Development of a High-resolution SSR-SPECT System for Preclinical Imaging and Neuroimaging

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Abstract

The utility of animal models in preclinical research has been increasing by the availability of methods for in vivo imaging. In particular, techniques like single photon emission computed tomography (SPECT) show high potential, which is usually limited by spatial resolution. This represents an important parameter influencing scanner design, given the small size of the anatomical structures to be investigated. The purpose of the present work was to assess the performance of a scintigraphic system with improved spatial resolution based on our previous detector by applying the Super Spatial Resolution (SSR). Our dual-head SPECT system is composed of gamma cameras based on the Hamamatsu H13700 position-sensitive photomultiplier tube (PSPMT). In each detector head, the PSPMT is coupled to a 28×28 array of CRY018 scintillation crystals. The pure Tungsten parallel square hole collimator ensures the position sensitivity, and a dedicated resistive chain readout so as an ADC board have been proprietary designed.

To finalize the mechanical development of the SSR-SPECT system several tests were carried out. Based on the results obtained in the test phase, a partial review of the mechanical design was performed. Then a dedicated machine handling software was developed, and in particular, a kinematic software debugging and testing was assessed. Finally, several experiments were carried out by using Derenzo phantoms and capillaries filled with radioactive sources. Finally, the performance of our system was evaluated performing small animal imaging studies.

The SPECT spatial resolution was experimentally determined to be about 1.6 mm. We reach a resolution of 1.18 mm by applying the SSR based on two images.

The results of this study demonstrated the good capability of the system as a suitable tool for preclinical imaging especially in fields like neuroscience for the study of small brain structures.

1. Introduction

Animal models are essential in biomedical research to study the causes of human and animal diseases and the underlying mechanisms involved, with the final aim of developing and testing new treatments able to improve both human and animal quality of life. The use of animal models allows controlling the confounding variables that characterize clinical research, mimicking biological conditions of human diseases. Thus, over the decades, animal models highly contributed to the most important knowledge advances in many biological fields. More recently, biomedical engineering techniques applied genetic and genomic tools to develop knockout or transgenic animals that are used in research. Noteworthy, in order to obtain reliable results, the health quality of the animal used as a model is fundamental, and thus, besides for obvious ethical reasons, it is in the interest of the researcher to have healthy and well treated animals. Over the past 20 years, molecular imaging emerged as a powerful approach in drug development and identification of new biomarkers of many pathologies¹. Conventional methods to study drug biodistribution or kinetic patterns are usually invasive and require high number of animals to obtain

a limited amount of data. In this context, the use of optical or nuclear medicine imaging has several advantages: i) they accelerate the time of investigation thus allowing the measurement of rapid changes in distribution; ii) many scans can be repeated on the same animal thus reducing the number of experimental groups; iii) they can be easily translated into humans. Therefore, the use of techniques like computed tomography (CT), positron emission tomography (PET) or single photon emission computed tomography (SPECT) became more frequent when conducting preclinical studies in animal models. However, these methodologies require dedicated equipment with a spatial resolution as high as possible. In fact, clinical scanners used for human imaging are bulky and provide a spatial resolution of the order of tens of mm inadequate to that required for studies in mice ($\leq 1\text{mm}$)².

Currently, various SPECT scanners dedicated to the small animal use scintillation detectors, in which the energy released from the incident gamma rays is converted into light photons through a scintillating crystal. Then, the scintillation light is detected by using proper photosensors, such as position-sensitive photomultiplier tubes (PSPMTs). Conversely, other scanners use semiconductor detectors that work through the direct conversion of gamma rays into electrical signals³. The latter, generally, allows achieving better energy resolutions and high spatial resolutions. Moreover, the use of semiconductors enables the design of more compact devices. However, semiconductor imagers are often subject to relatively low uniformity of spatial and energy response over the detector area, in addition, they usually suffer from slow mobility and a short lifetime of charge carriers, which constrain their maximum achievable thickness, hence the achievable sensitivity⁴. Finally, both types of detectors are equipped with high-density collimators, which select the incident gamma rays direction determining the imaging properties. One of the advantages of using scintillators is the relatively high achievable detection efficiency.

In the design of detectors for preclinical imaging, especially in the case of small structures such as brain tissues, the spatial resolution represents a key parameter. In this context, the need to develop imaging systems able to precisely visualize small details has arisen. Our research group has previously developed high-performance scintigraphic detectors for small animal imaging^{5,6}. The feasibility of our patented method named Super Spatial Resolution (SSR)⁷ has been simulated for its application on a scintigraphic system giving as a result planar or three-dimensional (3D) images with a resolution enhancement⁸. In this paper, we describe the application of the SSR method to a dual-head SPECT system evaluating the overall performance and its preclinical application, thus providing the rationale for its implementation in small animal imaging.

