# Awake Animal SPECT: Overview and Initial Results

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***Abstract*— A SPECT / X-ray CT system configured at Johns Hopkins University to image the biodistribution of radiopharmaceuticals in unrestrained, un-anesthetized mice has been constructed and tested on awake mice. The system was built by Thomas Jefferson National Accelerator Facility and Oak Ridge National Laboratory. SPECT imaging is accomplished using two gamma cameras, 10 cm x 20 cm in size based on a 2 x 4 array of Hamamatsu H8500 flat panel position sensitive photomultiplier tubes. A real-time optical tracking system utilizing three infrared cameras provides time stamped pose data of an awake mouse head during a SPECT scan. The six degrees of freedom (three translational and three rotational) pose data are used for motion correction during 3-D tomographic list-mode iterative image reconstruction. SPECT reconstruction of awake, unrestrained mice with motion compensation for head movement has been accomplished.**

## I. Introduction

THOMAS Jefferson National Accelerator Facility (JLab), Oak Ridge National Laboratory (ORNL), Johns Hopkins University (JHU) and the University of Maryland have been collaborating on the development of a SPECT / X-ray CT system for imaging un-anesthetized, unrestrained mice. Often small animal brain based studies are complicated when anesthesia is involved. The use of anesthesia has the potential to alter the neurological and physiological processes being studied [1]. The researcher has several anesthetics to choose from to permit SPECT imaging of mice.

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Cerebral blood flow is one example of a physiological function affected by anesthesia and is modified dependent on the type of anesthesia used. For instance, isoflurane greatly increases cerebral blood flow while propofol moderately reduces it and chloral hydrate has little effect. This variation can potentially skew brain pharmocokinetic studies involving brain specific ligands. There are obvious benefits to studying brain physiology in experimental animals while they are awake, i.e., to obviate the unpredictable and inconsistent effects of anesthesia on brain function. Similar benefits are applicable to research in oncology and is particularly true in studying angiogenesis, one of the most important targets of oncologic treatment. Imaging is a powerful way to study changes in blood flow in intact animals, it is non-disruptive and animals may serve as their own controls in longitudinal studies. As is the case with brain blood flow, flow to tumors undergoing anti-angiogenesis therapy will also likely be confounded by various anesthesia regimens.

Researchers have developed surgically implanted scintillator based probes to measure the uptake of radio-labeled tracers in the vicinity of the implanted intracerebral microprobes for anesthetized and un-anesthetized rodent studies [2, 3]. Others are pursuing a PET based system to image the brain of an un-anesthetized rat by use of a miniaturized PET detector intended for attachment to a rat's head but requiring the rat be attached to a counter balance to offset the weight of the detector and also requires minor surgery [4, 5, 6, 7, 8]. Reported applications of this method have been with rats that have been trained to stay confined. In addition, a group in Australia is also attempting to do head tracking on a mouse in a microPET system [9]. There are also commercially available fixtures for physically restraining rats and mice during studies but these can results in stress to untrained animals and this could affect experimental outcomes. Our project is the only effort involving SPECT imaging. We have reported earlier on the design and development of prototypes and sub-systems of our scanner [10, 11].

The fully operational system at Johns Hopkins University is composed of a precision SPECT / X-ray CT gantry, two high-resolution 10 cm x 20 cm gamma cameras and an infrared (IR)-based tracking system. We report here on the completion



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of the fully functional system including motion corrected SPECT reconstruction. The completed system is installed and being tested on awake mice at the Broadway Research Building at Johns Hopkins University (Fig. 1).

