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SPECT ASSAY OF RADIOLABELED MONOCLONAL ANTIBODIES

Progress Report

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Ronald J. Jaszczak

Radiology Department

Duke University Medical Center

Durham, North Carolina 27710

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SPECT Assay of Radiolabeled Monoclonal Antibodies

A. ABSTRACT

The accurate determination of the biodistribution of radiolabeled monoclonal antibodies (MoAbs) is important for calculation of dosimetry and evaluation of pharmacokinetic variables such as antibody dose and route of administration. A major long-term objective of this proposal is to determine the utility of single photon emission computed tomography (SPECT) for quantifying the biodistribution of monoclonal antibodies labeled with the clinically relevant radionuclides iodine-123 (I-123) and indium-111 (In-111). The specific aims of this project include the comparison of the effects of three methods of radiolabeling anticarcinoembryonic antigen (CEA-110)--iodination by Iodogen, the tin-containing ester methods and an indium chelate method--on the immuno-reactivity and affinity of the antibody. The biodistribution of anti-CEA labeled by the three methods will be compared in tumor bearing and normal mice. Subsequent studies of the pharmacokinetics of I-123 and In-111 labeled anti-CEA will be determined by SPECT in non-human primates. The errors associated with the SPECT measurements will be assessed with Monte Carlo simulations and by scanning phantoms containing I-123 or In-111 activity in regions of uniform and nonuniform attenuation. The ability of SPECT to quantify I-123 and In-111 distributions will be assessed, and new acquisition geometries and reconstruction algorithms for improved quantification will be evaluated.

B. Introduction

The hypothesis of this application is that the biodistribution of radiolabeled anti-carcinoembyonic antigen CEA 110 monoclonal antibodies (MoAbs) can be quantitatively determined using single photon emission computed tomography (SPECT). The clinically relevant radioisotopes of iodine and indium (I-123 and In-111) that are used to label anti-CEA 110 offer distinctive advantages and disadvantages. The method of radiolabeling will affect the immunoreactivity and affinity of the MoAb, and in vivo SPECT imaging of pure (I-124 free) I-123 and In-111 will require somewhat different data acquisition and processing methods. Major long term objectives of this proposal are to evaluate and develop appropriate SPECT imaging approaches to quantitatively compare the in

vitro and in vivo characteristics of anti-CEA 110 MoAb radiolabeled with I-123 and In-111.

B.1. Research Emphasis During First Budget Period

This project originally requested funds for a period of five years; however, funds were subsequently awarded for a period of three years. Furthermore, the acquisition of a dedicated research SPECT system has required some effort in validating its usefulness as a quantitative imaging device. Hence, we have emphasized the development of appropriate quantitative methods during the first 8 months of this project.

C. Specific Aims

1) To label anti-CEA antibody 110 and its $F(ab')_2$ fragment with I-123 via the ATE method and with In-111 via a thiocyanate-DTPA chelate and a) compare their immunoreactivity and affinity constants for CEA and CEA-positive cell lines in vitro and b) compare their ability to localize preferentially in LS174T human colorectal tumor xenografts in athymic mice.

2) To evaluate the capability of SPECT to quantify in vitro I-123 and I-111 radioactivity distributions. The errors associated with in vitro SPECT measurements of I-123 and In-111 will be estimated using Monte Carlo simulations and experimental scans of phantoms. SPECT measurements of radioactivity within uniformly and nonuniformly attenuating media will be evaluated.

3) To evaluate the ability of SPECT to quantify the in vivo biodistribution of I-123 and In-111 radiolabeled anti-CEA 110, and to determine the pharmacokinetics and biodistribution of the radiolabeled MoAb in non-human primates (Macaque fascicularis). These data will be used to estimate radiation absorbed dose in normal tissue.

D. Scientific Performance Report

D.1. Characterization of Triple-Camera SPECT System

The acquisition of a research Triad SPECT system required a careful evaluation of its quantitative capabilities. Although we had previously made measurements using Tc-99m, we had not evaluated this system using the radionuclides more relevant to MoAb imaging, I-123 and In-111.

During the current project period, we have evaluated this system using two parallel hole collimators: the low energy, high resolution collimator (LEHR-PAR) and the medium energy, general purpose collimator (MEGP-PAR). To quantitatively measure the performance of the system, five specific tests were made. These tests included the measurement of point source sensitivity in air, volume sensitivity in water, planar spatial resolution in air, and SPECT resolution in air and water. These basic measurements are prerequisites for any SPECT system that will be used quantitatively. The results are presented in Tables 1-3 and Figures 1-3.

This evaluation of the triple-camera SPECT system and parallel-hole collimators provided valuable data that will be used to guide our future research efforts. For example, we noted that the relatively large magnitude of septal penetration of high energy photons emitted by I-123 and In-111 limits the quantitative accuracy of low energy collimators. Although low energy collimation can be used for qualitative imaging of pure I-123, the broad tails observed in the point spread function resulting from septal penetration makes quantitative imaging more difficult.

