DEVELOPMENT OF A GAMMA CAMERA ARRAY FOR BIOLOGICAL IMAGING IN SMALL ANIMAL RESEARCH

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Development of a Gamma Camera Array for Biological Imaging in Small Animal Research

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One of the current problems facing biology is the need for real time imaging of biological processes in animals. William and Mary, in conjunction with Jefferson Lab, has developed a novel detector scheme to image a small animal *in vivo*. This paper describes the current imaging modalities for biological imaging, such as autoradiography and PET, and their advantages and disadvantages relative to the William and Mary system. The primary goal behind the William and Mary study is to develop a system that can reasonably be afforded by a small biology lab. There are numerous studies which can be performed by such a system. Current studies with this detector system have focused on the metabolism of the cocaine analog RTI-55 in a mouse. Some preliminary results are given below. This paper will also discuss future developments planned for this detector system.

Acknowledgements

There are a number of people whose help with this project has been invaluable. The first is my advisor, Dr. Bob Welsh, who has been there through every crisis I have had with this research. I also want to thank Dr. Drew Weisenberger, upon whose doctoral work this thesis is based. The entire detector group at Jefferson Lab, headed by Dr. Stan Majewski, contributed immense technical expertise to this project. The Biology professors, Dr. Bradley and Dr. Saha, and their undergraduate researcher, Brett Stetka, whose ability to secure grants to continue this research amazes me. I also have to thank them for their exhaustive patience with me when the detector system decided not to work. I must thank the Howard Hughes Medical Institute Small Research Grant and the HHMI Conference Travel Grant. I was able to use that money for machine work to the system and to present a paper on this work to a meeting of the Southeastern Section of the American Physical Society in November of 1999. I also must acknowledge the National Science Foundation Research for Undergraduates program for supporting me in the summer of 1999 to understand this research before I had to prepare for this thesis. Dave Leichtman, Chad Weiler, Theo Vecchione, Lisa Kaufman, Jen Wilkes, Ed Cox, Heather Faltin, and Brian Tighe are all physics people that have helped me; some during the summer with REU research and others that have helped throughout the whole year. Finally, I want to thank my brothers in Delta Phi for understanding my stress and hours locked in the lab.

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I. Explanation of Competing Imaging Methods

Any explanation of a new method requires one to explain the older methods and why one seeks to improve upon them. Therefore, we begin with a discussion of the traditional methods used to track the uptake of a variety of substances. This shall not be an extensive discussion; however the highlights of imaging shall be explained.

Since the discovery of radioactivity and of x-rays, the use of radiation in biological studies has grown rapidly. The invention of the gamma camera by H. Anger more than 35 years ago was perhaps the first application of full-scale *in vivo* biological imagery. (Anger 1964) Since then, the field has expanded rapidly. We describe here the status of several techniques used to image biological systems with radiation and the potential for future development in this field.

A very early method, by far the most common, is autoradiography. This procedure requires one to inject the animal with the radioligand of choice, wait an appropriate length of time, sacrifice the animal, and then slice the tissue into extremely small sections. One places these slices near a film containing silver bromide. The photon from the decay strikes the film and produces electrons that are trapped by specks of silver bromide crystals in the film. These negatively charged specks pair up with silver ions which form metallic silver. One then develops the photographic film; dark regions in the resulting picture indicate an area of radioactivity. (Glick 1998) This method boasts good resolution, as the photographic film can show fine detail. Some of the difficulties are rather obvious: One is required to sacrifice the animal before using this method so one can only look at the tissue at one point in time after the tissue is

dead. In order to do an accurate study of the uptake of a substance, one must use many animals to form a complete picture. The second detail is more subtle: there is a fixed amount of silver bromide per area of film. Once these grains are exposed by radioactivity, all other photons through that area are basically ignored. This leads to a limit on the range of radioactivity that can be discerned.

A second method with considerable potential is Positron Emission Tomography (PET). Positron Emission Tomography is a very powerful method used to track the location of a particular ligand. This method employs a ligand labeled with a positron emitting isotope. When that isotope decays, it emits a positron. This positron will annihilate at rest with an electron and usually produce an emission of two identical photons separated by 180 degrees. These photons are fairly energetic, 511 keV each, and can penetrate tissue easily. This high energy precludes traditional collimation; the photons can penetrate most collimators. However one can ring the animal with scintillators and the geometrical nature of the photon production allows for electronic collimation; one simply accepts only photon events that are detected simultaneously with 180 degrees separation.

There are limitations to this method; most due to the nature of positron-emitting isotopes. The commonly used isotopes for biological application (^{11}C, ^{13}N, ^{15}O and ^{18}F) must be produced with a particle accelerator, likely a cyclotron, and then used very rapidly because of their short half-lives. This requires a nearby cyclotron for isotope production and complex radiochemistry, something which most laboratories do not have.

Another method of considerable merit is Magnetic Resonance Imaging. One

places a specimen in a large magnetic field. The magnetic moments of the nuclei in the system will align with this field (ignoring thermal effects). Radiofrequency pulses are applied which 'tip' the spins; one can then measure the relaxation times until the magnetic moments realign with the magnetic field. This type of imaging can easily spot protons (Hydrogen) in a system. This is especially useful for biological systems where a large portion of the body consists of water. It is difficult, however, for this method to track a particular substance in a biological system. One would have to be able to know exactly the magnetic characteristics of the substance and be able to detect that through the surrounding tissue. It is this difficulty that makes uptake work difficult. (Wolbarst 1993) Some labs have produced exciting functional MRI work, however one is unable to study any arbitrary ligand of biological interest.

One thus turns to consideration of the gamma camera and a comparison to other methods available for research. A current trend in animal research is to design more accurate experimental techniques which minimize the use of animals. (Stokstad 1999) In order to follow the uptake of a substance using the autoradiography method, one must inject the animals, wait an arbitrary length of time and then sacrifice them. This must be done for every point on the curve; several animals are needed for each point to rule out individual variations and measurement error. The detector scheme discussed here can reduce the number of animals necessary for an experiment from approximately hundreds to a few dozen. (Mochizuki 1997) The gamma camera approach allows one to follow the same animal over the entire uptake cycle; the animals can easily be used in one set of experiments on one day and brought back later for another set. In addition, because one is able to follow the same animal over time, one does not worry about individual genetic

variations or other artifacts which can arise from using many animals. The method of Positron Emission Tomography also offers these benefits.

One of the biggest advantages of this technique is its dynamic nature. For autoradiography, one only views the sections of the body that one prepares. One has killed the animal for this technique; any tissue not viewed initially will decay and the information will be lost. In the method to be discussed here, one can image the animal and come back at any arbitrary future point to look at the entire animal. The data are therefore waiting on the computer for subsequent reanalysis over a different part of the specimen.

