: Springer, 2008.
- [14] P. P. Bruyant, "Analytic and Iterative Reconstruction Algorithms in SPECT," *Journal of Nuclear Medicine*, vol. 43, pp. 1343–1358, 2002.
- [15] L. Bouwens, de Walle Van, J. Nuyts, M. Koole, Y. D'Asseler, S. Vandenberghe, I. Lemahieu, and R. A. Dierckx, "Image-correction techniques in SPECT," *Computerized medical imaging and graphics: the official journal of the Computerized Medical Imaging Society*, vol. 25, pp. 117–126, 2001.
- [16] C.-L. Chen, Y. Wang, J. Lee, and B. Tsui, "Toward Quantitative Small Animal Pinhole SPECT: Assessment of Quantitation Accuracy Prior to Image Compensations," *Molecular Imaging and Biology*, vol. 11, pp. 195–203, 2009.
- [17] C. Wu, F. van der Have, B. Vastenhouw, R. Dierckx, A. Paans, and F. Beekman, "Absolute quantitative total-body small-animal SPECT with focusing pinholes," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 37, pp. 2127–2135, 2010.

- [18] C. Vanhove, M. Defrise, A. Bossuyt, and T. Lahoutte, "Improved quantification in single-pinhole and multiple-pinhole SPECT using micro-CT information," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 36, pp. 1049–1063, 2009.
- [19] F. Beekman and F. van der Have, "The pinhole: gateway to ultra-high-resolution three-dimensional radionuclide imaging," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 34, pp. 151–161, 2007.
- [20] N. Schramm, G. Ebel, and U. Engeland, "High-resolution SPECT using multipinhole collimation," *IEEE Transactions on Nuclear Science*, vol. 50, pp. 315–320, 2003.
- [21] F. P. DiFilippo, "Design and performance of a multi-pinhole collimation device for small animal imaging with clinical SPECT and SPECT/CT scanners," *Physics in Medicine and Biology*, vol. 53, pp. 4185–4201, 2008.
- [22] F. Kiessling and B. Pichler, *Small Animal Imaging: Basics and Practical Guide*. Berlin, Germany: Springer, 2011.
- [23] S. R. Meikle, P. Kench, M. Kassiou, and R. B. Banati, "Small animal SPECT and its place in the matrix of molecular imaging technologies," *Physics in Medicine and Biology*, vol. 50, pp. R45–R61, 2005.
- [24] M. A. King, P. H. Pretorius, T. Farncombe, and F. J. Beekman, "Introduction to the physics of molecular imaging with radioactive tracers in small animals," *Journal of cellular biochemistry*, vol. 87, pp. 221–230, 2002.
- [25] R. J. Jaszczyk, J. Li, H. Wang, M. R. Zalutsky, and R. E. Coleman, "Pinhole collimation for ultra-high-resolution, small-field-of-view SPECT," *Physics in Medicine and Biology*, vol. 39, pp. 425–437, 1994.

- [26] K. Ishizu, T. Mukai, Y. Yonekura, M. Pagani, T. Fujita, Y. Magata, S. Nishizawa, N. Tamaki, H. Shibasaki, and J. Konishi, "Ultra-high resolution SPECT system using four pinhole collimators for small animal studies," *Journal of Nuclear Medicine*, vol. 36, pp. 2282–2287, 1995.
- [27] Bioscan Inc, Washington, DC, *Technical Brochure and Specifications Guide: NanoSPECT/CT Preclinical Imager*, 2007.
- [28] M. Khalil, *Basic Sciences of Nuclear Medicine*. Berlin, Germany: Springer, 2010.
- [29] D. J. Rowland and S. R. Cherry, "Small-animal preclinical nuclear medicine instrumentation and methodology," *Seminars in nuclear medicine*, vol. 38, pp. 209–222, 2008.
- [30] M. Kupinski and H. Barrett, *Small-animal SPECT Imaging*. New York, NY: Springer, 2005.
- [31] Y. Du, E. C. Frey, and S. Member, "Simultaneous 99m Tc / 201 Tl / 123 I Triple-Isotope Cardiac SPECT Imaging," *IEEE Nuclear Science Symposium Conference Record*, vol. 6, pp. 4213–4216, 2007.
- [32] H. de Jong, F. Beekman, M. Viergever, and P. van Rijk, "Simultaneous 99mTc/201Tl dual-isotope SPET with Monte Carlo-based down-scatter correction," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 29, pp. 1063–1071, 2002.
- [33] S. C. Moore, R. J. English, C. Syravanh, D. E. Tow, R. E. Zimmerman, K. H. Chan, and M. F. Kijewski, "Simultaneous Tc-99m/Tl-201 imaging using energy-based estimation of the spatial distributions of contaminant photons," *IEEE Transactions on Nuclear Science*, vol. 42, pp. 1189–1195, 1995.

