

# Improvements for the Imaging of Small Animals

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Physics from the College of William and Mary.

by

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## Abstract

We have applied the techniques of scintillation imaging to studies of the mouse mammary tumor virus (MMTV). In these studies, the Sodium Iodide Symporter (NIS) transfers the radioactive  $^{125}\text{I}$  to the mammary glands of lactating mice and in particular to those mammary glands with visible tumors. These studies have principally been carried out using pixellated scintillators coupled to position sensitive photomultiplier tubes (PSPMTs). More recently, we have initiated such studies with a monolithic slab of  $\text{LaBr}_3$  scintillator coupled to an array of PSPMTs. A multi camera system was developed for planar imaging and Single Photon Emission Computed Tomography (SPECT).

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## **I. Introduction**

Cancer is one of the largest causes of death in the world. The disease can give rise to malignant tumors which invade and destroy surrounding tissues or it can lead to benign tumors which only grow but divert much of the body's resources. Early detection of tumors plays a vital role in treating the illness before the patient reaches the terminal stages. The purpose of this project is to improve the ability to detect tumors during their early stages by improving our detector equipment and analysis techniques. The hope is that the methods and detectors that are developed will be able to transition from small-animal imaging to the detection of tumors in humans. The focus of this research involves the testing of an innovative  $\text{LaBr}_3$  detector and the development of new multi-camera systems.

## **II. Multi-Camera System**

In small animal imaging it is most often necessary to anesthetize the animal during the imaging session. Two concerns arise when an animal such as a mouse is anesthetized. If one uses too much anesthesia then the mouse could die. But, if too little anesthetic is administered, the mouse could wake during the imaging session and its movements will distort the image. As a result it is desirable to develop a method to obtain the data needed while reducing the time that the mouse is anesthetized. To solve this problem a multi-camera system has been tested. By using multiple gamma cameras the sensitivity of the system can be increased substantially. As a result the amount of time required to obtain enough data will decrease allowing one to use less anesthesia. One concern with such a system is the potential for loss of spatial resolution when images from two or more detectors must be combined. Therefore, an arrangement has been

tested in order to attempt to minimize degradation in resolution from the simultaneous use of two or more detectors.

Once the data are obtained it is necessary to combine the images from each camera. Since the cameras will be placed equidistant from the subject animal, the images from each camera will be at different angles relative to the animal being imaged.

Interactive Data Language (IDL) is a programming language used frequently in data analysis. Its ability to deal with large volumes of data and the relative ease in implementing functions makes it an extremely useful tool in making quantitative analysis of the data. Therefore using IDL will allow for the manipulation of the images from each camera to align them. Once this is affected the images can be combined.

Prior to imaging one must develop the setup and method of analysis. The first steps in testing the system are generally based on the use of a radioactive phantom. Phantoms are various inanimate radioactive objects that have constant activity. They are created for preliminary tests and calibration purposes. Phantoms can be created to resemble a test subject such as a human brain or can be simple like a single capillary tube containing a radioactive sample. The phantom provides a known shape that will allow one to predict better the outcome of the imaging session and to correct accordingly.

The first part of the phantom imaging in this work involved the use of two gamma cameras positioned 180 degrees from each other. Once data are obtained one of the images will be flipped and combined with the other to form a composite image. Using IDL one can translate the images in order to overlap them. If image reconstruction can be carried out successfully with two cameras the work will be extended to three cameras. Each of those cameras would be placed 120 degrees from each other and during the

initial test imaging sessions they will each be rotated through as full 360 degrees so that each camera will obtain an image at each position. This is done to permit one to detect and correct any possible distortion for each camera.

The multi-camera system will then be used for single photon emission computed tomography (SPECT). SPECT can obtain data that will permit generating 3D images from a series of 2D views of the object at different angles. SPECT works by rotating gamma camera(s) 360 degrees around a target and allowing the camera to take data from various angles. The information from the system is then used to create a 3D data set that can be used to display 2D slices of the target. When imaging with two cameras placed 180 degrees apart, one can either overlay the images from each camera or gather half the data with one camera and the rest from the other one.