The gamma camera method requires equipment that can be affordably obtained by most laboratories. As opposed to PET, which requires a nearby cyclotron, this imaging method can be assembled for approximately 20,000 to 40,000 dollars. Photomultiplier tubes, assorted electronics, and computers (The primary tools of this method) are well understood and readily available to a scientific lab.

II. Basic Physics of System

The central physical principle involved is the decay of the radiotracer, Iodine-125 (^{125}I). ^{125}I has a characteristic decay pattern. It undergoes electron capture to ^{125}Te. From there, it often decays with the simultaneous emission of a 35 keV gamma ray and a 27-32 keV Kα or Kβ x-ray. (Lederer 1978) ^{125}I has a fairly long half-life, approximately 2 months, which allows for production off-site and easy delivery. The long half-life also allows for a level of ease with experiments; one can obtain the

radioligands, run several experiments and store the unused portion for future use. The decay also is of a fairly low energy so that the material can be easily stored and does not require a large detector. Commercial firms produce more than 100 ligands tagged with ^{125}I and can supply them to the biological researchers at modest cost.

The main detection apparatus in the experimental set-up is a photomultiplier tube (PMT). At the 'top' of the photomultiplier tube is a photocathode. When light strikes the photocathode, an electron is released via the photoelectric effect. The body of the PMT is a string of dynodes, which can be thought of as a series of steps in the electric potential. The photoelectron will be drawn to the first dynode because it is held at a positive potential. When the electron hits the dynode, it will give off a number of electrons via secondary emission. These electrons will then be drawn to the next dynode which is held at a higher potential than the first. This will continue until the electrons reach the anode, which collects all of the electrons. (Leo 1987) Photomultiplier tubes are both sensitive detectors, capable of detecting single photons, and efficient low noise amplifiers capable of providing a gain of 10^6 or more.

The photomultiplier used in most of the experiments described here is a Hamamatsu R3292 Position Sensitive Photomultiplier Tubes (PSPMT). These tubes are about 125 mm in diameter, which allows for a large viewing area. Traditionally, the anode of a PMT is similar to a cup; it simply collects the electrons. This is different for the position sensitive model; the anode of the R3292 photomultiplier tube is a 28 by 28 crossed grid of wires. It is this grid of wires that allows the experimenter to determine where the initial photon hit. However, the anode is not simply hit by one electron. As is evident from the previous discussion, a cloud of electrons is collected on the anode array

due to a single initial photon. Unfolding the information from the collected charge will be discussed below. Typically, this PMT is maintained at approximately 1 kV with a High Voltage Power Supply (Ortec Inc). This voltage is lower than the maximum allowed by the tube but gives lower electronic noise. The gain was increased by a low noise preamplifier.

Simultaneous use of two or more PSPMT detectors can allow accumulation of more data from a specimen. On the other hand, the larger photomultiplier tubes tend to interfere geometrically when positioned near a small animal. Thus, there is a limit to the number of detectors that can be placed near the animal. One solution we have begun to study is the use of a small one-inch PSPMT. This will allow closer examination of some areas, such as the brain of a mouse. There are some differences between the larger PSPMT tubes and the smaller ones. The Hamamatsu R5900-00-M64 with only 18 x 18 mm active area does not use wire anodes but instead uses small pads. This leads to a large number of output signals as each pad has an one output.

In order to convert the gamma ray photon into visible light that can be detected by the photomultiplier tube, a scintillator is required. A scintillation occurs when a burst of light is emitted as certain materials are hit by radiation. (Leo 1987) The ideal scintillator for such work will have very fast bright light pulses. The scintillator employed in this detector is a CsI(Na) crystal array segmented into 1 mm square by 3 mm thick. This implies a minimum pixel size to the spatial resolution of the gamma camera. This ideal 1 mm hardware resolution has not yet been achieved largely due to statistical variations in the Center of Gravity definition of the electron cloud in the data acquisition software.

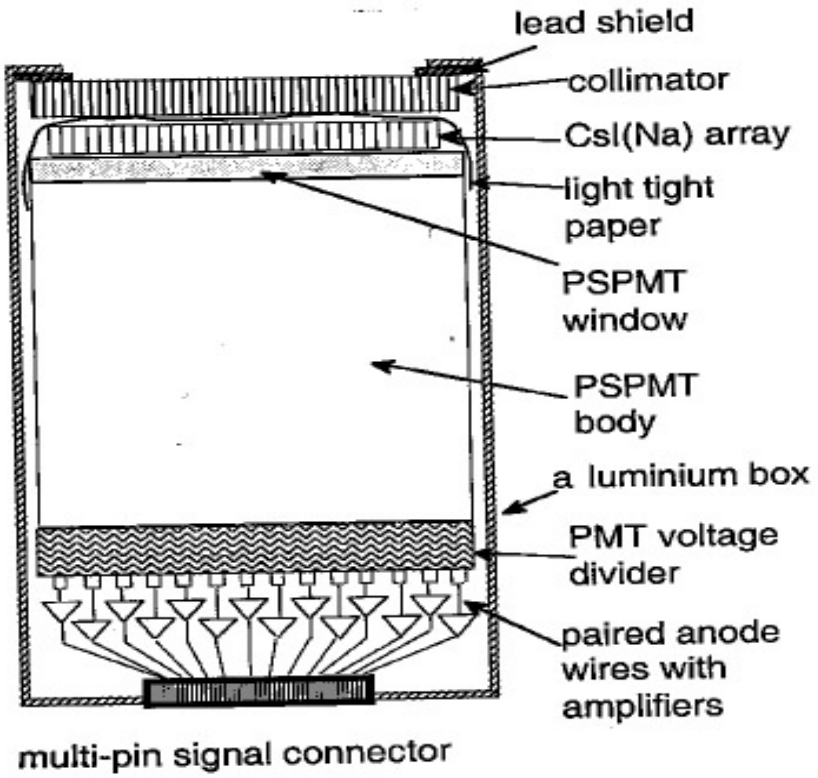


Figure 3.17: Cross sectional view of the 125 mm diameter R3292 position sensitive photomultiplier tube coupled to the CsI(Na) crystal array.

Figure 1: Picture courtesy of Weisenberger. This picture is a side view of the 125 mm R3292 position sensitive photomultiplier tube with collimator and scintillator.

One advantage to the scintillator-PMT combination is its energy resolution. The response of both the PMT and scintillator are linear to energy above a certain threshold. This allows the gamma camera to measure the energy of incoming events. This information allows one to discriminate against photons that are not in the energy range of interest.