2. Methods

The preclinical SPECT scanner presented in this work has been designed on the basis of an imaging system previously engineered by our group⁵. The detectors, named High-Resolution Imaging System (HiRIS2), have been developed using several Monte Carlo (MC) simulations to define the mechanical structure and compose the preparatory technical drawings⁸.

Each SPECT head, shown in Figure 1, is made up of a Hamamatsu H13700 Flat Panel PSPMT (Hamamatsu Photonics K.K., JP) coupled with an array of 28×28 CRY018 (Crytur spol. s.r.o., CZ) scintillation crystals with a size of 1.4 mm × 1.4 mm × 6.0 mm. The scintillator elements are separated by a reflective layer of white epoxy of 200 µm, obtaining a resulting pitch of 1.6 mm, and a field of view (FOV) of about 45 mm × 45 mm. Finally, a 36 mm parallel square hole collimator of pure tungsten with 200 µm thick septa and hole size of 1.4 mm × 1.4 mm, is matched with the scintillation crystals array. To better exploit the H13700 features, we have designed a dedicated resistive chain readout made up of a single miniaturized board connected directly on the PSPMT, which is read by a dedicated compact 4-channel ADC board (125 MSamples/s for each channel). A field-programmable gate array (FPGA) reads the ADC output and implements the digital pulse processing. Moreover, the FPGA is responsible for managing data transfer through a USB 2.0 transceiver that ensures a transfer data rate up to 8 Mbyte/s. The connection is master/slave, where the PC (master) sends a request to a detector (slave), and the detector responds to that request.

The HiRIS2 detector has overall dimensions of 69 mm × 69 mm × 165 mm, and a total weight of about 2 kg.

The HiRIS2 SPECT system, depicted in Figure 2, consists of two detector heads integrated within a rotating mechanical system. The detectors are respectively positioned at a distance of 5 cm and at 180° from each other, they are therefore assembled in opposition. The 360° full rotation of the gantry, the animal bed, and the SSR movements are provided by NEMA 23 stepper motors each controlled by a 1067_0B driver (Phidgets Inc., US) connected to a PC via USB.

According to the SSR method, the scanner allows managing the lateral shifts in the trans-axial plane of both detectors, while shifts along the rotation axis are carried out moving the animal bed. Since the crystal dimension is 1.6 mm by side, all the SSR shifts must be submultiples of this value, for instance, to perform a 2-step SSR along the lateral and/or axial directions, displacements of 0.8 mm must be carried out (half a pixel)⁸. Specifically, the sub-pixel movements may also be restricted to the tangential or axial direction only, depending on the ongoing study, thus limiting the number of projections required. For simplicity, we will use the notation N-step to indicate the number of steps performed by the SSR along the desired direction i.e., axial or trans-axial (e.g. 4-step trans-axial SSR). In the case that the SSR shifts are carried out in both directions, the direction will not be specified, e.g. 9-step SSR means 9 images acquired on a grid of 3 shifts along the axial and 3 along the trans-axial direction.

The prototype software was developed in C++ with Visual Studio 2019, using the Qt ver. 5.15 framework. It provides an automatic scanning procedure that performs image acquisitions through a sequence of successive angular steps around the rotation axis. Moreover, to implement the SSR required movements, for each angular step the detector heads are shifted by a number of displacements accordingly with the method. Finally, for SPECT image reconstruction the STIR software ver. 4.0.1 was used, which is an Open-Source consolidated and validated package, being widely used also as a reference in several scientific studies⁹⁻¹¹. Then image analysis was carried out using Mango - Multi-image Analysis GUI (Research

Imaging Institute, UTHSCSA) ¹² and AMIDE (Amide's a Medical Image Data Examiner) ¹³ image processing software.

2.1. Mechanical assessment

In order to fine-tune the SPECT scanner, the rotational axis of the gantry was assessed through the use of a mechanical centering tool aligned with the system. This tool holds a glass capillary positioned along the axis of rotation. The capillary was filled with a solution containing ^{99m}Tc-pertechnetate. This way, the position of the rotation axis was verified on the acquired images (Figure 3).