- [34] A. Tsuji, A. Kojima, M. Matsumoto, Y. Oyama, S. Tomiguchi, T. Kira, Y. Takagi, O. Shimomura, and M. Takahashi, "A new method for crosstalk correction in simultaneous dual-isotope myocardial imaging with Tl-201 and I-123," *Annals of Nuclear Medicine*, vol. 13, pp. 317–323, 1999.
- [35] J.-T. Yang, K. Yamamoto, N. Sadato, T. Tsuchida, N. Takahashi, and N. Hayashi, "Clinical value of triple-energy window scatter correction in simultaneous dual-isotope single-photon emission tomography with 123 I-BMIPP and 201 Tl," *European Journal of Nuclear Medicine*, vol. 24, pp. 1099–1106, 1997.
- [36] Y. Du and E. C. Frey, "Quantitative evaluation of simultaneous reconstruction with model-based crosstalk compensI dual-isotope simultaneous acquisition brain SPECT," *Medical Physics*, vol. 36, p. 2021, 2009-2033.
- [37] J. Ouyang, X. Zhu, C. M. Trott, and G. El Fakhri, "Quantitative simultaneous 99mTc/123I cardiac SPECT using MC-JOSEM," *Medical Physics*, vol. 36, pp. 602–611, 2009.
- [38] J. Ouyang, G. E. Fakhri, S. C. Moore, and M. F. Kijewski, "Fast Monte Carlo Simulation Based Joint Iterative Reconstruction for Simultaneous 99mTc/123I Brain SPECT Imaging," *IEEE Nuclear Science Symposium Conference Record*, vol. 4, pp. 2251–2256, 2006.
- [39] S. Shcherbinin, S. Chamoiseau, and A. Celler, "Quantitative image reconstruction for dual-isotope parathyroid SPECT/CT: phantom experiments and sample patient studies.," *Physics in Medicine and Biology*, vol. 57, pp. 4755–4769, 2012.
- [40] D. J. Kadrmas, E. C. Frey, and B. M. W. Tsui, "Simultaneous technetium-99m/thallium-201 SPECT imaging with model-based compensation for cross-contaminating effects," *Physics in Medicine and Biology*, vol. 44, pp. 1843–1860, 1999.

- [41] A. Larsson, M. Ljungberg, S. J. Mo, K. Riklund, and L. Johansson, "Correction for scatter and septal penetration using convolution subtraction methods and model-based compensation in ^{123}I brain SPECT imaging: a Monte Carlo study," *Physics in Medicine and Biology*, vol. 51, pp. 5753–5767, 2006.
- [42] J. Feng, B. C. Penney, and X. Pan, "Convolution estimation of spill-down and scatter in dual isotope ($^{99\text{m}}\text{Tc}/^{111}\text{In}$) SPECT," in *Medical Imaging 2002: Physics of Medical Imaging* (L. E. Antonuk and M. J. Yaffe, eds.), pp. 73–81, Proceedings of SPIE 4682, 2002.
- [43] T. Ichihara, K. Ogawa, N. Motomura, A. Kubo, and S. Hashimoto, "Compton Scatter Compensation Using the Triple-Energy Window Method for Single- and Dual-Isotope SPECT," *Journal of Nuclear Medicine*, vol. 34, pp. 2216–2221, 1993.
- [44] X. Zhu, M. A. Park, and S. C. Moore, "Quantitative Simultaneous $^{111}\text{In}/^{99\text{m}}\text{Tc}$ Planar Imaging of the Foot," in *Nuclear Science Symposium Conference Record, 2006. IEEE*, vol. 5, pp. 2684–2687, 2006.
- [45] K. Ogawa, Y. Harata, T. Ichihara, A. Kubo, and S. Hashimoto, "A practical method for position-dependent Compton-scatter correction in single photon emission CT," *IEEE Transactions on Medical Imaging*, vol. 10, pp. 408–412, 1991.
- [46] R. Jaszczyk, K. Greer, C. Floyd, C. Harris, and R. Coleman, "Improved SPECT quantification using compensation for scattered photons," *J Nucl Med*, vol. 25, pp. 893–900, 1984.
- [47] B. F. Hutton, I. Buvat, and F. J. Beekman, "Review and current status of SPECT scatter correction," *Physics in Medicine and Biology*, vol. 56, pp. R85–R112, 2011.