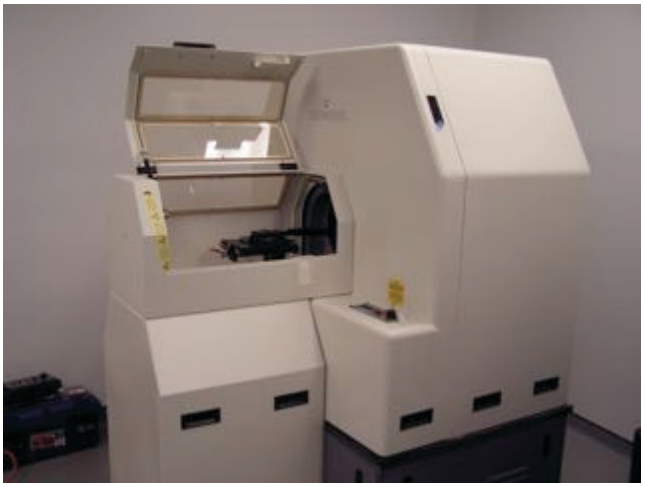

Fig. 1. Photograph of the system installed at Johns Hopkins University. Shown is the SPECT-CT with the x-ray shield in place.

The awake mouse is confined within a transparent burrow at the center of rotation of the gantry. The mouse head position is tracked by three infrared reflecting spheres attached to the front of the head; the spheres are imaged by three CMOS based infrared cameras.

## II. Methods

The SPECT-CT gantry is a custom modified Siemens MicroCAT II Small Animal Imaging System. The MicroCAT II is equipped with an 80 kVp (max), 40W x-ray source and a 2048 x 3096-pixel detector with up to 5-frame per second readout. The gantry includes a three-axis precision motion control system, vibration isolation, a fully interlocked FDA compliant x-ray cabinet with a removable mouse bed.

The SPECT 10 cm x 20 cm gamma cameras are built around a 2 x 4 array of the Hamamatsu H8500 flat panel position sensitive photomultiplier tubes (PSPMT) which are 5 cm x 5 cm in size and having 64 anode pads to be read out. For one of the gamma cameras the anode pads of a module made up of a 2 x 2 array of the H8500's are connected through resistors in rows and columns resulting in a 16 x 16 readout for each 2 x 2 module. Two modules are stacked together, resulting in a 20 cm x 10 cm detector and read out separately with a total of 64 channels required for readout. The signals from the modules are then combined in software to generate an image.

With the second gamma camera we were able to reduce the number of readout channels by connecting all PSPMTs through resistors resulting in 16 channels in the short dimension and 32 in the long dimension to be read out. Each gamma camera is read out by a separate JLab built FPGA based ADC module that is mounted on the gantry near each camera [12]. The JLab developed FPGA 12-bit ADC (2.5MHz triggerable). The ADCs were developed in three versions to provide 16, 32 or 64 channels with high-resolution time stamp capability and the ability to synchronize several units using timing distribution. The USB2 transfer rates are 30 MB/s to disk or 35 MB/s to memory.

In addition, we developed Java based control and gamma camera image formation software [13]. The control of the data acquisition is managed by a Java based data acquisition package running on the PC and communicating to the gantry mounted FPGA ADCs through USB2. Each FPGA USB2 module is first routed to a USB2–fiber optic repeater also mounted on the gantry. In this way a single fiber optic cable carries the USB2 signal to the PC dedicated to the gamma cameras.

One gamma camera has a 10 cm x 20 cm NaI(Tl) scintillator crystal array with individual crystal elements 1 mm x 1 mm x 6 mm in size and 0.2 mm septa between each element. The other is equipped also with a 10 cm x 20 cm NaI(Tl) scintillator crystal array but with individual crystal elements 0.8 mm x 0.8 mm x 6 mm in size and a 0.2 mm septum. The arrays were obtained from Saint Gobain in Lancaster, PA. One detector head can be equipped with various pinhole inserts while the other can accommodate various parallel hole collimators. The system is capable of imaging I-125, I-123 and Tc-99m.