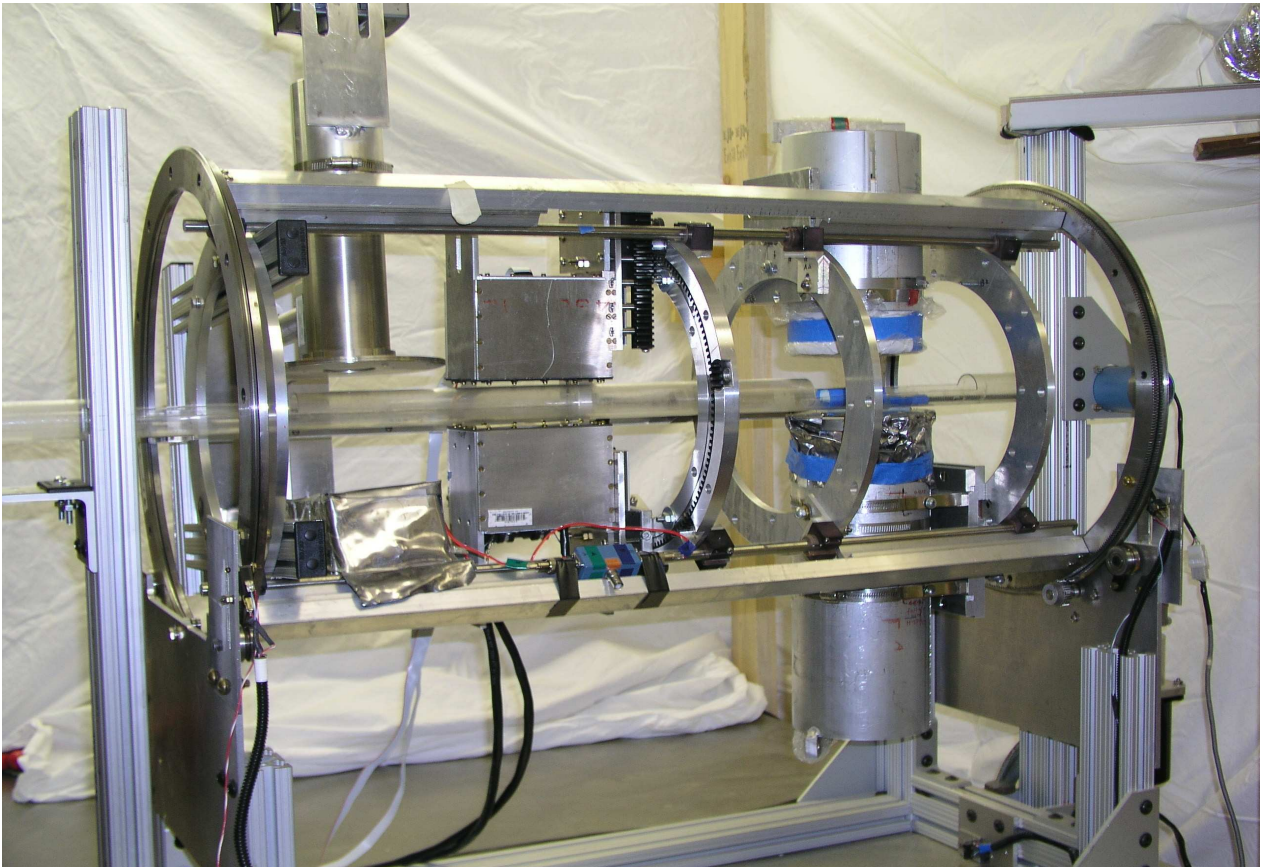


Figure 1: Multi Camera Setup

The first step in obtaining the images is to determine accurately the center of rotation of the system. This is done in order for the SPECT reconstruction program to have a common reference axis. Since the resolution of the detectors used here is on the order of 1-2 millimeter it is vital that center of rotation position be less than one millimeter off in either direction. Once the center of rotation is found a capillary phantom can be placed at the center of rotation and imaged by the multi-camera system 360 degrees around the phantom in three degree increments for 90 seconds to three minutes depending on the activity of the phantom. Once this has been completed a phantom of three capillaries at varying distances from each other was created. These were imaged for 90 seconds at each point by both cameras using a modified data acquisition program. The data acquisition software for the system is Kmax which was developed by the Sparrow Corporation. Kmax has the ability to obtain data as a histogram or as a time distribution.



Figure 2: Phantom



The SPECT reconstruction program creates 2D slices at various points of the test subject. This program was written in IDL by scientists from Jefferson Labs, William and Mary and the University of Sydney. The program first reads the center of rotation correction data that was obtained earlier. Then the data from the phantom are read. The program has the ability to allow one to compare multiple slices and to change other aspects of the image.

The data from each detector were read by the program and used to produce the expected image. Next the data from one detector were matched with the center of rotation correction data from the other detector. The 2D slices for each one was highly distorted, enough to cause serious problems. This was done to see if only the phantom data from the two detectors needed to be combined. The first step was to check how the data between each detector should be combined. Each image at each point is saved as an individual Kmax file for a total of 120 files per camera. The first method was to take the data for the first 60 images for each detector and treat them as the data from a single detector. The image produced was highly distorted and no image could be discerned. The next method was to take the first 60 images for one detector and combine those with the second 60 images from the second detector. The reconstructed image was still too distorted.

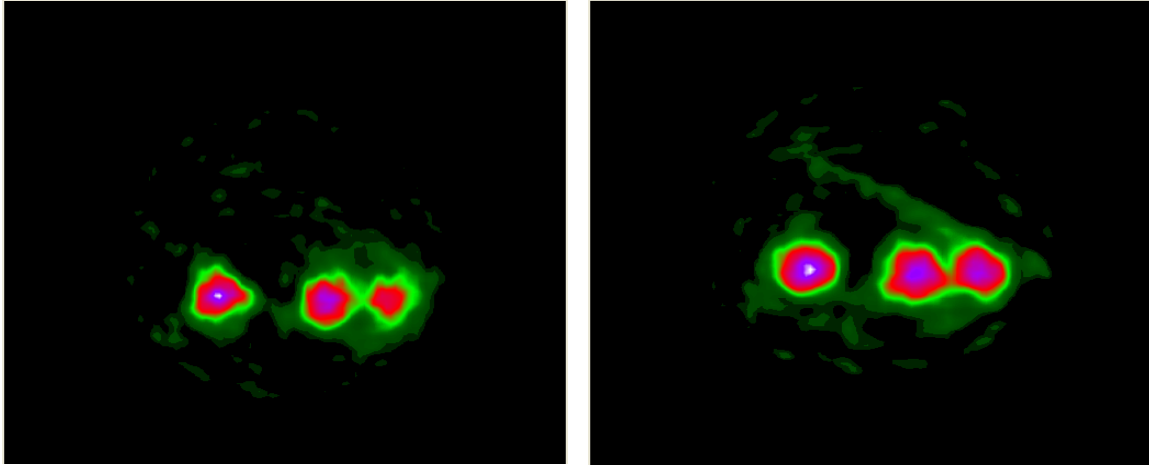


Figure 3: Reconstructed Images for Detector C(left) & Detector D(right) with their respective COR data