In order to achieve good imaging, a collimator must be employed. In the basic sense, a collimator is a set of holes drilled into an absorbing material such as lead or copper. In our case, the holes are very small and they have been chemically etched into thin sheets which are then stacked and cemented together. The holes in the collimator limit the directional acceptance of the photons, as a photon that is not in the correct direction will be absorbed by the walls, or septa, of the collimator. This obviously reduces sensitivity (count rate). Without it, however, it would be impossible to perform imaging. There are several different types of collimators. The ones used in our experiments are parallel-hole collimators, which have evenly spaced parallel holes. The choice of collimator is not simple; there are several trade-offs. The material used for the collimator has to be chosen with regard to the energy of the photons; some materials can absorb higher energy photons but are not able to be formed with very small holes. As the area of the apertures decreases, the position resolution improves but there is a corresponding decrease in sensitivity. The walls between the holes of the collimator must be thick enough to absorb stray photons; but thinner walls increase sensitivity. (Sprawls 1995) Using a lead collimator, our system reaches a spatial resolution of approximately 3.5 mm FWHM. The system allows one to change the collimator to optimize the system.

III. Detector Set-up

The original set-up for the detector used one 125 mm PSPMT tube in the vertical position. This arrangement was used for the earlier RTI-55 studies. This allowed planar imaging of the animal while allowing the investigators to optimize the detector and data acquisition system. Once the software and hardware were well understood, we added a second detector. As mentioned earlier, the 125 mm tubes interfere geometrically. Therefore, different arrangements have been tested to overcome this. One set-up was to put one detector at the vertical with another at 135 degrees to the first. This allowed both detectors to be very close to the animal. The disadvantage to this is that the views produced are not standard; they are not easily recognizable compared to other work. The current set-up is a rotatable gantry with two 125 mm PSPMTs at 90 degrees to one another, one at the vertical and one at the horizontal. However, this means that only one detector is able to be flush to the animal.

As a very large number of data lines proved unwieldy, we made tests to determine if signal wires from the 125 mm PSPMTs could be summed in groups of two. This reduction in the number of channels was found to have a minimal effect on the resolution of the device.

As mentioned earlier, I^{125} often has a two photon decay. This suggests the possibility of coincidence detection; one would only accept position events when one detected a corresponding second photon event on a coincidence detector. The equipment set-up for such a configuration is displayed in Figure 2. The same main detector would be used, a 125 mm position sensitive tube, and the coincidence detector

would use a simple PMT, one that is not position sensitive. The problem with this arrangement is the coincidence detector; it subtends such a small solid angle that in preliminary trials it reduced the count rate by a factor of 80. (Weisenberger 1998) For the present system, this loss of statistics has proved unacceptable. The noise reduction was encouraging but the loss of statistics is still not tolerable. We will continue these studies in an attempt to further exploit this technique.

To overcome this simple geometric limitation, we are planning to use a 18 mm x 18 mm phototube. This small detector can be placed flush to the brain of a mouse, for example, allowing the investigator to get a close image of that area. The 18 mm square tubes have never been incorporated into a small animal biological imaging system; this is a novel use of these tubes.

The main difficulty with modifying such a system is the complexity involved in making the system recognize and interpret data from new hardware. In order to reduce such problems with the interchange of a 18 mm square PSPMT (with pad anodes) for a 125 mm PSPMT (with crossed wire anodes), an adapter circuit has been designed at Jefferson Lab for insertion into a NIM card. It accepts the outputs from the small PSPMT, converts them to a resistive read-out, and then splits the channels into X and Y, so that they can be sent to the two ADCs normally used for the larger PSPMT tube.

The NIM system performs some simple electronic discrimination. To decrease noise, the anode signal is required to coincide with the dynode signal. With the addition of a second tube, the NIM electronics are used to form an OR gate for the dynode signals so that it can be fed to the CAMAC crate.

The CAMAC electronic standard was originally created to deal with digital data.

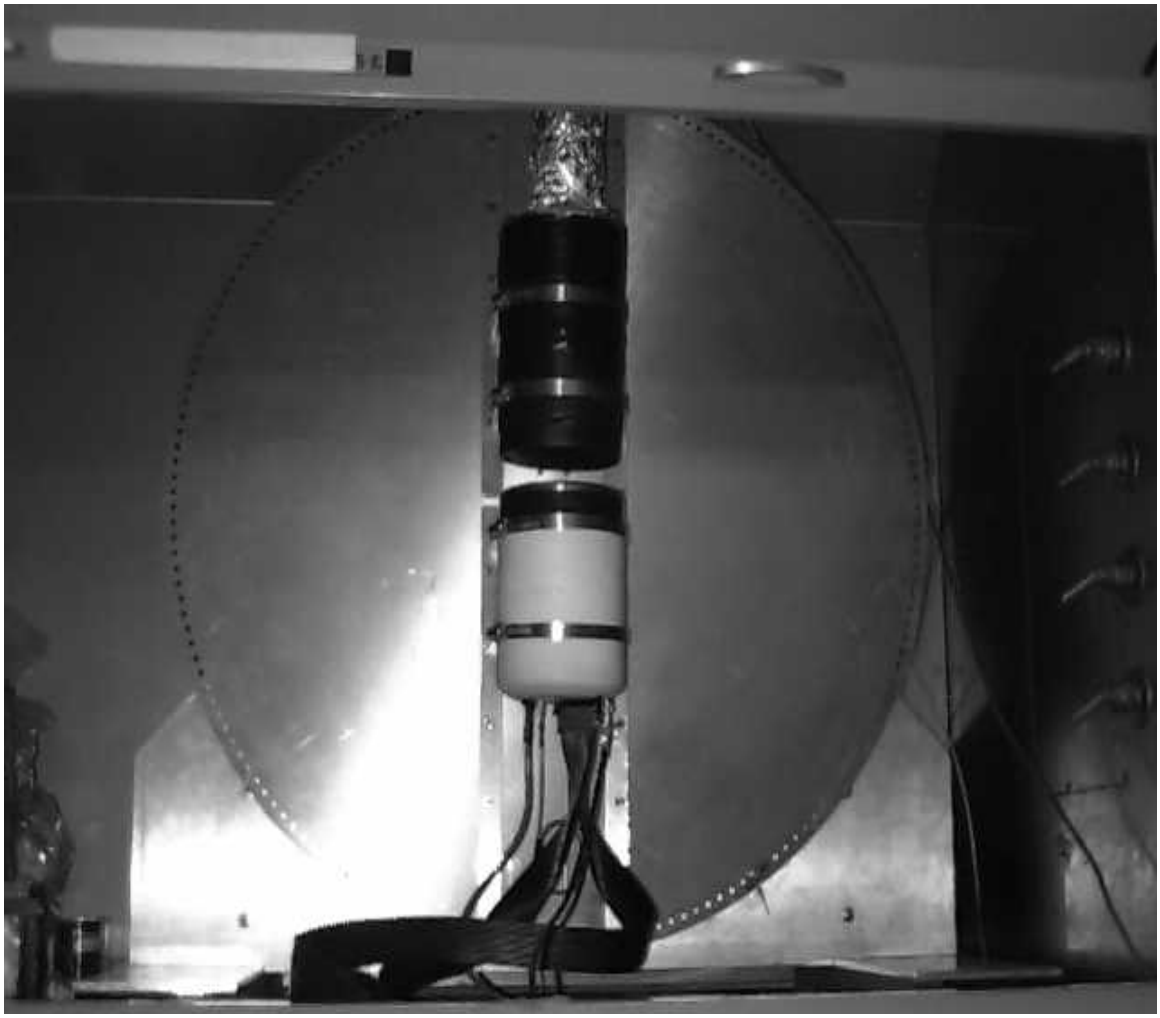
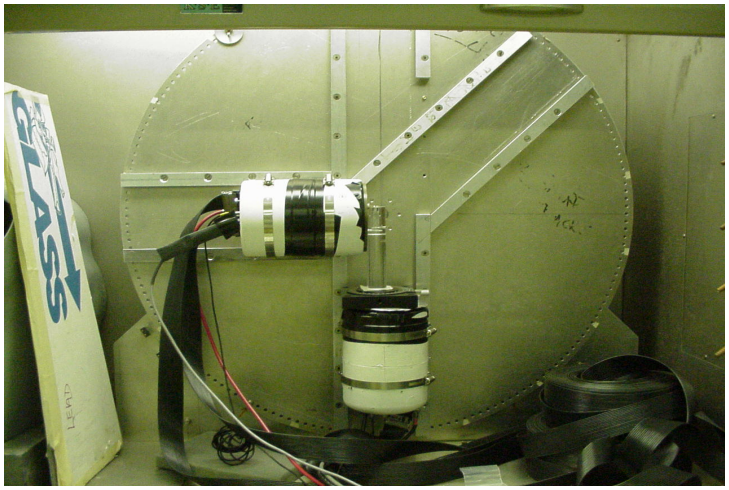