TABLE 1. Point Source Sensitivity in Air

Radionuclide	Energy Window (keV)	Sensitivity (cnts/sec)/μCi	Collimators Type
Tc-99m	126-154	11.4	LEHR
Tc-99m	126-154	8.3	MEGP
I-123	143-175	7.9	MEGP
In-111	157-191	6.2	MEGP
In-111	216-264	4.1	MEGP

TABLE 2. Volume Sensitivity in Water of 20.7cm Diameter Cylinder

Radionuclide	Collimator Type	Energy Window (keV)	Sensitivity [(cnts/sec)/(μCi/ml)]/cm
Tc-99m	LEHR	126-154	1831
Tc-99m	MEGP	126-154	1269
I-123	MEGP	143-175	1415
In-111	MEGP	157-191	1431
In-111	MEGP	216-264	801

TABLE 3. On-Axis SPECT Resolution (in Air) at 15cm from collimator surface

Radionuclide	Collimator Type	Energy Window (keV)	FWHM (mm)	FWTM (mm)
Tc-99m	LEHR	126-154	11.8	20.6
Tc-99m	MEGP	126-154	13.1	23.4
I-123	MEGP	143-175	14.5	26.7
In-111	MEGP	157-191	13.4	24.7
In-111	MEGP	216-264	14.9	27.0

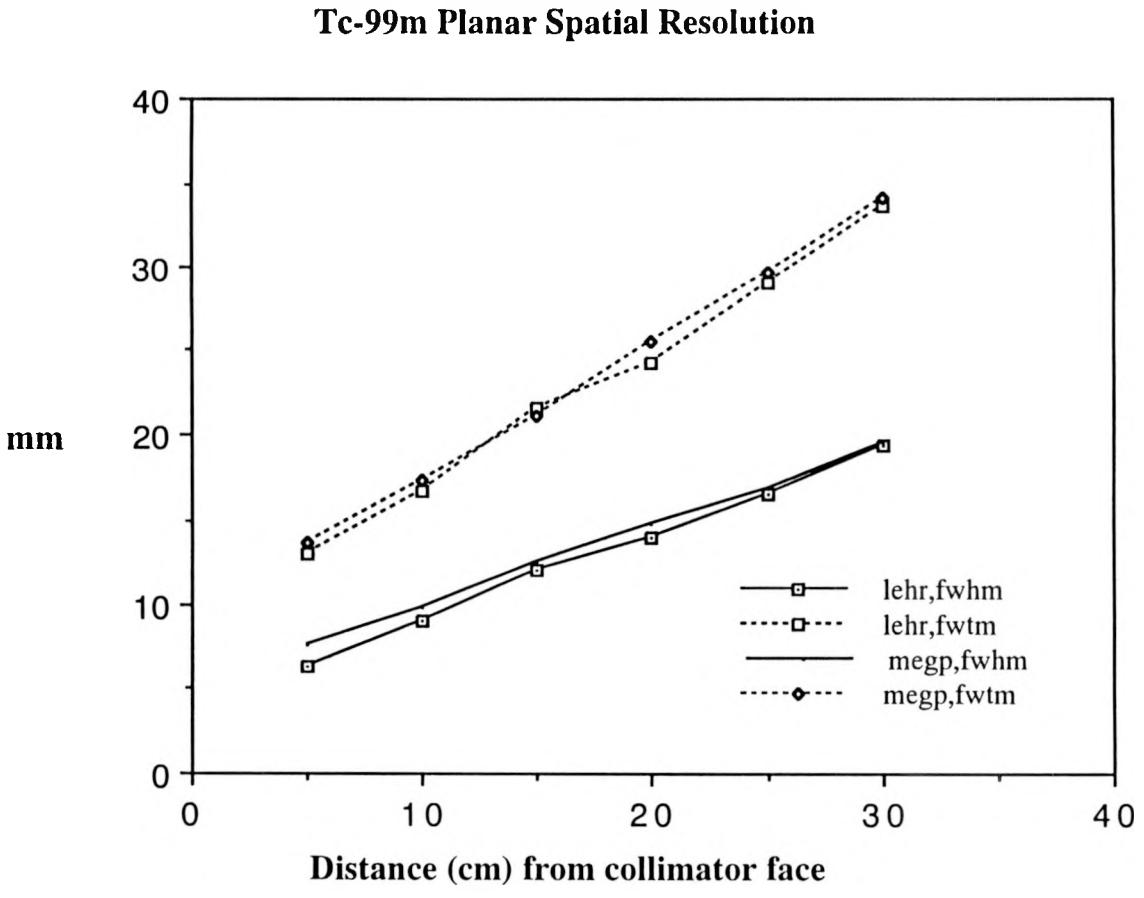

FIGURE 1. Tc99m planar resolutions of triple camera SPECT system using low energy, high resolution (LEHR) and medium energy, general purpose collimators (MEGP).