2.2. Phantoms evaluation

The characterization of the sole HiRIS2 detector has been performed in our previous work by using complex patterns allowing the assessment of the spatial linearity and the overall imaging capability ^{5,6}. In this study, the HiRIS2 SPECT system performance was determined experimentally using a custom-designed polymethyl methacrylate (PMMA) phantom, and a glass capillary, both filled with a solution of ^{99m}TcO₄⁻ (γ-ray 140.5 keV). This Derenzo-like phantom was used to assess the spatial resolution. It consists of a block of PMMA with a thickness of 20 mm that contains several cylindrical holes grouped into three sectors according to their diameter, which is 1.6 mm, 1.8 mm, and 2.2 mm respectively. In addition, for each sector, the distance between the centres of two adjacent cylinders is twice their diameter. The activity of ^{99m}TcO₄⁻ used for these measurements was 37 MBq. Two acquisitions were performed: a 4-step planar SSR, and a 2-step trans-axial SSR acquisition. In the former, the phantom was positioned at contact with the surface of one detector, and aligned orthogonally with it. Meanwhile, regarding the SPECT acquisition, the phantom was positioned nearby the centre of the system FOV, aligned with the axial direction orbiting the detectors over 360° from the phantom in 24 steps. The 1 mm glass capillary filled with a ^{99m}TcO₄⁻ solution, positioned along the axis of rotation, was used to assess the SPECT system spatial linearity, the SPECT spatial resolution, and the centering of the rotation axis (Figure 3B). Specifically, the SPECT spatial resolution was measured in terms of full-width-at-half-maximum (FWHM) of the point spread function (PSF) obtained at the corresponding source's positions. All SPECT images were obtained in step-and-shoot mode by collecting 48 projection views in 24 steps over a 360° arc. Generally, each projection view was acquired for a time of 1 min, then the full standard SPECT scan required 24 min, while the 2-step SSR acquisition has needed 48 min. Finally, the tomographic images have been reconstructed using the Software for Tomographic Image Reconstruction (STIR) framework with the order subsets expectation maximization (OSEM) algorithm ¹⁴.

2.3. Animal studies

The HiRIS2 SPECT system was evaluated in a proof-of-principle animal study, in accordance with international and local guidelines. In these studies, different clinical radiopharmaceuticals radiolabelled with ^{99m}Tc and ^{123}I (γ -ray 159 keV) were administered.

2.3.1 $^{99m}\text{TcO}_4^-$

^{99m}Tc -pertechnetate (18.5 MBq in 50 μl), a well-known anion, used for clinical imaging of thyroid function, was injected under isoflurane anesthesia in the tail vein of a female, 6 weeks-old Balb/c mouse. SPECT images without SSR were obtained at 1 h after injection by collecting 48 projections (24 steps) over an arc of 360° focusing on the thyroid. Each acquisition lasted 1 min per step, for a total of 24 minutes. Subsequently, the 2-step trans-axial SSR was carried out, performing 24 steps. The total scan time was 48 minutes.

2.3.2 Technetium-99m-methyl diphosphonate

Technetium-99m-methyl diphosphonate (^{99m}Tc -MPD, 7.4 MBq in 50 μl) for bone imaging was injected under isoflurane anesthesia in the tail vein of a female, 6 weeks-old Balb/c mouse. Planar and 16-step whole body SSR images were acquired at 4 h post-injection according to the kinetics of the tracer to obtain the maximum radiopharmaceutical concentration in the bone.

2.3.3 ^{123}I -ioflupane (DaTSCAN®)

^{123}I -ioflupane (DaTSCAN®, 21.1 MBq in 100 μl) was injected under isoflurane anesthesia in the tail vein of a female, 6 weeks old Balb/c mouse for brain dopamine active transporter (DAT) imaging (Figure 4). The SPECT scan was performed at 1 h after injection and then a 2-step trans-axial SSR SPECT acquisition was carried out for a total acquisition time of 48 min (1 min acquisition time for each step).

3. Results

3.1. Detector performance evaluation

Our group already described an outlook of the HiRIS2 detector in the past, where the performance assessment resulted in a sensitivity of about 76 cps/MBq, an energy resolution of about 18% (FWHM) at 140 keV (^{99m}Tc), and an intrinsic spatial resolution of about 1.6 mm ^{5,6}.

Figure 5A shows four low-resolution images of a Derenzo-like phantom obtained by planar shifts of a half-pixel (0.8 mm). Figure 5B shows the high-resolution reconstructed planar image resulting from the application of the SSR technique on the above images. In the resulting image, the three sectors with respectively 1.6 mm, 1.8 mm and 2.2 mm hole sizes are well defined. Moreover, also the 2-step trans-axial

SSR SPECT reconstruction, depicted in Figure 5C, shows that the holes with 1.6 mm diameter are well resolved. This is clear from the intensity profile, displayed in Figure 5D, where the average peak to valley ratio is about 4:1. Actually, the images exhibit good spatial linearity proved by the regular arrangement of the elements and the proper separation of the holes.