Figure 2: Equipment set-up for the coincidence detection system. The black tube is the five inch regular PMT and the lower tube is the main five inch PSPMT.



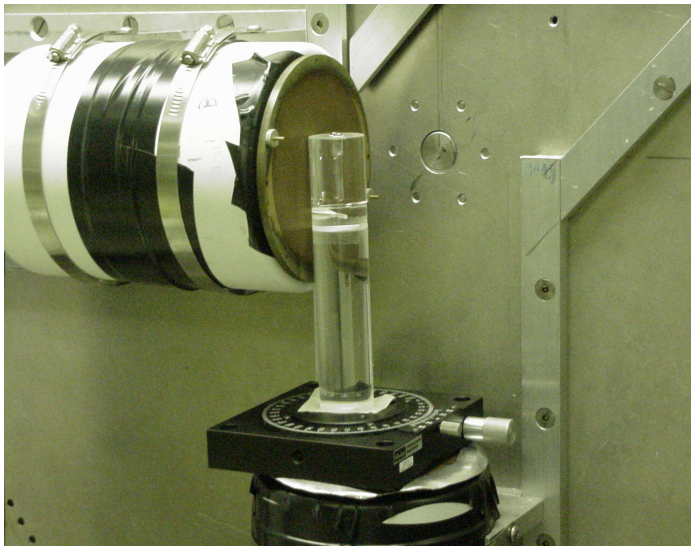
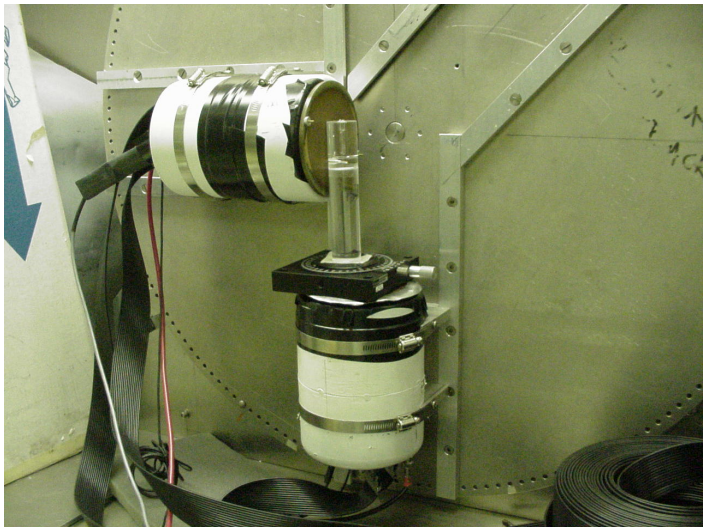


Figure 3: These three pictures represent the current detector arrangement. Both detectors are Hamamatsu 125mm PSPMTs, positioned at 90° to one another. In the center is a phantom currently being used for tomography studies. The black plate underneath allows one to turn the phantom by a set number of degrees. The side detector, using a highly position sensitive collimator, performed the imaging.



A CAMAC system is easily compatible with computers so that detector data can readily be transferred to the computer. (Leo 1987) Outputs from the PMT are fed into analog to digital converter (ADC) cards. As is evident in the name, the ADC cards convert the analog pulse height from the PMT into digital data which can then be stored in the computer. In our configuration, the x outputs and the y outputs each are fed into a separate ADC card. The information received in that card is saved to a memory module which stores events temporarily. The CAMAC crate may contain a number of different circuit cards and is controlled by a crate controller which oversees all card operations. The CAMAC crate is connected to the computer via a Small Computer Systems Interface (SCSI) cable. The computer thus controls the operations of the crate.

The digital data are brought to the SCSI port of a Blue and White G3 Macintosh computer. The data software used during acquisition has been written in KMAX 6.5 (Sparrow, Inc.). The bulk of the data processing occurs in a C program linked to the KMAX software. This C program performs a centroid calculation on each event to determine where the initial photon struck the scintillator and photomultiplier tube and to exclude events that do not occur in a specified energy acceptance window. Events are then scaled by the energy look-up table in which are stored variations of the phototube-scintillator combination. The computer then saves all events to a hard disk drive in sequential form. From there, a DVD writer has been used for permanent data storage.