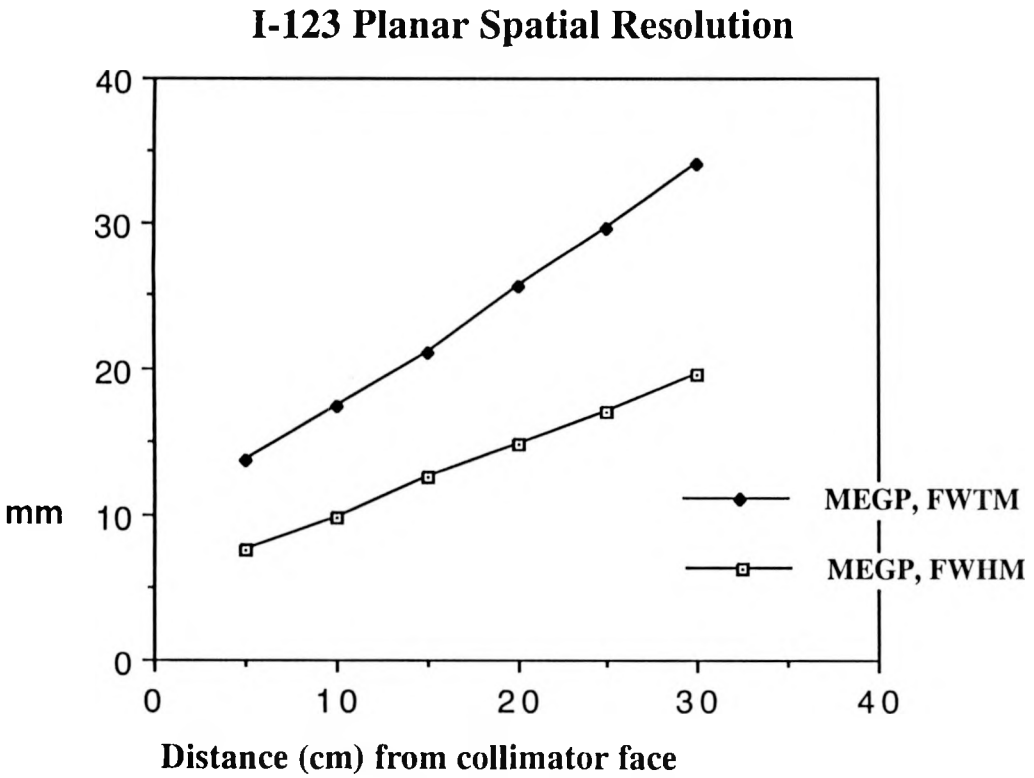

FIGURE 2. I -123 planar resolutions of triple camera SPECT system using medium energy, general purpose collimators (MEGP).