Figure 6 highlights that the SPECT reconstructed capillary is de facto aligned with the effective system mechanical centring. Indeed, the reconstructed 45 mm long and 1 mm inner diameter capillary source, filled with a 3.7 MBq $^{99m}\text{TcO}_4^-$ solution, is perfectly aligned with the axis of rotation. Moreover, the proper geometry reconstruction points out the correct linearity response of the system.

The achieved spatial resolution in SPECT reconstruction was assessed by evaluating the FWHM of the reconstructed capillary profile. In particular, for the standard SPECT, this value was estimated to be 1.6354 ± 0.1488 mm, which reduces to 1.1806 ± 0.1420 mm, for the 2-step trans-axial SSR.

3.2. Small Animal Imaging measurements

Figure 7 shows the SPECT reconstruction of the thyroid gland of a mouse obtained with the administration of ^{99m}Tc -pertechnetate. In the standard SPECT reconstruction (Figure 7A and 7C) the shape of the gland is roughly recognisable. Instead, as displayed in Figure 7B and 7D, the 2-step SSR SPECT images allows discriminating both lobes of the thyroid.

The whole-body images of the ^{99m}Tc -MDP injected mouse are represented in Figure 8, where, in panel A a low-resolution planar image is shown, resulting from the fusion of two images obtained from the animal bed shift of 40 mm of due to the detector FOV size limitation. Panel B represents the high-resolution version of the same image obtained through the application of the 9-step SSR technique. In this case, the spine, the bones, and even the ribs of the mouse are clearly imaged. This is indicative of the achievable SSR SPECT spatial resolution.

^{123}I -ioflupane binds with high affinity to the presynaptic dopamine transporter (DAT) located on the presynaptic nerve endings (axon terminals) in the striatum, a cluster of neurons in the subcortical basal ganglia of the forebrain. This radiopharmaceutical is, thus, indicated for detecting loss of functional dopaminergic neuron terminals. Figure 9 shows the accumulation of ^{123}I -ioflupane due to the dopaminergic activity in the striatum. Since no thyroid blocking agents were administered to the mouse, images also show the ^{123}I thyroid uptake. The 2-step SSR method application provides considerable improvement of image quality in DAT SPECT. The imaging of small cerebral structures such as the corpus striatum provides an excellent map of regional cerebral function.

4. Discussion

The present study reports the development of a preclinical SPECT system based on parallel hole collimation detectors exploiting a super resolution method to enhance the imaging performance. To this

purpose, both planar and SPECT measurements have been performed on both phantoms and small animals, then the acquired images have been reconstructed with OSEM algorithm in 3D to fully characterize the system configuration, and to test the SSR technique. In general, using parallel holes collimators, the overall spatial resolution cannot be better than the intrinsic one. This limitation is overcome by using the imaging magnification provided by the pinhole collimation. However, although pinhole or multi-pinhole collimators provide better spatial resolution and sensitivity, due to their FOV limitation, the inhomogeneity of the spatial resolution, and the axial blurring effect^{15,16}, the parallel hole collimation appears to us to be the most suitable option for whole-body imaging of mice achieving good sensitivity and good spatial resolution in the full FOV.^{17,18}

Preclinical SPECT systems are often technically challenging and expensive machine based on the use of multi-pinhole collimators¹⁹⁻²³. In this paper we reported the development of a system based on parallel hole collimation, which can be built at a reasonable cost, has a homogeneous resolution over the entire area due to the parallel collimator and, last but not least, due to its high sensitivity low doses are required. Besides the application of the SSR method resulted in the improved anatomical delineation of the structures in the reconstructed images and provides higher contrast recovery. Another advantage of our SSR SPECT system over the conventional ones consists of the possibility to apply the SSR method both in planar and in 3D. This makes the system extremely versatile because it is possible to decide the acquisition time in line with the ongoing study and to adjust the resolution by increasing or decreasing it according to the anatomical details to be examined. Through simulations, we have estimated an improvement in terms of spatial resolution of about 30% using the SSR technique⁸. However, beyond a certain number of steps, the cost-benefit becomes marginal because choosing a high number of steps would increase acquisition times, which makes the examination incompatible with anaesthesia duration. For this reason, we suggest the application of SSR in tomography not beyond three steps, compatibly with acceptable doses and acquisition times. Our experimental tests have shown that good results are achieved with small animals even though there are limits on the FOV, which is currently 45 mm square. For this reason, we thought to use the system, specializing it for imaging on small organs such as the brain, having a FOV compatible with the area of investigation. As the visual interpretation of DAT SPECT performed with conventional preclinical systems already provides useful diagnostic information, it is expected that enhanced images with SSR DAT SPECT would be particularly useful in borderline cases and for experienced readers. Additionally, improved image quality allows for a more realistic determination of tracer uptake so as for early detection of underlying psychiatric disorders.