- [48] H. de Jong, E. Slijpen, and F. Beekman, "Acceleration of Monte Carlo SPECT simulation using convolution-based forced detection," *IEEE Transactions on Nuclear Science*, vol. 48, pp. 58–64, 2001.
- [49] F. Beekman and M. Viergever, "Fast spect simulation including object shape dependent scatter," *IEEE Transactions on Medical Imaging*, vol. 14, pp. 271–282, 1995.
- [50] E. Frey and B. Tsui, "A new method for modeling the spatially-variant, object-dependent scatter response function in SPECT," in *Nuclear Science Symposium, 1996. Conference Record., 1996 IEEE*, vol. 2, pp. 1082–1086, 1996.
- [51] R. Wells, A. Celler, and R. Harrop, "Analytical calculation of photon distributions in SPECT projections," *IEEE Transactions on Nuclear Science*, vol. 45, pp. 3202–3214, 1998.
- [52] A. B. Hwang, B. L. Franc, G. T. Gullberg, and B. H. Hasegawa, "Assessment of the sources of error affecting the quantitative accuracy of SPECT imaging in small animals," *Physics in Medicine and Biology*, vol. 53, pp. 2233–2252, 2008.
- [53] J. H. Strydhorst, F. H. Leenen, T. D. Ruddy, and R. G. Wells, "Reproducibility of Serial Left Ventricle Perfusion, Volume, and Ejection Fraction Measurements Using Multiplexed Multipinhole SPECT in Healthy Rats and Rats After Myocardial Infarction," *Journal of Nuclear Medicine*, vol. 52, pp. 1285–1292, 2011.
- [54] J. Strydhorst and R. G. Wells, "Attenuation correction of multiplexed multipinhole microSPECT reconstruction," in *Nuclear Science Symposium Conference Record (NSS/MIC), 2010 IEEE*, pp. 2841–2843, 2010.
- [55] J. A. Patton and T. G. Turkington, "SPECT/CT Physical Principles and Attenuation Correction," *Journal of Nuclear Medicine Technology*, vol. 36, pp. 1–10, 2008.

- [56] J. Strydhorst and R. G. Wells, "Quantitative reconstruction of microSPECT data," in *University of Ottawa Heart Institute's Molecular Function & Imaging Symposium*, p. 32, 2012. (abstract).
- [57] Y. Seo, "Calculation and validation of the use of effective attenuation coefficient for attenuation correction in In-111 SPECT," *Medical Physics*, vol. 32, pp. 3628–3635, 2005.
- [58] J. H. Hubbell and S. M. Seltzer, "Tables of X-Ray Mass Attenuation Coefficients and Mass Energy-Absorption Coefficients from 1 keV to 20 MeV for Elements $Z = 1$ to 92 and 48 Additional Substances of Dosimetric Interest," in *Physical Reference Data*, 1996.
- [59] H. O. Anger, "Scintillation camera with multichannel collimators," *Journal of Nuclear Medicine*, vol. 5, pp. 515–531, 1964.
- [60] H. O. Anger and D. H. Davis, "Gamma ray detection efficiency and image resolution in sodium iodide," *Review of Scientific Instruments*, vol. 35, pp. 693–697, 1964.
- [61] J. Bai, J. Hashimoto, K. Ogawa, T. Nakahara, T. Suzuki, and A. Kubo, "Scatter correction based on an artificial neural network for Tc-99m and I-123 dual-isotope SPECT in myocardial and brain imaging," *Annals of Nuclear Medicine*, vol. 21, pp. 25–32, 2007.
- [62] S. Brown, D. L. Bailey, K. Willowson, and C. Baldock, "Investigation of the relationship between linear attenuation coefficients and CT Hounsfield units using radionuclides for SPECT," *Applied Radiation and Isotopes*, vol. 66, pp. 1206–1212, 2008.
- [63] G. J. Hademenos, M. Dahlbom, and E. J. Hoffman, "Simultaneous dual-isotope technetium-99m/thallium-201 cardiac spet imaging using a projection-dependent