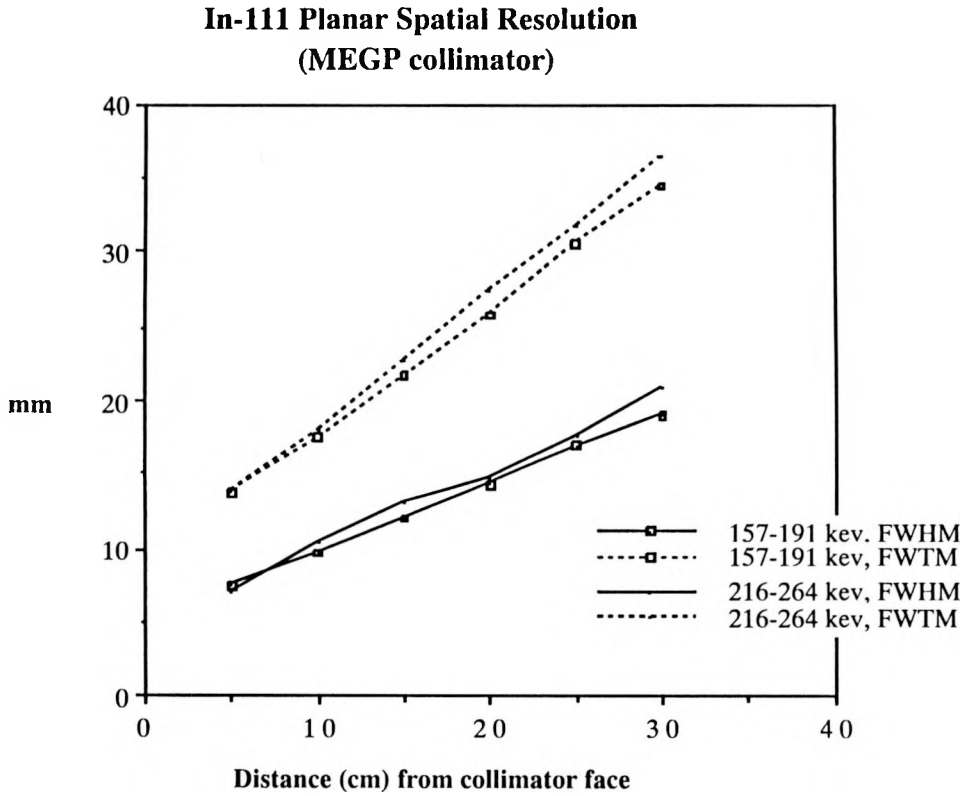

FIGURE 3. In-111 planar resolution of triple camera SPECT system using medium energy, general purpose collimators (MEGP).

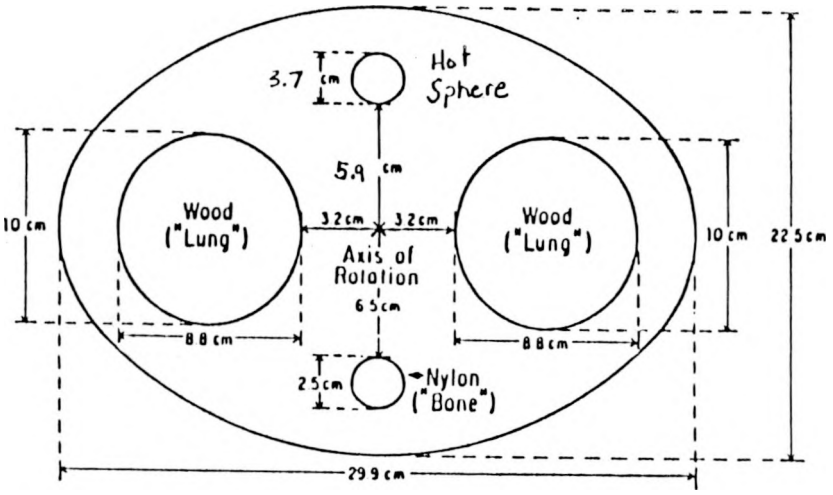

Figure 4. Diagram of experimental phantom containing region of nonuniform attenuation.

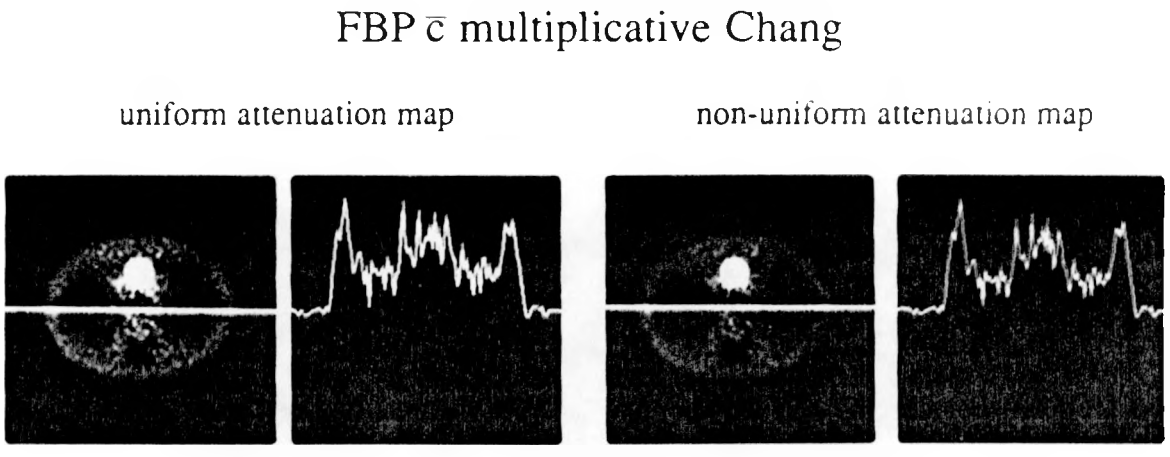

Figure 5. SPECT scan of nonuniform phantom reconstructed using the filtered backprojection (FBP) algorithm and multiplicative correction for attenuation.