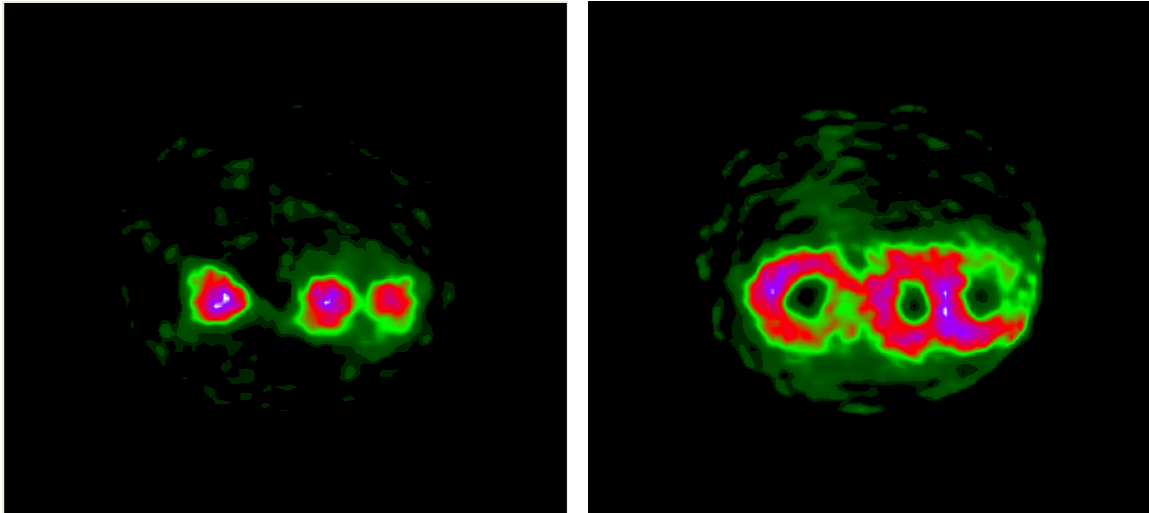


Figure 4: Phantom Data reconstructed using COR data from other detector. Detector C (left) & Detector D (right)

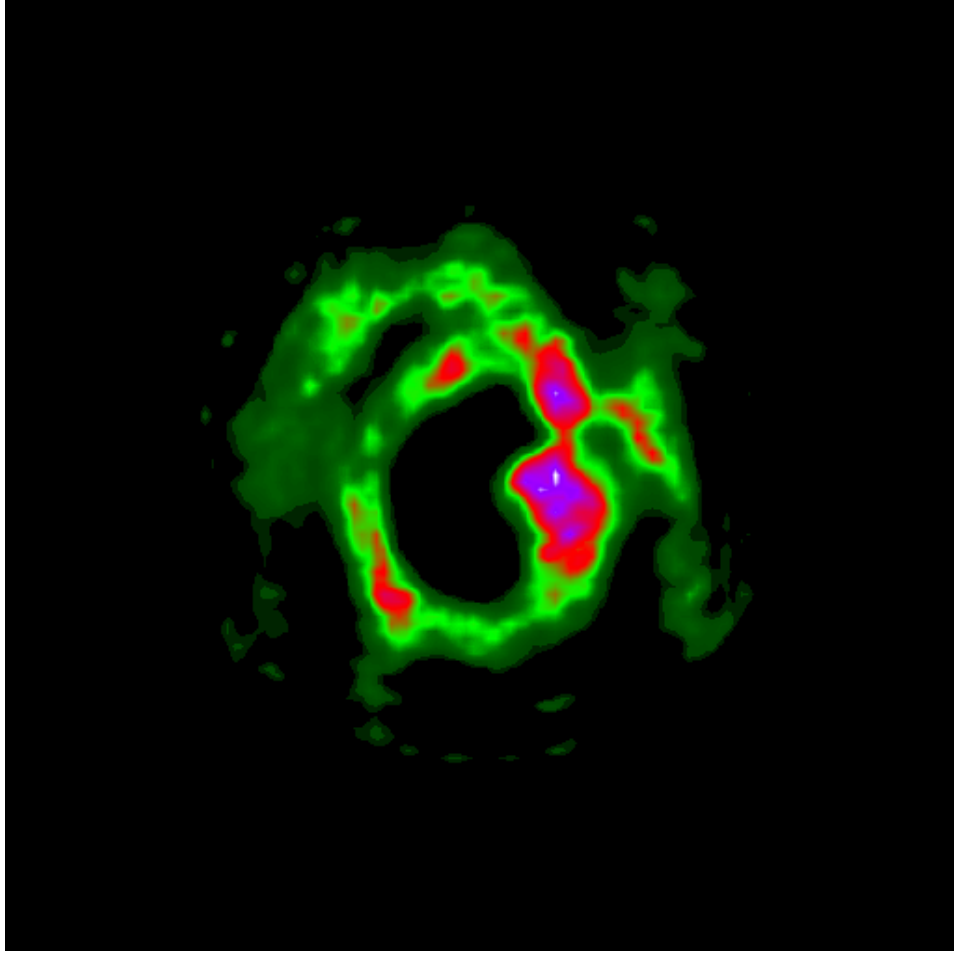


Figure 5: Reconstructed Image data for positions 1 through 60 for Detector C and positions 1 through 60 for Detector D using COR data from Detector D