Tracking of the mouse's head is achieved by imaging three spherical retro-reflectors glued in a triangular arrangement on the head of the mouse. The tracking apparatus measures the pose of the mouse's head with 6 degrees of freedom at a rate of 10-15 frames per second with sub-millimeter accuracy. The optical tracking system is composed of three CMOS optical cameras and each camera has a concentric mounted circular array of infrared emitting LEDs such that the light output is coaxial to the detected reflections off of the spherical retro-reflective markers [14]. Please see Fig. 2.

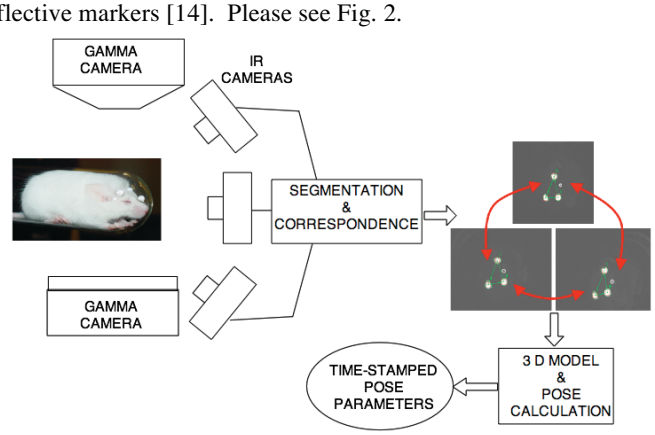


Fig 2. Schematic diagram for motion tracking and SPECT data acquisition.



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The setup provides simultaneous (stereo) blur-free images of the markers. Our initial system made use of only two IR cameras but the three camera system provides improved accuracy, range of motion and robustness. The CMOS camera image acquisition, processing and algorithm development is handled by a Dell workstation.

Three Dell workstations are used to control the three sub-systems (gantry, IR tracking and gamma cameras) and record three separate data sets that are later used to correct for motion in image reconstruction. The three data sets that are time tagged via a common clock are: gantry location, mouse pose and gamma camera list mode data. The system architecture is illustrated in Fig. 3 and a photograph of the completed system with x-ray shield removed is shown in Fig. 4.

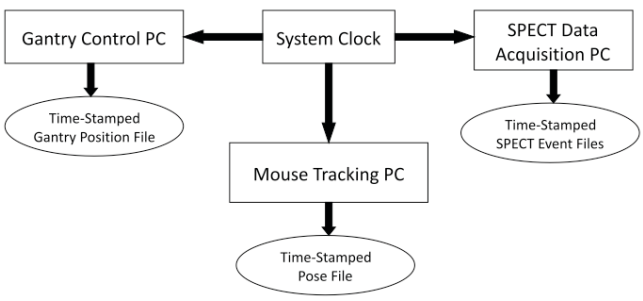


Fig. 3. System architecture of SPECT scanner showing the PC's associated with each sub-system and time synchronization via a common system clock.

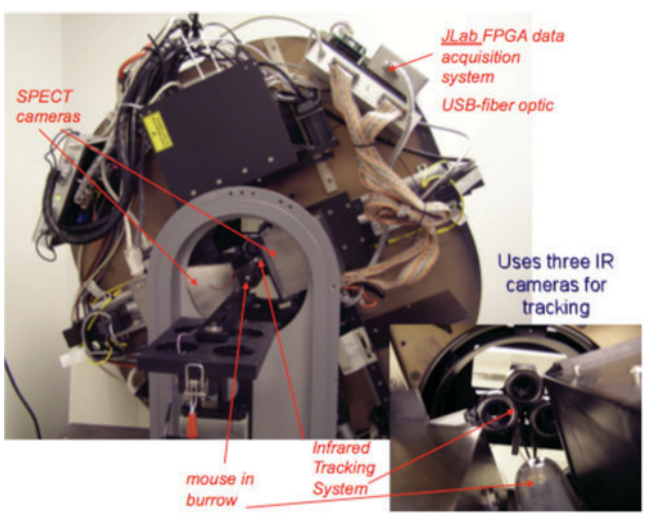


Fig. 4. Siemens MicroCAT II based gantry with the x-ray source and detector and with mounted optical tracking system at JHU. Three CMOS IR cameras are seen in the enlargement.