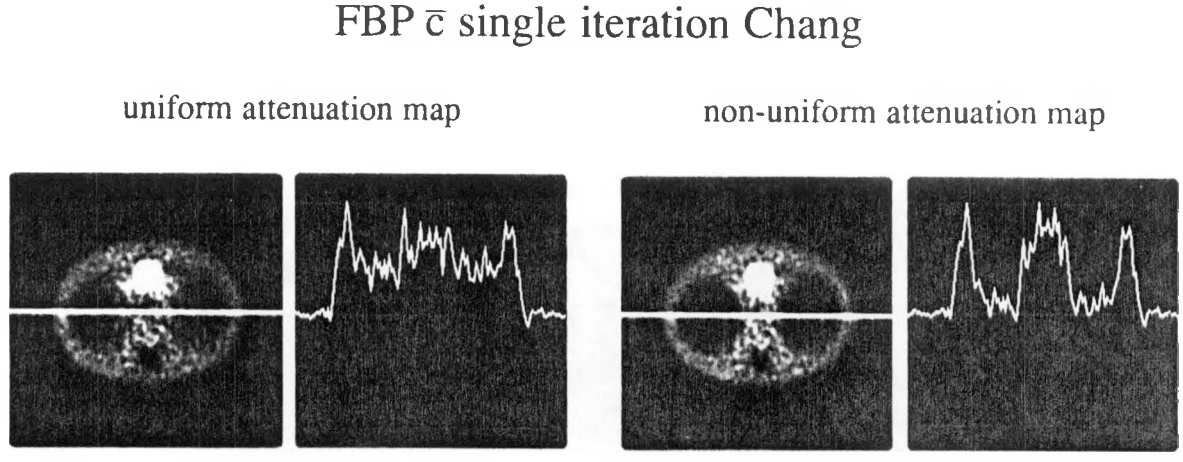

Figure 6. SPECT scans of phantom reconstructed using filtered backprojection (FBP) algorithm and single iteration Chang correction.

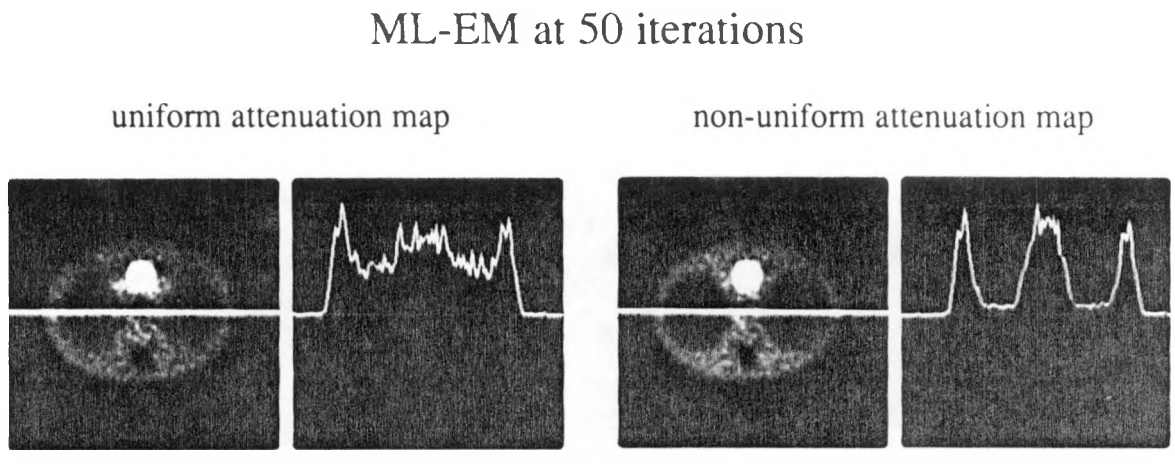

Figure 7. Maximum likelihood (ML) reconstructions using expectation-maximization (EM) method.

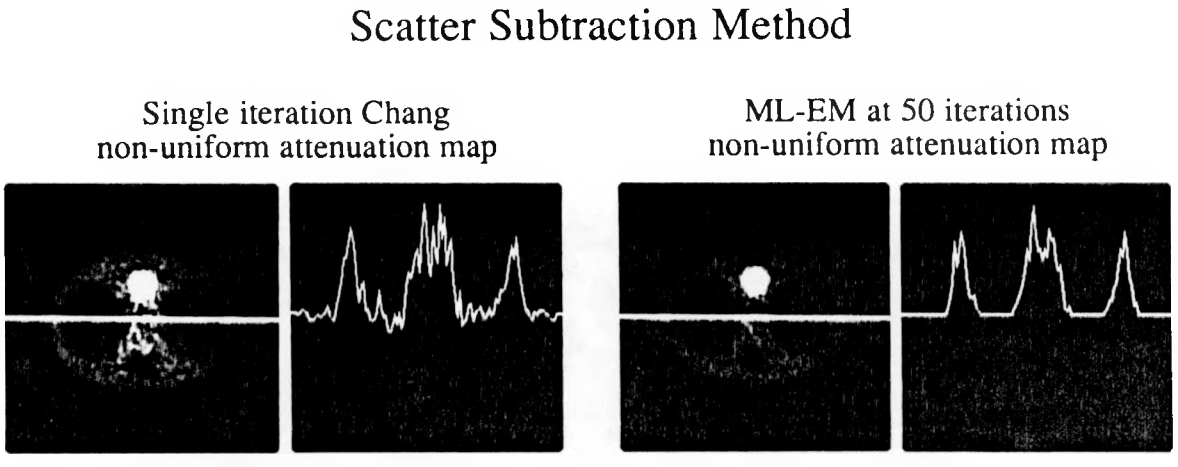

Figure 8. Effect of dual energy, scatter subtraction method on SPECT quantification.