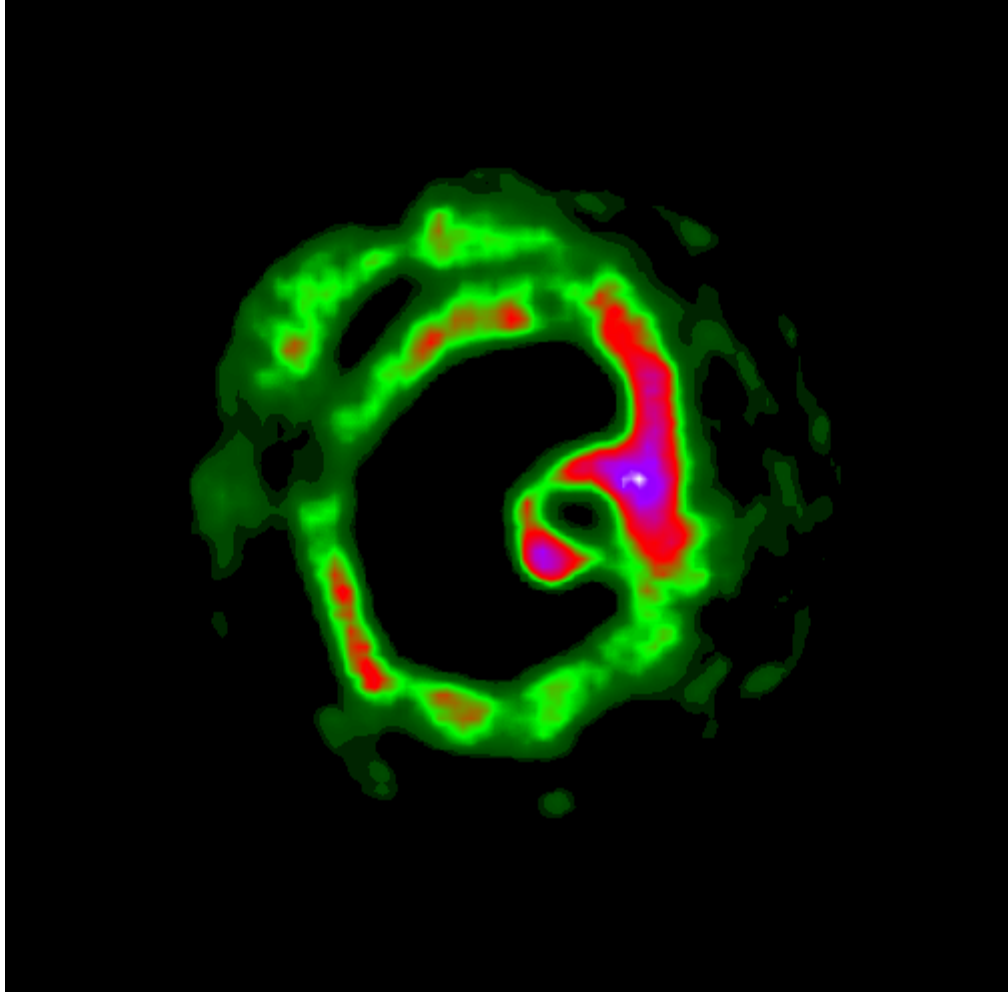


Figure 6: Reconstructed Image using data from positions 1 through 60 for Detector C and positions 61 through 120 for Detector D

Using a program created in IDL each set of data can be compared to find the parameters needed to align each image. It was found that the images from each detector were separated by three pixels. After the images were shifted they were compared to see how they align with each other.

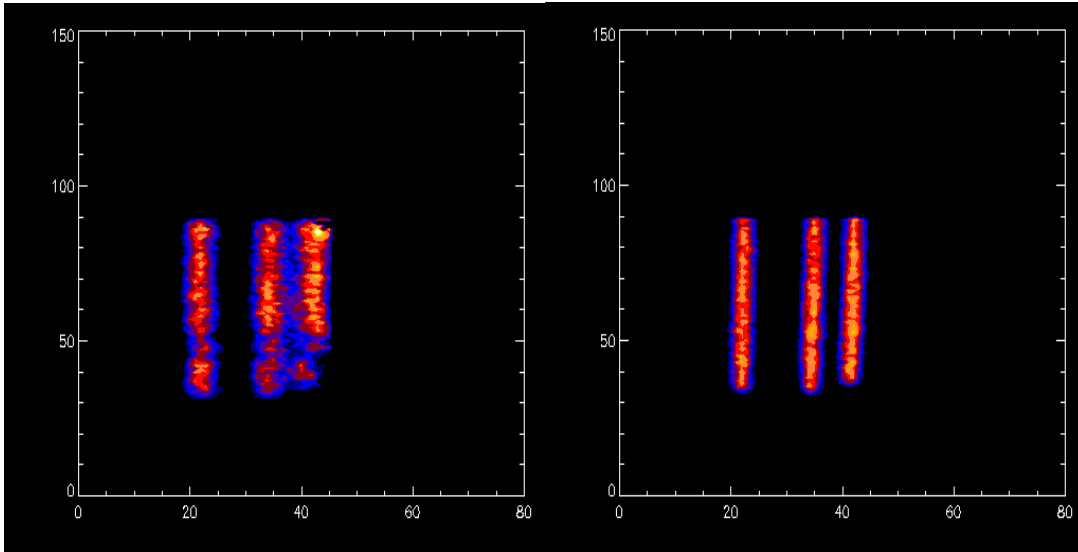


Figure 7: Shifted Phantom from Detector C(right) & Detector D(left)

