A METHOD FOR THE ASSESSMENT OF SPECIFIC ENERGY
DISTRIBUTION IN A MODEL TUMOR SYSTEM

by

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Michael A. Noska. A Method for the Assessment of Specific Energy Distribution in a Model Tumor System.

ABSTRACT

Due to the short range of alpha particles in tissue, the calculation of dose from internally deposited alpha emitters requires a detailed analysis of the microscopic distribution of the radionuclide in order to determine the spatial distribution of energy emission events and, from this, the spatial distribution of dose. In the present study, we used quantitative autoradiography (QAR) to assess the microdistribution of a radiolabeled monoclonal antibody (MAb) fragment in human glioma xenografts in mice. Mice were injected with ^{125}I -labeled Mel-14 F(ab')₂ MAb and killed at various timepoints after treatment with hyperthermia (HT). HT was used to alter the distribution of labeled MAb and thereby attempt to improve its uptake in tumors in order to increase the therapeutic index (TI). Tumors were excised, sectioned and exposed to x-ray film. The images obtained then were digitized and analyzed for activity distribution (i.e., emission density) in three dimensions. This information was used to quantify the absolute uptake of MAb in tumor and to compare the effect of HT on distribution relative to non-heated controls. Digitized autoradiographs were compared to sections stained by hematoxylin and eosin (H&E) to assess the activity associated with tumor cells alone. An attempt was made to determine specific energy distributions, however, the resolution of the system was not sufficient to obtain true microdosimetric quantities. A dose was calculated for a sample tumor using biodistribution data from the ^{125}I -labeled MAb along with the physical data for ^{211}At , a cyclotron-produced alpha emitter with 5.9-7.5 MeV particles and 7.2 hr. half-life. This radionuclide is of interest for the treatment of tumors using labeled MAbs and other carriers.

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LIST OF ABBREVIATIONS

MAb	monoclonal antibody
QAR	quantitative autoradiography
Mel-14 F(ab')₂	specific antibody fragment
RPC-5 F(ab')₂	non-specific antibody fragment
ROI	region of interest
i.p.	intraperitoneal route of injection
i.v.	intravenous route of injection
TCA	trichloroacetic acid
H & E	hematoxylin and eosin
HT	hyperthermia
OD	optical density
TV	transmission value

CHAPTER I

INTRODUCTION

Dose assessment following an uptake of radioactive material is a complex process which requires the dosimetrist to enumerate the total disintegrations that have occurred or will occur in organs of interest or the body as a whole. This information is needed to calculate the density of energy deposited in tissue - the radiation absorbed dose. Only after arriving at an estimate of the dose can the investigator make a judgement concerning the risk and/or benefit from the uptake, whether it be accidental from an occupational or environmental source or intentional as from a diagnostic or therapeutic procedure in Nuclear Medicine. In order to calculate a dose, one must specify both the physical characteristics of the radionuclide and its biological distribution in the body. The physical properties of radionuclides are well-established and numerous decay tables and references are available. However, due to inherent intersubject variations in physiology and biochemistry, the challenge of internal dosimetry is to describe the spatial distribution of activity in tissues. As interest increases in the dose from short range emitters of radiation such as alpha emitters, it is necessary to develop new methods for measuring the pattern of uptake at the microscopic level.

This chapter provides an overview of internal dosimetry techniques and introduces the specific model which was used in this work to study a method for assessing the microdistribution of radioactivity as a first step in calculating the dose or specific energy

distribution from an internally deposited radionuclide.

Internal Dosimetry Models

Formal techniques for calculating dose from internal radionuclides first arose from medical uses of radioactive materials in the 1940's.¹ Although radionuclides were used previously in medicine, calculation of dose was not routinely performed. The field of internal dosimetry has continually expanded over the last 50 years as a result of the increasing use of radioactive materials in medicine, industry and research. Much of the growth in dosimetry has been driven by technological advances in computers and instrumentation for measuring radioactivity.

With the formation of the International Commission on Radiological Protection (ICRP) and the National Council on Radiation Protection and Measurements (NCRP), the work of investigators became formalized and standardized. Although these two groups only made recommendations on radiation matters, the works of their various committees have become the standards for radiation protection and measurements. Further, in many instances, their conclusions have been integrated into the regulations governing the use of radioactive materials. Numerous reports from both groups have established the techniques for radiation dosimetry. One of the most influential works was that of ICRP Committee II which published ICRP Report Number 2 (1) outlining that group's recommendations for standards on internal dose including methods for calculating dose based on the concepts of Maximum Permissible Organ Burden (MPOB) and Maximum Permissible Body Burden (MPBB). The

¹For an excellent review of the history of internal dosimetry, see: A.T. S.-Stelson *et al.* Health Physics, 69: 766-782, 1995.

recommendations of this committee eventually became the basis for 10 CFR Part 20. This work also presented the concept of Standard Man which provided the dosimetrist with a set of standard physiological parameters that could be used in the determination of doses.