D.2. Development and Evaluation of Quantitative Reconstruction Strategies

The objective of these experiments was to compare several reconstruction methods in terms of quantitative accuracy, image noise, and artifacts with Iodine-123 data. The methods were based on either the Chang algorithm (Chang, IEEE Trans. Nucl. Sci. NS-25:638-643, 1978) or the maximum likelihood-EM algorithm (Shepp and Vardi, IEEE Trans. Med. Imaging MI-1; 113-122, 1982). Within each algorithm, different approaches to attenuation and scatter compensation were studied. These approaches included the use of non-uniform vs. uniform attenuation map and scatter subtraction (Jaszczak, et al, J. Nucl. Med. 25:893-900, 1984) vs. deconvolution vs. broad beam attenuation map.

D.2.1 Methods

The phantom study was designed to model a hot lesion in background activity and non-uniform tissue density typical of the chest region. The phantom consisted of an elliptical plastic cylinder containing balsa wood "lungs" and a hollow sphere (Figure 4). The inside of both the cylinder and the hollow sphere were filled with I-123 solution. The relative concentrations of I-123 in the sphere and background were approximately five and one, respectively.

Projection images were acquired using the TRIAD SPECT system. The projections were 128x128 and were acquired at 120 angles over 360 degrees for a total scan time of 20 minutes.

A total of 11 reconstruction methods were examined. These included:

1. Filtered backprojection without compensation (FB)
2. FB with multiplicative Chang attenuation compensation, uniform, broad beam attenuation map (FB1U)
3. FB, mult. Chang, non-uniform, broad beam atten. map (FB1N)
4. FB, single iteration Chang, uniform, broad beam atten. map (FB2U)
5. FB, single iteration Chang, non-uniform, broad beam atten. map (FB2N)
6. FB, single iteration Chang, non-uniform, narrow beam atten. map, with scatter subtraction (FB2S)
7. FB, single iteration Chang, non-uniform, broad beam atten. map, with Metz deconvolution (FB2M)

8. Maximum likelihood-EM method, uniform, broad beam atten. map (MLU)
9. ML-EM method, non-uniform, broad beam atten. map (MLN)
10. ML-EM method, non-uniform, narrow beam atten. map, with Jaszczak's scatter subtraction (MLN)
11. ML-EM method, non-uniform, broad beam atten. map, and Metz deconvolution.

The attenuation maps were computer simulated based on the known object dimensions and attenuation coefficients. The broad beam attenuation coefficients were obtained empirically. The scatter subtraction was performed by subtracting the data acquired in a scatter energy window from that acquired in the photopeak energy window. The photopeak window was from 143 keV to 75 keV, and the scatter window was from 100 keV to 140 keV. All ML-EM reconstructions were stopped after 50 iterations to reduce noise.

D.2.2 Results

The results of the phantom experiments are summarized in Table 4. Columns 2 and 3 show the quantitative accuracy in the sphere and background regions, respectively, for the 11 methods tested. The true concentrations were determined from planar measurements of syringe samples of each solution. Column 4 shows the reconstructed activity in the lungs where there was actually no I-123 present. Finally, column 5 gives the ratio of the reconstructed activity in the three regions.

TABLE 4

Recon. Method	R[1]		μCi/ml in lungs[2]	sphere:bckgrd: lungs[3]
	Sphere	Bckgrd.		
FB	.28	.27	.17	5.4:1:0.49
FB1U	.67	.73	.39	4.8:1:0.41
FB1N	.72	.73	.50	5.1:1:0.52
FB2U	.69	.74	.33	4.8:1:0.35
FB2N	.76	.94	.12	4.2:1:0.1
FB2S	.94	.88	0	5.5:1:0
FB2M	1.02	.91	-.02	5.8:1:-.002
MLU	.69	.68	.52	5.2:1:0.58
MLN	.78	.91	.11	4.4:1:0.1
MLS	.98	.90	.03	5.7:1:0.03

[1] R = ratio of reconstructed μCi/ml to true μCi/ml.
[2] True μCi/ml in lungs = 0.
[3] True ratio = 5.2:1:0

These results suggest that the most accurate compensation technique within either the FB or ML-EM reconstruction method does scatter subtraction and uses the narrow beam attenuation coefficients (FB2S and MLS). These methods did best at removing activity from the lung regions and estimating the activity in the sphere and background. The Metz deconvolution technique also performed well although some over-compensation was observed in the lungs and the sphere. The methods which used the uniform attenuation map were worst at estimating the activity in the lungs.