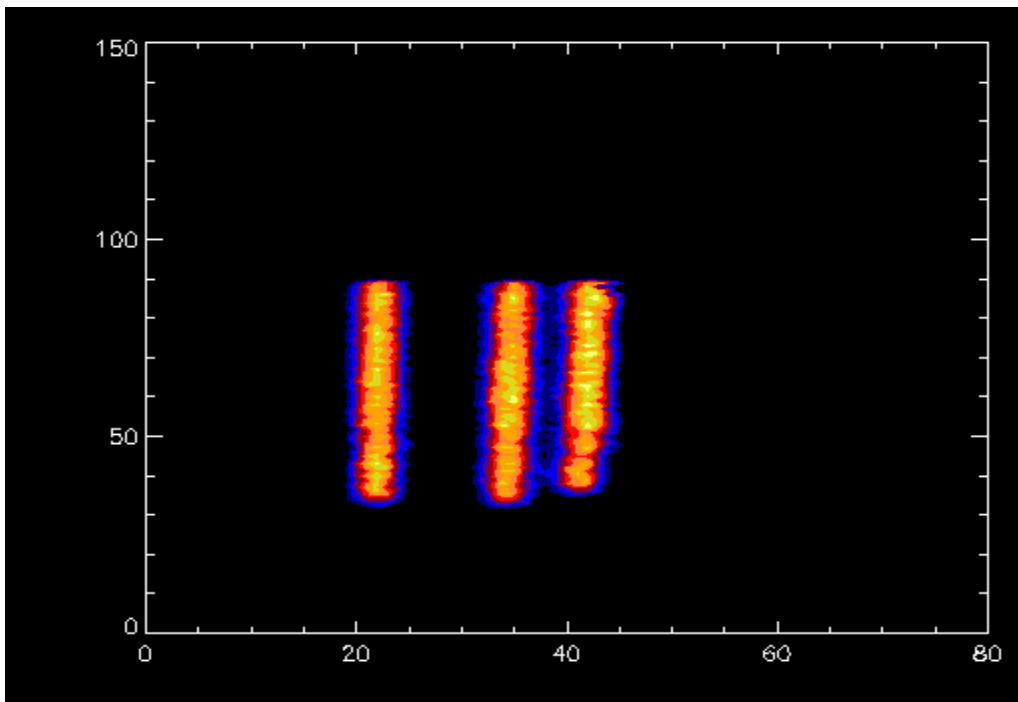


Figure 8: Combined Planar Image

After subtracting each data set from the other and setting the negative value to zero the data set was contour plotted and it was found that the images matched up well with each other.

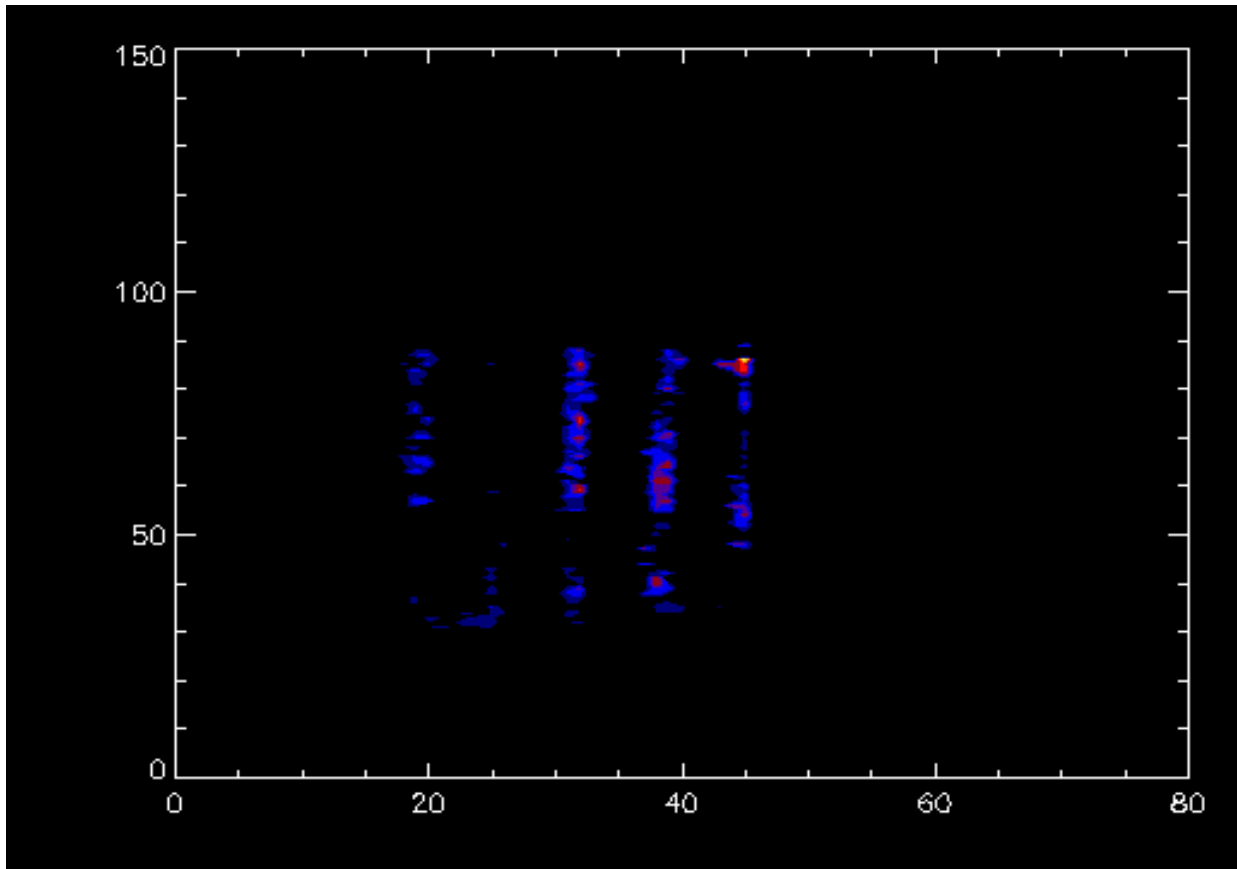


Figure 9: Difference plot of the two planar images (Detector C's data subtracted from Detector D's data)

Unfortunately when the data were corrected, IDL was unable to save the data as a Kmax file and the IDL files could not be converted into Kmax files. As a result the SPECT reconstruction program will require some additional modification before the multi-camera system can become functional.

Since we have determined that images from both detectors can be combined without substantial loss in spatial resolution the multi camera system can be used for small animal imaging.

### III. Lanthanum Bromide Detector

One of the problems of tumor detection is that many tumors during their early stages are extremely small. As a result many detectors are unable to detect these small

tumors. In order to improve the imaging of small animals a novel detector is being tested. The detector uses a continuous slab of Lanthanum Bromide ( $\text{LaBr}_3$ ) scintillator in contrast to the pixilated scintillator previously used based upon Thallium doped Sodium Iodine ( $\text{NaI}(\text{Tl})$ ).