Image reconstruction is accomplished using an iterative list-mode maximum likelihood expectation maximization algorithm that incorporates time-stamped event data, pose information, and gantry rotation position. Further details and examples are provided in a separate submission to this conference [15].

## III. Results

We have tested the system at Johns Hopkins University, first with moving phantoms and then with several awake mice. Please see Fig. 5 for an example result of the tracking of an awake mouse injected with Tc99m-MDP. In this example a white female BALB-C 32.1g mouse was injected through a tail vain with 3.3 mC of Tc99m- methylene diphosphonate (MDP). Tc99m-MDP is used for bone scintigraphy where it is typically taken up by healthy bone. The resulting SPECT images are, therefore, similar to a low resolution x-ray CT of the skeleton. About three hours after injection, the mouse was anesthetized for ~10 minutes with the inhalant isoflurane. This allowed placement on the mouse's head using instant adhesive the IR reflectors used for tracking. The mouse was imaged ~3hr post injection using a 1 mm diameter pinhole collimator with 1.5x magnification. List-mode data were acquired at 120 gamma stops over 360 degrees, at 20 sec /position. The list mode projection data with mouse head tracking was reconstructed using OSEM with tracking data incorporated. Our first results are shown in Fig. 5. Additional mouse studies are planned.

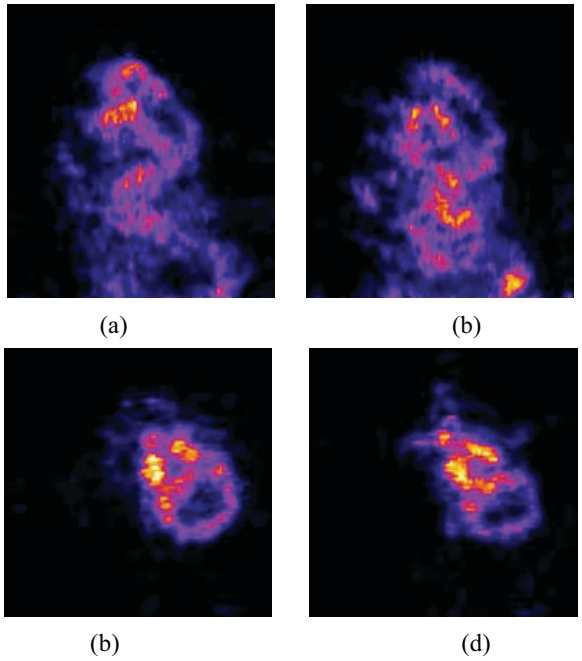


Fig. 5. Slices through the mouse volume: sagittal with corrections (a) and without corrections (b), axial with (c) and without corrections (d).

## IV. Discussion

We have developed and implemented a SPECT / X-ray CT based imaging methodology that uses IR tracking with motion



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compensation reconstruction code that allows for imaging awake unrestrained mice. This system provides researchers the ability to study complicated brain chemistry without the obscuring influence of anesthesia. Anesthesia can affect blood flow in tumor anti-angiogenesis therapy, and other aspects of tumor biology, including drug delivery can also be studied in the context of awake animals. This provides a unique tool for research using rodent brain models in addiction research, neuro-degeneration (i.e. Alzheimer's disease, Parkinson's disease), brain inflammation (i.e. HIV, MS) and stem cell trafficking. The system is installed at Johns Hopkins University where it is being used for animal studies.

It is clear that the system could be improved for researchers to use particularly since they are dealing with awake radioactive mice. In its present configuration three separate computers are needed to control the three sub-systems: gantry control, IR tracking and SPECT data acquisition. This makes for less than an ideal arrangement as each control program on each computer system needs to be dealt with manually during a scan. We are investigating ways to consolidate the three separate tasks to a single computer.

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