Profiles were drawn through images reconstructed by the various methods to observe the presence of noise and other artifacts. Examples of these images are shown in Figures 5, 6, 7, and 8.

D.3. Biodistribution Studies

The major long-term goal of this research is to be able to use SPECT to quantify both normal tissue and tumor uptake of monoclonal antibodies labeled with In-111 or I-123. These data will form the basis for dosimetry calculations to estimate the feasibility of antibody-mediated radiotherapy in patients. A critical issue is how well the pharmacokinetics

of the diagnostic nuclide mimic those of the therapeutic nuclide when used as antibody labels. Since there is some disagreement about whether the tissue distribution of ^{111}In-labeled antibodies reflects that which occurs when the beta-emitter ^{90}Y is used as the label, initial studies were performed with C110 labeled with radiohalogens.

Our facilities for the athymic mouse studies are located in the new addition to the Cancer Center Isolation Facility (CCIF). Unfortunately, there have been some major problems with the addition to the CCIF. Thus, instead of performing our biodistribution measurements in athymic mice with subcutaneous LS174T human colon carcinoma xenografts, normal BALB/c mice were used. Since in the clinical setting, limiting radiation dose to normal tissue is the critical factor, this should not be a major problem.

An advantage of radioiodine nuclides is that ^{123}I can be used to predict the dosimetry of the beta-emitter ^{131}I without concern about differing stabilities and catabolic pathways for the diagnostic and therapeutic label. Moreover, because of the radiobiological advantages of alpha emitters such as the heavy-halogen ^{211}At, it would be of interest to determine whether imaging with ^{123}I could be used to estimate the dosimetry of ^{211}At-labeled antibodies. To investigate this possibility, paired-label comparisons of the pharmacokinetics of ^{211}At and ^{131}I coupled to C110 IgG antibody and its F(ab')$_2$ were performed. Details of this study are contained in an accompanying manuscript. The results are summarized as follows:

a) The anti-CEA monoclonal antibody C110 IgG and its F(ab')$_2$ fragment were labeled successfully using the ATE method. Immunoreactive fractions greater than 90% versus the colorectal carcinoma LS174T cell line could be obtained routinely for all labeled proteins.

b) For radioiodinated C110 IgG and F(ab')$_2$, thyroid uptake was only 0.2-0.6% of the injected dose at all time points, indicating that use of the ATE metod for labeling this MAb and fragment decreased dehalogenation <u>in vivo</u>.

c) Use of the ATE method for labeling C110 IgG with ^{211}At and ^{131}I resulted in similar biodistribution patterns for me two nuclides. Thus, it may be possible to use SPECT imaging of ^{123}I-labeled C110 IgG to predict the dosimetry of ^{211}At-labeled C110 IgG. However, in contrast,

loss of ^{211}At from the $F(ab')_2$ was considerably more rapid than ^{131}I. These data suggest that different labeling methods must be develped if SPECT imaging with 123I-labeled $F(ab')_2$ fragments can be used to predict the dosimetry of ^{211}At-labeled C110 $F(ab')_2$.

E. Research Plans for Coming Year

E.1. System and Collimator Characterization

As a result of the performance evaluation using parallel hole collimators, a design specification for a medium energy, fan-beam (MEFB) collimator has been completed. One of these collimators will be ordered from the vendor (Nuclear Fields, Inc.) next month. Following an evaluation of its performance capabilities, it is anticipated that two additional MEFB collimators will be ordered in the fall of this year.

E.2. Phantom Experiments

The investigation of alternative reconstruction strategies will be continued. Particular areas of investigation that are being emphasized include the problem of non-uniform attenuation, the use of fan beam collimation, and the value of iterative reconstruction.

Hardware and software modifications are being made to our Triad research SPECT system to allow the acquisition of transmission computed tomography (TCT) using a sheet source of radioactivity. The reconstructed TCT images will be used to estimate a non-uniform attenuation map of the emission source. This map will then be used to quantitatively improve the SPECT reconstruction, if necessary, for regions such as the thorax.

The TCT data may not be necessary for regions of the body where the attenuation is relatively uniform, such as the brain and abdomen.

Our preliminary phantom experiments indicate that iterative reconstruction techiques based on Maximum likelihood or Bayesian image processing approaches may provide an improvement compared with conventional filtered-backprojection methods (at least with regions having non-uniform attenuation). However, since it is computationally impractical to directly incorporate scatter compensation into the iterative approaches, we intend to use a modification of our dual energy subtraction method (Jaszczak, et al., J. Nucl. Med. 25:893-900, 1984) to provide a first-order

correction for scatter. With this approach the memory and computational requirements result in realistic reconstruction times.