Scintillators are transparent materials that absorb radiation such as x-rays or gamma-rays and emit light with wavelengths ranging from the visible range to the UV range. Decay time for a scintillator is defined as the time needed for the emission to reach  $1/e$  of the maximum value. Scintillators with short decay times can maximize the amount of data collected [1]. Two categories for scintillators are organic and inorganic scintillators. Organic scintillators have extremely quick decay times and are well suited for detecting charged particles. At the same time inorganic scintillators have longer decay times but they are able to better detect a range of photon energies. Therefore for imaging studies, inorganic scintillators such as Lanthanum Bromide are the most appropriate choice.

Lanthanum Bromide is a recent discovery with the first paper investigating its properties published in 2001 [2]. The continuous scintillator has higher sensitivity compared to the pixilated one because there are no separators which fail to scintillate. One of the most promising features of the new scintillator is its high light output which has been measured to be approximately 61,000 photons/MeV for gamma rays of energy 662 keV as opposed to NaI whose energy output is 40,000 photons/MeV against gamma rays of the same energy [3]. This is useful for imaging since electronic noise is a significant problem when processing events. A higher light output will minimize the effect of noise and it will be easier to detect random signals that might distort the image.

In addition,  $\text{LaBr}_3$  has an excellent energy resolution of 2.8% versus 5.6% for NaI when detecting gamma rays of 662 keV[4]. This feature will allow the new detector to yield better signal to noise. Finally  $\text{LaBr}_3$  has an improved decay time of 35 nanoseconds versus 230 nanoseconds for NaI [5]. The quicker decay allows for the detector to process more events in a given which allows the system to be more discerning of the events that it processes with less pileup of detected events.  $\text{LaBr}_3$  also improves the spatial resolution owing to its greater light output and has constant photoemission rates over a wide range of temperatures. One problem is that  $\text{LaBr}_3$ , like NaI, is hygroscopic; as a result one needs to be careful in packaging and installing the detector. [6]

The complete detector makes use of an entrance window, collimator, scintillator, position sensitive photomultiplier tubes and a data acquisition system. The entrance window is a thin, light-tight graphite composite which protects the system.

Collimators control the number and direction or orientation of the detected gamma rays. Tungsten and lead are popular materials for making collimators due their high Z. Some types of collimators that are used in medical imaging include parallel hole, rotating slit and pinhole collimators. Pinhole collimators allow for magnification of the target while sacrificing some efficiency. The experiment described here uses parallel hole collimators made from Copper Beryllium since one can etch holes easily into that material. Also thin sheets of etched Cu-Be can be stacked on top of one another until the desired collimator thickness is achieved [7]. Since stray sources of gamma rays can distort images one needs to minimize the effects of these stray gamma rays. As a result a collimator serves as a useful tool in limiting the stray rays and ensuring that gamma rays from the source hit the scintillator perpendicularly.



Photomultiplier Tubes create an electrical signal when visible light hits the photocathode which emits electrons. The electrons then strike dynodes of increasing positive voltages and secondary electron emission can eventually result in gains of  $10^8$  when those secondary electrons finally reach the anode. A data acquisition system is then used to process the electrical signal. Position Sensitive Photomultiplier Tubes follow the same principle as normal photomultiplier tubes except that the dynodes and anodes are arranged so that there are X anodes crossed with Y anodes and the pattern of electrons striking the anodes follows accurately the pattern of photons which initially strike the photocathode[8].

Innovative electronics designed and built at Jefferson Lab employs a resistive matrix array to reduce the number of readout channels from the 1024 anode pads in four H9500 phototubes to 64 lines to the data acquisition system.

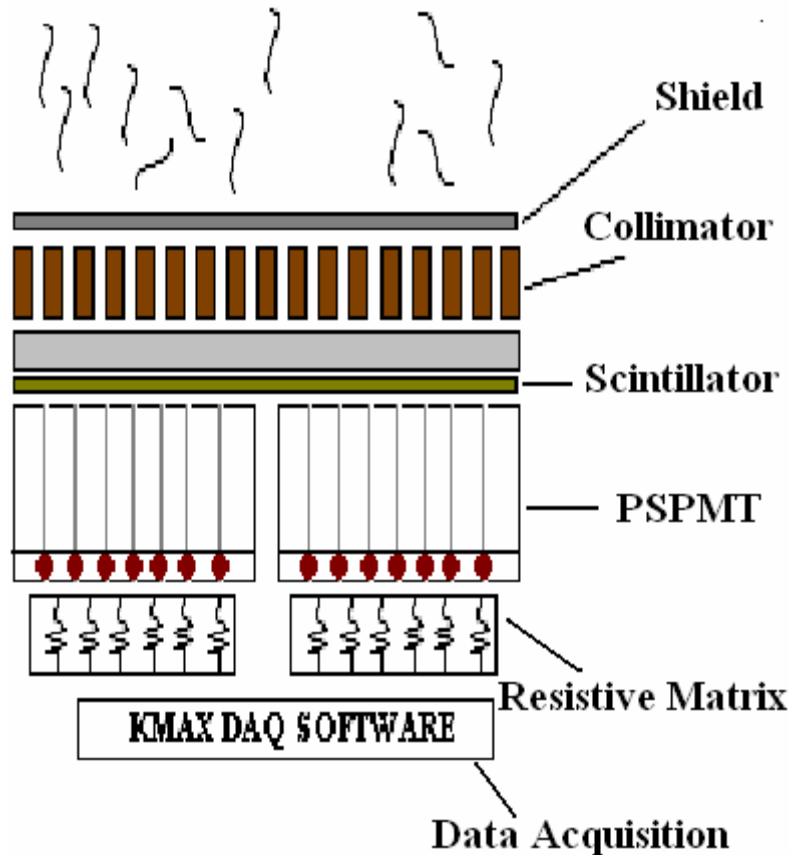


Figure 10: Detector Setup

The dimensions of this detector (100 mm x 100 mm) allow the imaging of two mice simultaneously when using parallel-hole collimation. This permits one to obtain data more quickly and compare the activity in mice more effectively. This detector size is also suitable for pinhole collimation which can easily magnify the image by a factor of two to four.