The good experimental results reported in this work, have proved that the anatomical structures are well visible, and with the application of the SSR technique, the images obtained are improved. In particular, in our opinion, the tomograph is indicated to be used for in vivo study of smaller organs, such as the brain or thyroid of a mouse. However, several actions may be undertaken for the future development of a system that overcomes the limitations of the current one. In the current configuration, the tomograph uses two detector heads mounted in opposition: to increase the detection efficiency, in a subsequent configuration, it will be equipped with a third detection module placed at 120° with respect to the others.

In this way, by increasing the sensitivity of the system, it will be possible to reduce the time of acquisition *in vivo*. Another interesting future modification may be to change the detector technology using Silicon Photomultipliers (SiPMs) and increase the FOV (e.g., 10 × 5 cm) so that a whole body of the mouse can be obtained in faster acquisition times. Furthermore, taking into account the simulation results of our previous work, with a better intrinsic resolution (i.e., 1.2 mm) and the application of the 2-step SSR method it can be achieved resolutions below 1 mm⁸. Finally, the integration with CT is also desirable to co-register the images and perform a correction of absorption effects and obtain a prior image in the reconstruction algorithms. In this class of preclinical systems, it is very important to reach a compromise between administered dose, resolution, and measurement time. In particular, the ability to monitor fast changes in DAT expression *in vivo* may be crucial to better understand the behaviour of this transporter. Applying our intended improvements, our SSR SPECT system, due to its ability to enhance the spatial resolution, will offer the opportunity to characterize novel SPECT tracers for potentially all types of receptors. Indeed, the application of the SSR technique could improve our understanding of the pathophysiology of neuropsychiatric diseases, so as may also be of value in evaluating new potential diagnostic tracers and monitoring new treatments, which will be of great value for future neuropsychiatric disease research.

In this work, we presented the HiRIS2 SSR SPECT system for applications where spatial resolution plays an important role such as radiopharmaceutical and neuroimaging *in vivo* studies with small animals. The SSR method is promising to be applied to small animal SPECT, it allows to overcome the trade-off between spatial resolution and count sensitivity in preclinical SPECT of “small” organs. This methodology may raise the opportunities for studies in brain imaging focusing on inflammatory processes underlying psychiatric pathologies.

Declarations

Author contributions

AD: conceptualization, experimentation and writing - original draft preparation. AS experimentation. FG experimentation and data analysis. SS draft preparation. GDS and AB system mechatronic assessment. VT and RM methodology, writing-review, and editing and supervision. All authors contributed to the article and approved the submitted version.

Ethical approval

This study was performed and reported in compliance with the ARRIVE guidelines²⁴. All applicable Institutional and National guidelines for the care and use of animals were followed. This study was carried out in accordance with the recommendations in the guide for the care and use of laboratory animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Ministry of Health of Italy.

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Conflict of interests

The authors declare no conflict of interest associated with this manuscript.

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Figures

Figure 1

Schematic drawing of the detector head assembly.

Figure 2

Overall view of the HiRIS2 SPECT system.

Figure 3

(A) Centering tool with radioactive capillary. (B) Radioactive capillary filled with ^{99m}Tc pertechnetate. (C) One of the acquired images.

Figure 4

An acquisition step during the imaging of the mouse brain functions.

Figure 5

Derenzo 2-step planar SSR reconstruction. (A) Low resolution images obtained by the SSR planar shifts of a half pixel. (B) Final high resolution reconstructed image. (C) 2-step trans-axial SSR SPECT Derenzo reconstruction.

Figure 6

System evaluation. SPECT reconstruction of a capillary placed along the HiRIS2 system axis of rotation.

Figure 7

SPECT OSEM reconstruction of the distribution of ^{99m}Tc -pertechnetate uptake in a mouse thyroid. (A) Coronal view without SSR. (B) Coronal view with SSR2. (C) Volumetric rendering without SSR. (D) Volumetric rendering applying the 2-step trans-axial SSR technique.

Figure 8

Results of the small animal bone imaging measurements in a mouse injected with ^{99m}Tc MDP at 4 h post injection (36 min acquisition time). (A) Low resolution measured planar image without SSR. (B) 9-step SSR image.

Figure 9

High resolution in vivo imaging of both striata of a mouse brain with the SSR SPECT system using ^{123}I -DaTSCAN. Axial, coronal and sagittal views, and SPECT slice co-registered with an anatomic brain atlas of the mouse 25,26.