- spillover correction factor," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 22, pp. 465–472, 1995.
- [64] W. R. Hendee and E. R. Ritenour, *Medical imaging physics*. New York, NY: Wiley-Liss, 4th ed., 2002.
- [65] G. T. Herman, "Image reconstruction from projections," *Real-Time Imaging*, vol. 1, pp. 3–18, 1995.
- [66] R. J. Jaszczyk, C. E. Floyd, and R. E. Coleman, "Scatter Compensation Techniques for SPECT," *IEEE Transactions on Nuclear Science*, vol. 32, pp. 786–793, 1985.
- [67] J. Feng and B. C. Penney, "Accurate spill-down and scatter correction for Tc-99m/In-111 SPECT," in *Nuclear Science Symposium Conference Record, 2002 IEEE*, vol. 3, pp. 1562–1566, 2002.
- [68] J. Ouyang, S. C. Moore, M.-A. Park, and G. E. Fakhri, "Fast Monte Carlo estimation of patient and detector scatter and crosstalk contamination in SPECT imaging," in *Nuclear Science Symposium Conference Record, 2005 IEEE*, vol. 3, pp. 1560–1562, 2005.
- [69] T. Kaneta, H. Kurihara, T. Hakamatsuka, H. Ito, S. Maruoka, H. Fukuda, S. Takahashi, and S. Yamada, "Scatter and cross-talk correction for one-day acquisition of 123I-bmipp and 99mTc-tetrofosmin myocardial spect," *Annals of Nuclear Medicine*, vol. 18, pp. 647–652, 2004.
- [70] K. Knesaurek and J. Machac, "Comparison of correction techniques for simultaneous 201 Tl/ 99m Tc myocardial perfusion SPECT imaging: a dog study," *Physics in Medicine and Biology*, vol. 45, pp. N167–N176, 2000.

- [71] K. Knesaurek and J. Machac, "A transformation cross-talk technique for simultaneous dual radionuclide imaging: a myocardial perfusion $^{201}\text{Tl}/^{99}\text{Tcm}$ sestamibi dog SPECT study," *British Journal of Radiology*, vol. 72, pp. 872–881, 1999.
- [72] K. Knesaurek and J. Machac, "Three-window transformation cross-talk correction for simultaneous dual-isotope imaging," *Journal of Nuclear Medicine*, vol. 38, pp. 1992–1998, 1997.
- [73] A. Larsson and L. Johansson, "Scatter-to-primary based scatter fractions for transmission-dependent convolution subtraction of SPECT images," *Physics in Medicine and Biology*, vol. 48, pp. N323–N328, 2003.
- [74] Y. Lemoigne, A. Caner, and G. Rahal, *Physics for medical imaging applications*. Dordrecht, Netherlands: Springer, 2007.
- [75] S. C. Moore, J. Ouyang, M.-A. Park, and G. E. Fakhri, "Monte Carlo-based compensation for patient scatter, detector scatter, and crosstalk contamination in In-111 SPECT imaging," *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers*, vol. 569, pp. 472–476, 2006. Proceedings of the 3rd International Conference on Imaging Technologies in Biomedical Sciences Innovation in Nuclear and Radiological Imaging: from Basic Research to Clinical Application.
- [76] P. Pretorius, M. King, T.-S. Pan, and B. Hutton, "Attenuation correction strategies for multi-energy photon emitters using SPECT," *IEEE Transactions on Nuclear Science*, vol. 44, pp. 1323–1328, 1997.
- [77] S. Shcherbinin and A. Celler, "Simultaneous crosstalk compensation for a ROI activity estimation in dual-isotope SPECT imaging," in *Nuclear Science Symposium Conference Record*, pp. 3256–3261, 2009.

- [78] S. Shcherbinin and A. Celler, "A physical phantom evaluation of absolute quantitation of simultaneous Tc-99m/In-111 SPECT," in *Nuclear Science Symposium Conference Record (NSS/MIC), 2009 IEEE*, pp. 3736–3740, 2009.
- [79] M. F. Smith and R. J. Jaszczeni, "Generalized dual-energy-window scatter compensation in spatially varying media for SPECT," *Physics in Medicine and Biology*, vol. 39, pp. 531–546, 1994.
- [80] S. Vallabhajosula, *Molecular Imaging: Radiopharmaceuticals for PET and SPECT*. Berlin, Germany: Springer, 2009.
- [81] P. Weinmann, M. Faraggi, J. Moretti, and P. Hannequin, "Clinical validation of simultaneous dual-isotope myocardial scintigraphy," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 30, pp. 25–31, 2003.
- [82] R. Weissleder and U. Mahmood, "Molecular imaging 3+6 32++2," *Radiology*, vol. 219, pp. 316–333, 2001.