The detector was tested using a special mask drilled with about 1000 small holes along with both high energy and a low energy gamma sources. A second small mask was also used bearing small holes in the shape of a stick figure image with a halo. Its dimensions were .65mm x .9mm x .8mm. Against the higher energy source (166 keV) the spatial resolution was 0.7 mm to 0.8 mm. The spatial resolution using the low energy source (30 keV) was 0.9mm.

During testing a small crack developed down the middle of the scintillator. The position corresponds to the region between pairs of PSPMTs. Possible causes for the crack include an overly thin scintillator or effects caused by rapid temperature change. The detector has been returned to the manufacturer for repairs. Unfortunately the detector has not yet been returned and as a result no further tests have yet been done.

#### **IV. Tumor Analysis**

Detecting the presence of tumors early is one of the primary motivations for developing new detectors. At the same time the ability to understand how a tumor will develop is an extremely important tool. This knowledge would allow one to know where to expect tumor formation which could lead to more precise and efficient treatment.

Mouse Mammary Tumor Virus (MMTV) is a virus that causes tumor formation in mice mammary glands. MMTV is a retrovirus meaning that it contains RNA and the enzyme reverse transcriptase. Reverse transcriptase allows the virus to convert its RNA into DNA. Once this happens the retrovirus becomes a provirus, in that it will integrate its DNA into the host's genome and therefore forces the host to replicate the virus. The virus can be transmitted directly from the mother's genes or it can be transmitted through her milk [9].

Their tumors were analyzed quantitatively by comparing the percent increase in tumor area versus time and original size. Next the tumors were qualitatively analyzed to see if the original size and shape would have any bearing on the subsequent shape of the tumor. Nine tumors were imaged multiple times among six mice.

Before imaging the mice were anesthetized and injected with Iodine 125. Iodine 125 ( $Z=53$ ) serves as an important radioisotope for the imaging of small animals. Iodine

$^{125}\text{I}$  has a half life of 60.2 days. When it decays into  $^{125}\text{Te}$ , it emits gamma rays and/or x-rays with energy of about 30 keV [10]. The relatively long half life of the isotope allows one to manufacture the isotope and store and/or transport it without concern about same day usage as with the positron-emitting imaging isotopes. Also the energy of the emitted gamma rays makes Iodine 125 attractive since in small doses there can be very little tissue damage to the mouse or other animals.

Sodium Iodine Symporter (NIS) is a protein that transports Iodine from extra cellular fluid (i.e. blood) to the thyroid region in a mouse. High levels of the protein have been found in the breast tissue of lactating mice. Since mice suffering from MMTV will have more blood going to the tumor, the levels of NIS will generally be higher. Also of note is that NIS can easily be tagged with radioactive Iodine making it and Iodine 125 an ideal pairing in studying mammary tumors [11].

Each mouse was imaged for one hour using a pixellated NaI scintillator coupled with two Hamamatsu 8500 PSPMTs and combined with a parallel hole collimator. Occasionally, data are accumulated from a large uniformly illuminated “flood” source of  $^{125}\text{I}$  and those data form a “flood filter” which is used to correct the images for non-uniform sensitivity across the detector.

One of the first steps carried out is to determine the location of any tumors. Using a special “region of interest” (ROI) program one is able to view the flood corrected image of the mouse and detect a tumor. Once a tumor is found, one can use the ROI program to determine how large an ROI needs to be in order to encompass the tumor. The program will also give the location for the center to the ROI.

The next step is establishing the threshold level for counts detected in a tumor. The threshold is determined by using a small array of 2x2 pixels and placing them in areas that do not have unusually high amounts of activity. Once the three 2x2 pixel regions are selected, one can use the program to analyze the counts for the region over time and exclude positions that have extremely high or very low counts. In the case of regions whose average counts per pixel are close to each other, it is necessary to check the values of the array in order to prevent discounting areas that are part of the tumor. Once the position for the threshold ROI is determined, the total number of counts for that ROI will be summed from the first five-minute "time cut" to the last one (at one hour). After all of the threshold ROIs have been summed up we can divide the total by the number of time cuts multiplied by the area of the ROI. This number will serve as the threshold number for all time cuts.

Using a program created in IDL one can correct the image with the flood filter, isolate the tumor region from the whole mouse image and display it as an array whose values correspond to the number of counts in each pixel. One can then subtract the threshold from the array and set all negative numbers to zero. After that one can apply a function that displays a contour image of the tumor that highlights regions by designating colors based on the number of counts. The scheme used in this function goes from black (lowest) to yellow (highest). One thing to note is that the color scheme for the contour plots is based on a relative scale and not on an absolute scale. This is done to make it easier to identify the most active region during a time cut if the counts are all low or if all are high. The contour plots at the 45 to 50 minute period for each imaging session were

then used for the qualitative analysis of the tumors. The area of the tumor was defined as the number of pixels that have counts greater than the threshold.

Throughout of the course of the MMTV studies nine tumors were imaged twice among six mice. The number of days between imaging sessions ranged from four days to 27 days.

The first quantitative method was carried out by comparing the amount that the tumor grew versus the number of days between imaging sessions. That graph was extremely scattered and no discernable pattern could be detected. For mice with multiple tumors, each of their tumors was studied and it was noted that the ratio of their old size versus the new size varied greatly.