IV. Sources of Error

When the radioisotope decays, it normally does not affect the remaining

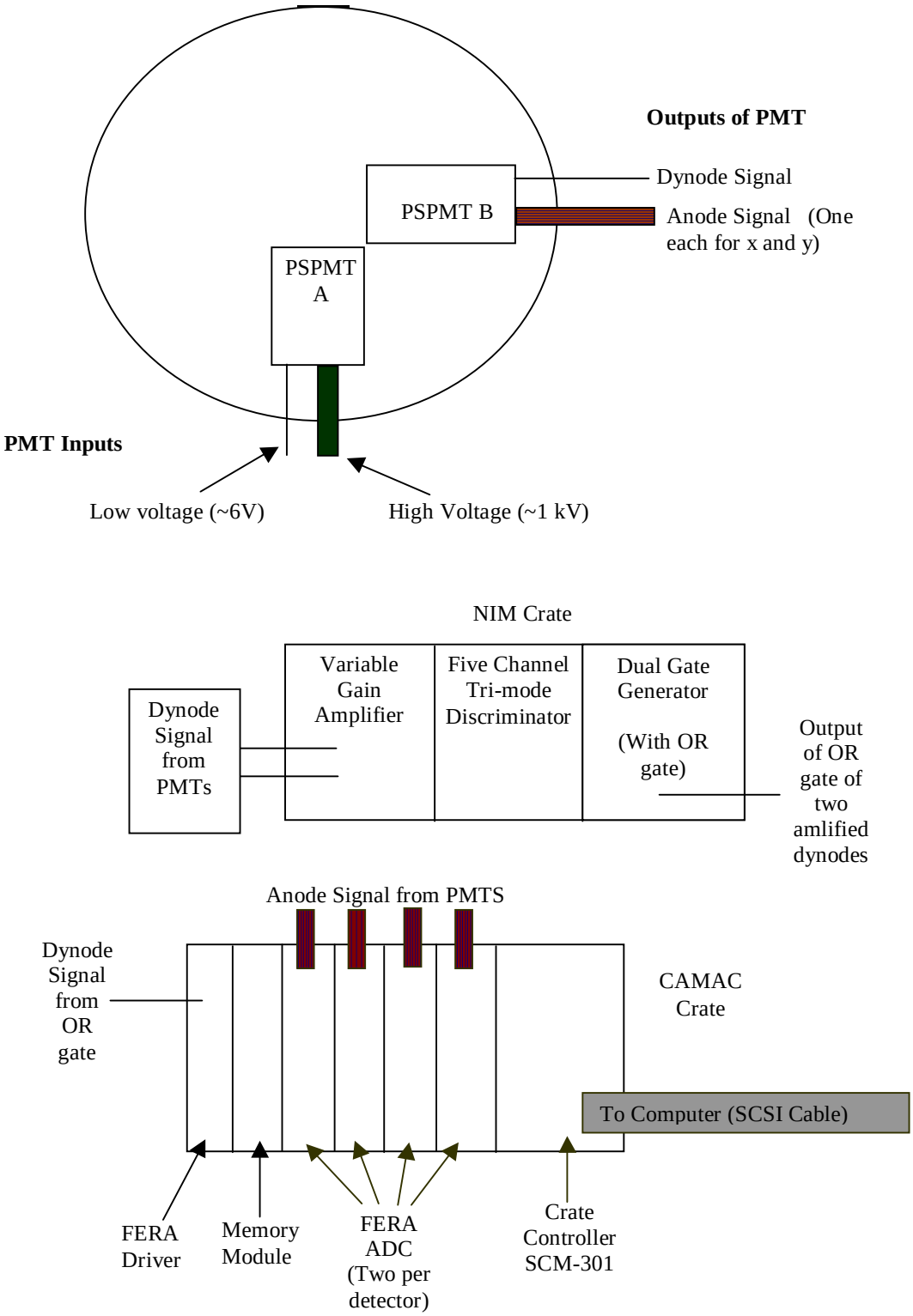


Figure 4: This diagram represents the current electronic configuration of the detector system. The upper portion of this schematic shows the gantry with the two detectors. The detector outputs are fed into a NIM electronic module and a CAMAC crate before finally fed into the computer.

chemical structure of the ligand. Therefore, as the study progress, one has increasing amounts of cold ligand which can block the uptake of the tagged ligand. The amounts detected, therefore, can be less than the actual amounts present in the animal as time progresses.

A second chemical problem is that the radioisotope may detach from the ligand. This means that one will detect radioactivity where the ligand is not. In the case of iodine, the thyroid will be an obvious repository for the free iodine.

Another problem is the fraction of solid angle that the detector subtends. Iodine-125 does not decay in a correlated geometric fashion. The ^{125}I photons are emitted in all directions isotropically. Therefore, the efficiency of the system is strongly linked to how many detectors surround the animal or, more specifically, to the total solid angle subtended by detectors about the animal being studied.

Another problem is simply the blur that occurs in the image; the blur becomes considerably worse as the separation between the animal and the detector increases. One knows the flux is proportional to the inverse square of the distance ($\Phi \propto r^{-2}$) simply from the geometry of a radiation decay. It would seem that this would account for this blurring, However, a careful study of the collimator reveals that the parallel hole type only accepts photons over a very specific range of angles, those perpendicular to the detector face. The number of photons within this range of angles does not change with separation between detector and animal. (Sprawls 1995) The cause for this blurring is in fact due to a number of problems. The first is simply that the scintillator pixels are of a finite size; a point on the animal will not correspond to a point on the scintillator. Also, while the gamma-ray photons from the animal are limited in angular acceptance; the

same is not true of the visible photons emitted from the scintillator. These are allowed to move in any direction before they reach the phototube. A second difficulty is the finite nature of the collimator holes. These also will prevent one point on the animal from corresponding to a single point in the final image. (Sprawls 1995)

Additional problems may arise within the measuring equipment. Photomultiplier tubes are sensitive to variations in temperature. As time progresses, temperature changes may cause drift effects in the PMT. As the PMTs in this configuration are stored under a draft hood owing to the radioactive substances involved, this problem can become significant.

V. Data Analysis

Data Analysis for the system has been written in IDL, an image analysis software package. (RSI, Inc) Data acquisition records each event to an event log. After an experimental run is completed, the first analysis is carried out by a simple sorting program which sorts through the event log and arranges events into arrays. The sorting can be determined by the detectors of interest and the time window desired. The user can set the time windows to any arbitrary length. The one most often used in this experiment is a 15 minute time cut of the data. The resulting arrays, or histograms, simply reflect the number of counts recorded by a particular detector during the chosen time period. It is valuable to display these histograms as they provide a visual time sequence of the pharmacological behavior of the injected substance. In order to be displayed pictorially however, the histograms are scaled to the two hundred fifty-five

colors that the computer supports. The underlying histograms can range in value from zero to one hundred at early times to a maximum of five thousand during a six hour run. This results in some data compression as two similar pictures can have different underlying arrays.

Any such ambiguity can be removed however using the Region of Interest program. This program provides the majority of the data analysis for the system. This program allows the user to graphically select and plot a region of interest. The program then runs through the time-cut files and produces a plot of counts in this region versus time. These plots give the uptake curves of the injected substance for the animal.

In order to allow the computer to correct for variations in efficiency with the detector, a "flood filter" is employed. A flood image is formed by placing a large uniform source of activity near the detector for a long period of time. This allows one to find possible sensitivity variations of the detector-collimator combination. If an area exhibits a low sensitivity, the data from that area will be corrected accordingly so that they more accurately reflect the true activity.