Due to the ever increasing use of radionuclides in medicine, the Society of Nuclear Medicine formed the Medical Internal Radiation Dose (MIRD) Committee in 1965 to improve methods for calculating absorbed dose from internal emitters. Their work was enhanced by the development of computers and the use of phantoms which represented various organs and the whole body. Using sophisticated Monte Carlo codes, the dosimetrist could "place" radioactivity into an organ (the source organ) and the code would statistically follow the history of thousands of emissions based upon the stochastic nature of radiation to determine the total deposition of energy (dose) in any other organ in the body (the target organ). The first report of the MIRD Committee authored by Loevinger and Berman in 1968 (2) established the basic formulae and techniques which are used in internal dosimetry today. The work of this Committee has continued to expand the field through MIRD Pamphlets and Dose Estimate Reports. The basic formula for mean absorbed dose in a target organ r_k from a source organ r_h is given by (3):

$$\bar{D}(r_k - r_h) = \tilde{A}_h \sum \Delta_i \phi_i(r_k - r_h)/m_k,$$

where $\tilde{A}_h$ is the cumulative activity or total number of disintegrations in the source organ, Δ_i is the mean energy emitted per nuclear transition of type i , ϕ_i is the fraction of energy absorbed in the target from radiation in the source and m_k is the mass of the target. The absorbed fraction and mass terms can be combined to create the specific absorbed fraction,

Φ_i , which simplifies the formula to:

$$\bar{D} = \tilde{A} \sum \Delta \Phi.$$

Further, the terms for specific absorbed fraction and mean energy per transition can be combined to yield the mean absorbed dose per unit cumulative activity or what is more commonly known as the S factor. The equation for mean dose then becomes:

$$\bar{D} = \tilde{A} S.$$

Tables of S factors for a number of radionuclides were published in 1975 based on the work of Snyder *et al* (4). For calculating doses from point sources of electrons or beta particles, Berger (5) developed the concept of the point kernel which provides the distribution of absorbed dose as a function of distance from the source. His work has been expanded by Kwok *et al* (6) and Prestwich *et al* (7) for the use of beta emitters in radioimmunotherapy. Cumulative activities can be determined either directly by *in vivo* measurements of radioactivity in patients or animals or by the use of multicompartiment mathematical models for uptake, retention and excretion which describe the distribution of activity using multiexponential functions.

The methods of the MIRD Committee have been incorporated into more recent recommendations of the ICRP and the NCRP which in turn have led to revisions in 10 CFR Part 20 regulations governing the activities of radiation workers. ICRP 26 and 60 modified the concepts of cumulative activity and the S factor and introduced organ weighting factors to derive formulae for the dose equivalent, effective dose equivalent and committed dose (8,

9). Other publications which provide data and methods for calculating doses in the occupational and medical settings include ICRP 30 (10), ICRP 53 (11) and International Commission on Radiological Units and Measurements (ICRU) Report 32 (12). The data for the model of Standard Man have been updated and renamed Reference Man. This revision was published in 1975 as ICRP 23 (13).

For work involving the use of short range emitters such as alpha particles and Auger electron emitters, some investigators have concluded that the traditional concepts of mean absorbed dose are not sufficient to determine the spatial distribution of energy deposition events in tissue (14-26). The dose from short range, high LET radiations is highly dependent on the distribution of radioactivity in tissue. When radioactivity is homogeneously distributed in tissue, the deposition of energy may be approximated by the mean absorbed dose. However, when a high LET radionuclide is distributed in a heterogeneous fashion, local (microscopic) doses to cells within the range of the emissions can be significantly higher than would be predicted by traditional dose averaging techniques, while doses outside of this range would be zero. In this scenario, microdosimetric methods must be employed to calculate doses at the cellular and subcellular levels. Rossi *et al* first developed the theory and methodology of microdosimetry in the 1950's based on stochastic properties of radiation at the microscopic level. The ICRU in Report 36 (27) has summarized the principles and techniques of microdosimetry.

Monoclonal Antibodies

The Nuclear Medicine community has studied a variety of agents for the diagnosis and treatment of cancer. One of the most promising technologies of the last 20 years has been the

use of radiolabeled monoclonal antibodies for radioimmunoscinigraphy and radioimmunotherapy (RIT). The concept of using antibodies as carriers of radioactivity dates back to the 1950's (28), however, early attempts with polyclonal antibodies were hampered by a lack of specificity. The first use of a labeled antibody for treatment of a patient with glioma was reported in 1965 by Day *et al* (29). This effort was greatly enhanced by the groundbreaking work of Kohler and Milstein who first reported a technique for creating an immortal cell line capable of producing a homogeneous supply of antibodies that were highly specific for the antigens to which they were raised (30). Since then, many attempts have been made to treat and diagnose tumors both in humans (31-42) and in animal studies (43-56) with varying success.