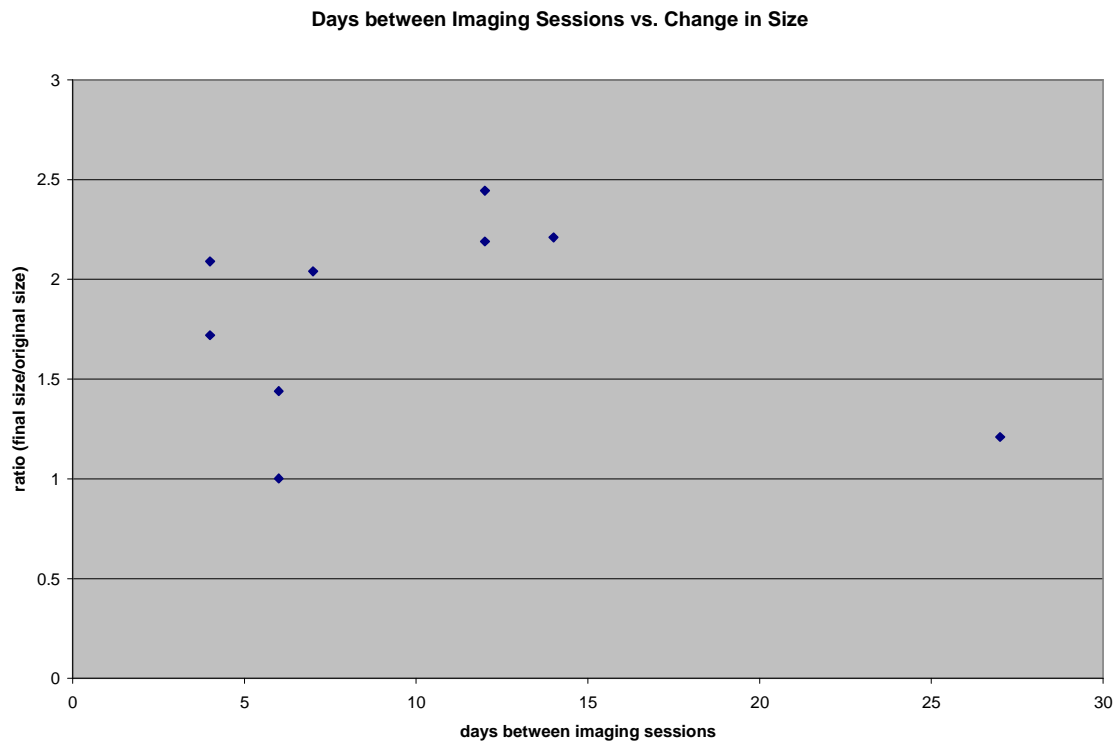


Figure 11: Days between Imaging Sessions vs. Ratio

The second method involved comparing the original size of the tumor versus the ratio of final size to initial size. When those data are plotted a noticeable pattern emerges. With the exception of one outlier, the data indicate that percent change in size decreases as the original tumor size increases.

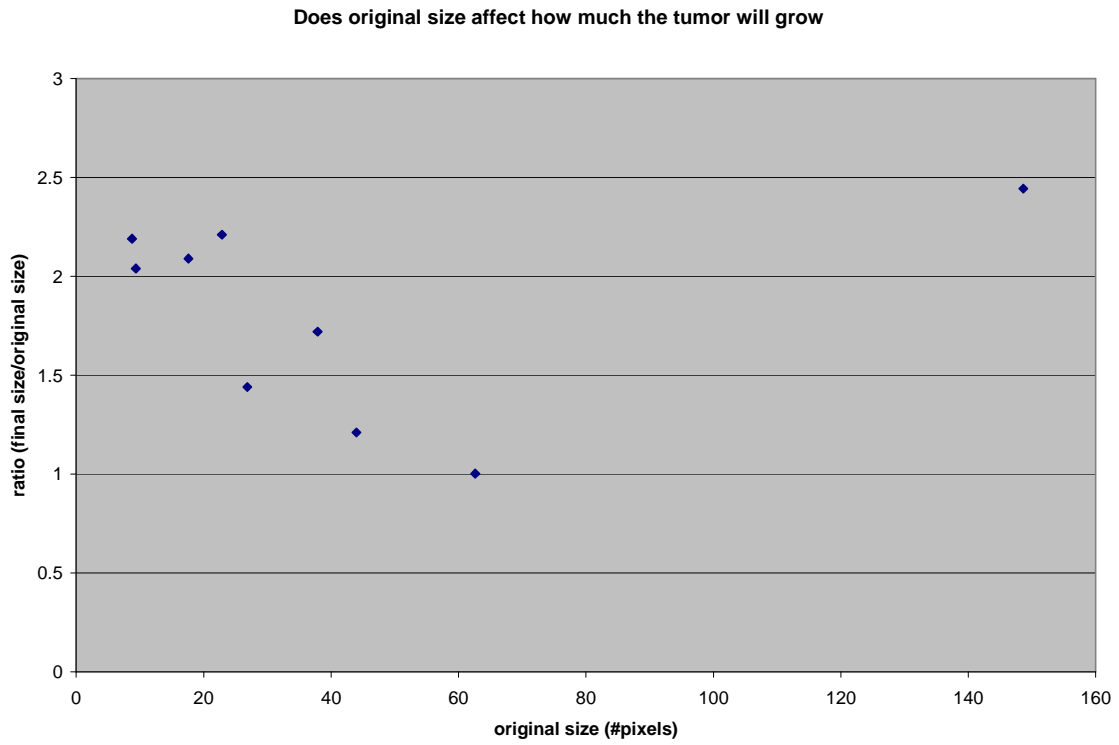


Figure 12: Original tumor size vs. Ratio

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