VI. RTI-55 Studies

RTI-55 is a cocaine analog that has recently been synthesized. It naturally acts on certain receptors in the brain and is later processed by the liver, kidneys, and stomach as part of the body's toxin removal process. This substance has been fairly well studied. The aim of the William and Mary research study has been simultaneously to enhance the detector system, while gaining insight on RTI-55's true pharmacological characteristics.

Researchers at Johns Hopkins University did the pioneering work to analyze the pharmokinetics of RTI-55 using a gamma camera. The group utilized a small photomultiplier tube coupled to a NaI scintillating crystal attached to a pinhole collimator. This apparatus was positioned on the left side of the mouse's head and recorded count as a function of time. The study confirmed earlier studies done with *ex vivo* methods. (Mochizuki 1997)

The pinhole collimator is a different concept than the parallel collimator. The pinhole collimator is simply one small hole in the middle of an absorbing material. When placed close to a subject, it has a magnifying effect. The sensitivity for such a collimator is generally less than the parallel variety because there is only one hole that admits radiation. (Sprawls 1995)

The limitations to the above probe is that only one portion of the body is examined. The experimenters only know the amount of radiation that is present in the detector's line of sight and cannot see data from other biological structures. However, the Johns Hopkins study did confirm the validity of the gamma camera approach as its results agree with traditional methods of autoradiography.

The studies at William and Mary use a twenty to fifty gram CD-1 mouse injected with approximately twelve to fifteen microCuries of the substance (^{125}I – RTI-55) into the tail vein. The animal was first anesthetized with chloral hydrate before the injection of the radiolabeled ligand. The amount of anesthesia is crucial to the accuracy of the study, for if the mouse moves during the run subsequent analysis will be severely compromised. In addition, the anesthesia influences the metabolism of the animal, which can affect the uptake of the substance being studied.

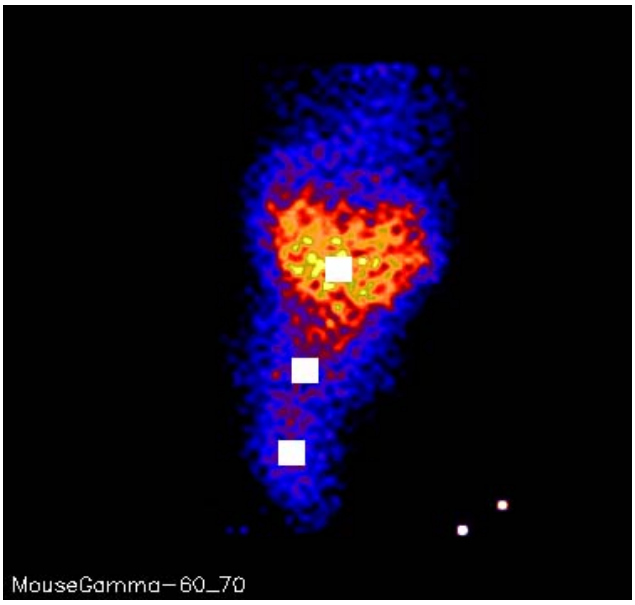
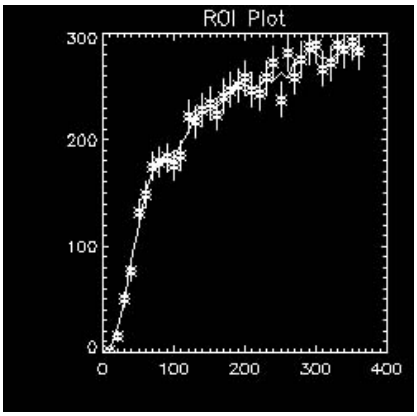


Figure 5: View taken by a 125 mm PSPMT of Mouse Gamma with 7.5 μCi ^{125}I-- RTI-55. Black regions indicate no detector activity. Blue, red, orange, yellow colors show progressively more activity respectively. The head is down in this figure and the tail is at the top of this diagram. The white squares in its brain, liver and thorax are user-defined regions of interest. This image is the time cut histogram holding events from 60 minutes after the start of imaging until 70 minutes. The uptake curves for the brain and liver are below.



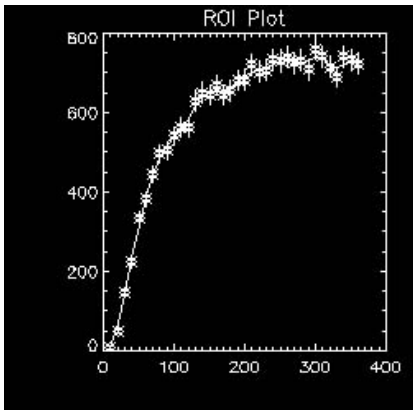


Figure 6: The left graph shows the uptake curve of ^{125}I-- RTI-55 in the brain of Mouse Gamma (Shown Above). Note that it is still accumulating RTI-55 at the 6 hour mark.
The graph on the right shows the uptake curve of the liver. Note that this curve has leveled off around 4 hours.