These algorithms will be extended to fan beam geometry, and the new medium energy fan beam collimator will be used to acquire appropriate experimental scans of phantoms. A quantitative comparison of the fan beam and parallel hole collimators will be performed.

E.3. Monte Carlo Studies

A preliminary investigation of the potential usefulness of Monte Carlo modeling to improve dose estimates is in progress. Similarly, we are investigating the modifications that would be required of our existing Monte Carlo code to accurately model the acquisition of a larger class of source geometries. These preliminary studies will be used to guide our future research efforts in applying Monte Carlo modelng to improve the quantification of I-123 and In-111 SPECT scans, or to improve dose estimation.

E.4. Biodistribution Studies in Mice

These studies will be performed in order to a) demonstrate that antibody 110 radioiodinated via the ATE method is taken up preferentially in CEA-secreting LS174T xenografts and b) to compare the normal tissue uptake of In-111 labeled antibody to an I-125-labeled antibody which is relatively inert to dehalogenation in vivo. In the first set of experiments, antibody 110 will be labeled with I-125 using the ATE reagent and with I-131 using Iodogen. Thyroid uptake will be used as an indicator of loss of radioiodine label in vivo. Subsequent experiments will be performed with antibody 110 labeled with I-125 using ATE and with In-111 using the SCN-Bz-DTPA chelate. These studies will be performed both in tumor-bearing athymic mice and normal mice in order to differentiate between liver and spleen uptake resultant from the formation of labeled antibody 110-CEA immune complexes and accumulation due to other processes. Livers from selected animals will be homogenized and the form of the radiolabels will be analyzed by size-exclusion HPLC. These research efforts depend upon the availability of the Cancer Center Isolation Facility (CCIF).

E.5. Quantitative Studies in Nonhuman Primates

A selected set of studies using 2 nonhuman primates (Macaque fascicularis) will be performed to evaluate the effectiveness of our SPECT system and reconstruction algorithms to extract quantitative

measurements of in vivo radionuclide distributions. One primate will be studied with microspheres labeled with 2mC of I-123 or 2mCi In-111.

SPECT images will be obtained with our Triad Research SPECT system fitted with the collimators deemed appropriate from our phantom studies. SPECT images of the entire body will be obtained at 1 hour prior to sacrifice. The activity in the lungs, heart, liver, thyroid, spleen, kidneys, bladder, and bone marrow (iliac crest) will be quantitated and compared to the results of specimen counting.

At sacrifice, tissues of interest will be weighed and counted for I-123 or In-111 (for animals also received In111 in a gamma counter. The percent injected dose per organ and per gram of tissue will be calculated. The weight of the organ and the absolute amount of the activity in the organs at the time of sacrifice will be used to compare with the organ volumes and uptakes determined from the SPECT study.

E.6. Related Research

During the next year, the quantitative methodology being developed under this DOE grant will be used to extract quantitative data for several monoclonal antibody studies in patients. These studies have been approved under a separate protocol and are funded by separate resources. Hence, these patient studies are not part of this DOE grant, although our research results will be of value to those investigations.

F. Publications and Presentations for Project Period

F.1. Publications Supported by This Grant

1. Jaszczak RJ: SPECT: State-of-the-art scanners and reconstruction strategies. In Radiopharmaceuticals and Brain Pathology Studied with PET and SPECT. Eds. R.C. Reba and M. Dirksic (CRC Press, New York, 1990) in press.

2. Garg PK, Harrison CL, Zalutsky MR: Comparative tissue distribution of the alpha emitter ^{211}At and ^{131}I as labels of a monoclonal antibody and $F(ab^1)_2$ fragment. Cancer Research, 1990, in press.

3. Liang F, Jaszczak R, Coleman R, Johnson V: Simultaneous reconstruction segmentation and edge enhancement of relatively piecewise continuous images with intensity level information. Med Phys, 1990, submitted.

4. Gilland DR, Jaszczak RJ, Greer KL, Zalutsky MR, Coleman RE: SPECT Quantitation with Iodine-123. Abstract to be presented at the 37th Annual Meeting of the Society of Nuclear Medicine, Washington, June, 1990.

F.2. Related Publications

1. Mendez GE, Jaszczak RJ, Greer KL, Gilland DR, Coleman RE: ROC evaluation of cone beam and parallel beam collimators. Abstract to be presented at the 37th Annual Meeting of the Society of Nuclear Medicine, Washington, June, 1990.

2. Smith MF, Jaszczak RJ, Floyd CE, Greer KL, Coleman RE: Evaluation of SPECT images using interactive three-dimensional display. Abstract to be presented at the 37th Annual Meeting of the Society of Nuclear Medicine, Washington, June, 1990.

3. Jaszczak RJ: The history of SPECT and new advances in SPECT instrumentation. Invited presentation at the 37th Annual Meeting of the Society of Nuclear Medicine, Washington, June, 1990.