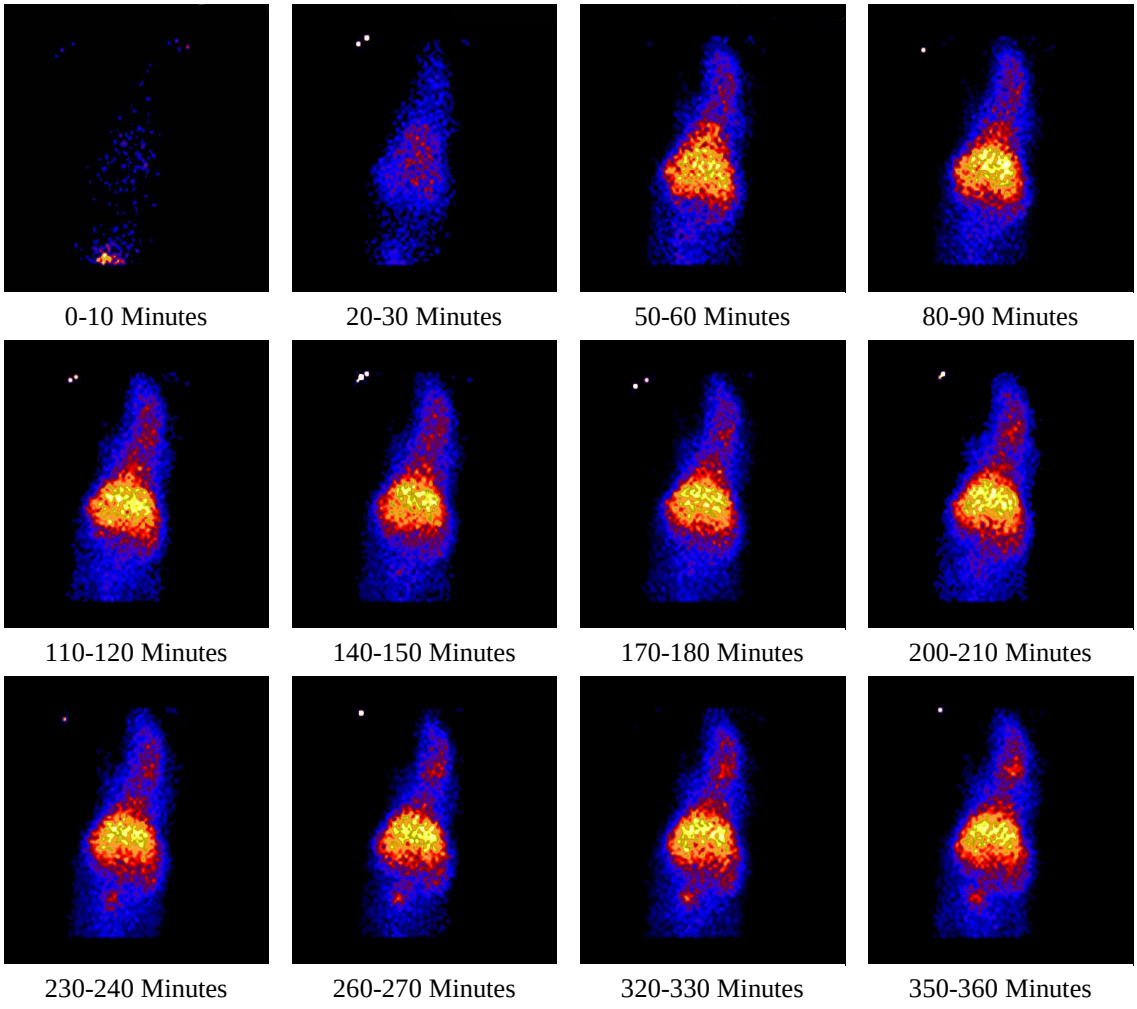


Figure 7: The above diagrams show the distribution of RTI-55 in mouse Gamma. One can see the spread of hot sites from the tail vein, the injection site, to the rest of the body. This is especially true if one looks at the difference between 0-10 minute image and the 20-30 minute image. The brain (top of image) is obviously absorbing the RTI along with the liver and kidneys (the larger yellowish site in the middle of the image). These images allow an investigator to accumulate quantitative pharmacological data as the pictures show the uptake of the substance through the entire body, as opposed to systems which may show the uptake at a specific site only.

The first five mice were run for three hours on this detector array. We then compared the amounts of radiation detected by the gamma camera array to that measured by more traditional methods. It was found that the mouse had a fairly uniform uptake of the substance during this time period. The liver and the brain both showed high rates of change.

Two later mice were imaged for six hours. In this longer time period, the liver reached a maximum accumulation while the brain continued to absorb RTI-55. Both of these time periods showed static amounts of activity in the liver while the brain continued to accumulate the tagged ligand. The advantage of this longer run is that one can observe the plateau in the liver. The liver uptake curve is initially rather steep but later flattens out.

VII. Tomography Experiments

In the hope of performing tomography on mice in the future, the team has started to experiment with a phantom. The phantom consists of four 30 mm long tubes, each of diameter 2 mm, drilled into a Lucite cylinder, 50 mm long by 32 mm in diameter. Those holes were filled with approximately 20 μCi of radioactive material. A center of rotation cylinder was also constructed out of a Lucite cylinder of the same diameter, however this cylinder simply held a single tube drilled along the cylinder axis. A calibrated rotatable microscope stage was used to rotate the specimens in front of the detector. The center of rotation cylinder showed, quite aptly, the center of rotation that the phantom used. This allowed a correction if the phantom was not centered on the

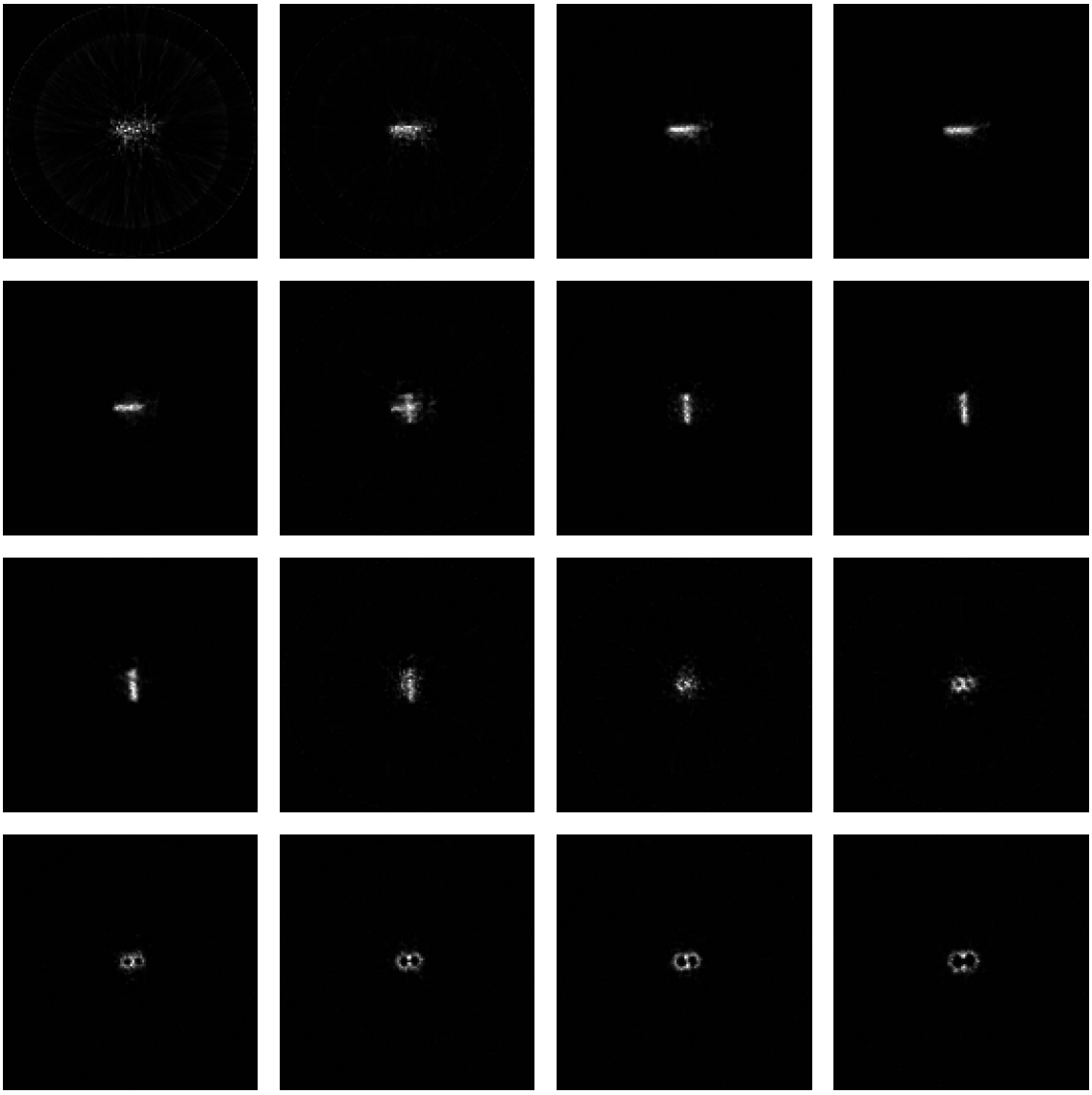
rotation disk. The phantom was placed 3 mm from the detector and imaged for three minutes per view, with one image every six degrees for a full 360 degree rotation. The length of the image was not arbitrary. In order for tomography to be performed effectively, one must have good statistics. A highly position sensitive copper collimator was used to perform imaging (Teco-met Corp.) with 0.2 x 0.2 mm square openings and septa walls 0.05mm thick.

The images must be processed before they can be used in a tomographic algorithm. The data acquisition software corrected sensitivity variations using a "flood filter". The analysis software then returned and masked out the area outside of the phantom. The anodes toward the outside of the detector show considerable noise which could produce artifacts in the tomography. As the software prefers a uniform background, the software also smoothed the image and insured no pixels had zero counts.

The tomography algorithm used is one known as One Step Late (OSL) algorithm, invented by Peter Green. This algorithm produces a much cleaner image than that produced by conventional backprojection. (Green 1990) This algorithm was translated from its original C code into the IDL programming language by Dr. Steve Meikle of the Department of Radiology at the Royal Prince Alfred Hospital in Sydney Australia. As one may notice on the next page, the tomographic slices have very little noise.

The main difficulty in applying tomography to the mice studies lies primarily with the quantity of radiation. In order to produce an image suitable for tomographic runs, the phantom, filled with 20 μCi of activity, was imaged for three minutes a view

for 61 projections. A typical mouse is injected with 15 μCi, therefore views for tomography work must be of similar length. However, this system is designed to study the uptake of a substance, which is necessarily time-dependent. One is thus forced to obtain 61 views of a mouse in 10 to 15 minutes for tomography to be useful for this work. One solution to this problem is to surround the animal with the smaller 18mm PSPMT, as the large number of detectors will allow the full set of views to be acquired in a short amount of time.



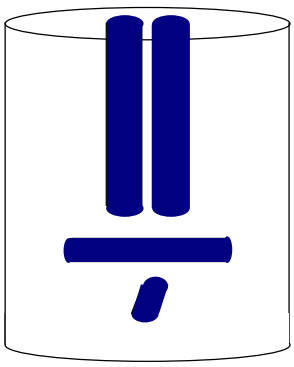


Figure 8: The upper twelve panels represent sampling of the tomographic slices obtained while imaging the phantom shown to the right. (Blue in the phantom indicates a radioactive region) The panels on the top left represent the slices near the bottom of the phantom. These progress up the phantom from left to right along each row, with the panel on the bottom right indicating the highest slice of the phantom. (Some slices are omitted in the interest of brevity)

VIII. Conclusions

The primary accomplishments of this researcher in the previous year were rewriting the data analysis software, performing tomography experiments and building adapter electronics for the 18 mm tubes. A considerable amount of time was needed to keep the detector system in good working order, especially during experimental runs. One of the final tasks was assisting the biologists, the end users of the system, in data analysis.

While this study has produced valuable results, there are a number of potential improvements to this imaging method. These improvements are motivated by the desire to obtain more information from the animal and to improve the quality of the information that currently is obtained.

A large hurdle to overcome is noise. While the photomultiplier tube is a fairly low noise device, the picture display is very sensitive to the noise that occurs. Currently, one often uses a mask to separate the image of interest or a flood filter to account for variations in sensitivity of the detector. One possible way to overcome this is through the Maximum Entropy Method. The Maximum Entropy Method (MEM) has been well known in information processing; however it has only recently been applied to problems of image processing. One finds a Point Spread Function (PSF) which characterizes the imaging equipment used. MEM treats the image as incomplete data and seeks to fill in the missing data using the tools of thermodynamics. (Hollis 1992)

The ultimate goal of this project is Single Photon Emission Computed Tomography (SPECT). Such tomography can provide valuable knowledge of internal

structures. Currently, with only two fixed 125 mm PSPMTs, more views would have to be acquired to perform accurate tomography. The other goal is the implementation of the necessary hardware; a stepping motor controller will be implemented into the data acquisition system along with additional PMTs. Finally, all the data analysis software will be rewritten to allow generation of three-dimensional tomographic images. This work will likely be accomplished with the smaller 18mm square tube as several are able to be placed close to the animal. These tubes have excellent position resolution on the scale of 0.7 mm FWHM. This microSPECT work will allow one to closely examine interesting areas such as the brain.

It can be difficult to extract specific information on the uptake of a substance when one does not have a simultaneous image of the animal's structure. This is especially true of the brain, where it is difficult to label the sections of the brain from a gross image of uptake. A second modality, such as an x-ray device, can provide this information. The use of complimentary modalities is encouraged by the imaging community with each modality providing different information. We have thus tested and will soon install a small x-ray system capable of providing precise structural information on a specimen simultaneously with the data obtained with the PSPMTs.

During one data run, there was an odd dip in the middle of the uptake cycle. This was puzzling as there seemed to be no biological reason or equipment failure. It was later discovered that these dips correlated with a dose of anesthesia. We have concluded that the anesthesia was causing the animal's metabolism to drop and therefore the uptake cycle was interrupted. The addition of biological monitoring will allow one to monitor the animal's physiological state. Recently, the addition of a thermometer has

allowed us to monitor the animal's temperature. In addition, the bed for the animal has been lined with resistive heating wire which can be controlled to maintain the animal's temperature throughout a study. In this fashion, we intend to limit changes in metabolism which might affect the imaging results.

The double photon from the iodine decay allows for the possibility of coincident detection to screen out background radiation. However, previous attempts at applying coincidence techniques has resulted in a loss of statistics by a factor of 80. For the present, this type of noise screening is not used.

The gamma camera detector system discussed here provides useful images of the uptake of various substances. Limitations to this system are its lack of structural information about the mouse, issues with noise, and its planar imaging. However, with the improvements discussed above, this detector system could produce valuable information about the pharmacological characteristics about a substance.

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