Much of the success in animal models has not been translated into humans due in part to the relatively low uptake of labeled MAbs and the inability to achieve a homogeneous distribution of radioactivity throughout the volume of the tumor. In patients, the uptake of labeled MAb has been exceedingly low, on the order of 0.001-0.05 % of the injected dose per gram (57,58). The issue of distribution is particularly relevant to the use of short range emitters for therapy. As with any radiolabeled compound, the biodistribution of radiolabeled monoclonal antibodies depends upon a number of factors including the chemistry and physical characteristics of the labeled protein and the physiology of the host. MAbs also present unique challenges due to the nature of the antibody/antigen interaction.

The delivery of MAb to a tumor can be impeded by binding of the labeled MAb to antigen which has been shed from the tumor and is freely circulating (47). Methods to overcome this problem include the use of two antibodies or a split dose of the same antibody:

one to bind with circulating antigen, the second for specific tumor binding (59). Other groups have investigated the use of plasmapheresis as a means of removing unbound antibody (60). Since current technology is limited to hybridomas of murine origin, there is a potential for patients to develop an immune response to the MAb known as Human Anti-Mouse Antibody or HAMA (32,61). This can result in rapid clearance from the blood and uptake by normal tissues such as the liver and spleen. The development of chimeric (humanized) MAbs may help to overcome this problem (36). Binding to normal tissues also reduces the availability of labeled MAb for tumor binding (41). This necessitates the development of new antibodies or pre-dosing with "cold" antibody to occupy sites of non-specific binding. The use of bifunctional MAbs also has been studied to improve the specificity of tumor binding (62).

The rate of antibody uptake is an important factor in the use of these molecules for diagnosis and therapy. The rate of uptake must be compatible with the half-life of the radionuclide in order for the method to succeed. For example, if it were to take a period of several days for sufficient labeled MAb to be delivered to a tumor, then the use of a radionuclide with a physical half-life of several hours would render the treatment ineffectual since much of the radioactivity would decay by the time the MAb reached the tumor, reducing the radiation dose to the tumor. Thus, the physical half-life of the radionuclide must match the biological half-life of the carrier. The size of the antibody molecule plays a large role in the kinetics of distribution. Work in our laboratory (63) and others (50) has shown that MAb fragments exhibit more rapid blood clearance and uptake by tumors. However, the total uptake is somewhat lower for $F(ab')_2$ fragments than for intact IgG.

The radionuclide and labeling method also can have a profound effect on the

distribution of labeled MAbs (64-67). The most commonly used radionuclides for MAb imaging and therapy are ^{131}I , ^{111}In and ^{90}Y . Radioiodinated antibodies have been shown to suffer from catabolism *in vivo* by dehalogenases when labeled by traditional methods (40,48). This is thought to be due to the structural similarity between the labeled protein and naturally occurring thyroid hormones. New labeling methods have been studied which would theoretically reduce this structural similarity (68) and our group has reported a method which successfully reduces loss of label *in vivo* while maintaining immunoreactivity (48). MAbs labeled with ^{111}In and ^{90}Y (both transition metals) have a tendency to accumulate in the liver and spleen. In addition, ^{90}Y is known to be taken up in bone which can result in dose limiting toxicity to the bone marrow due to its very energetic beta particle (69).

The use of gamma and beta emitters in therapy has limitations due to cross irradiation of normal tissues. In fact, one of the necessary ingredients for successful imaging with MAbs is contraindicated for use in therapy. In nuclear medicine imaging, the radionuclide must possess emissions of sufficient energy (and range) to be detected outside of the body. However, if this same nuclide is used in therapy, the relatively long range of its emissions can result in irradiation of normal tissue which surrounds the tumor. Iodine-131 was one of the first and most widely used radionuclides for labeling MAbs because of its seemingly favorable emissions. The 365 keV gamma ray is suitable for imaging while its 606 keV beta particle can be exploited for therapy. However, studies have shown that significant doses can be delivered to normal tissues with therapeutic levels of radioiodinated MAb (69).

This has led to a great deal of interest in the use of short range, high LET alpha emitters for the treatment of tumors. In theory, the specific nature of the antibody/antigen

complex combined with the high LET and short range of alpha particles in tissue could result in a significant dose to tumor cells while sparing normal tissues. Two nuclides which have engendered the greatest interest recently are ^{212}Bi and ^{211}At . Bismuth-212 has a half-life of 60.6 minutes and alpha emissions ranging from 5.6-8.8 MeV. This nuclide has shown promise in the treatment of leukemia and lymphoma (43,46,70). However, due to its extremely short half-life, it probably would not be suitable for the treatment of solid tumors where uptake kinetics are much slower. Our group has studied the efficacy of MAbs labeled with ^{211}At (44,53,71,72). Astatine-211 has a half-life of 7.2 hours. Alpha particles of 5.9 and 7.5 MeV are associated with 100% of its decays (Figure 1.1). The range of these particles in tissue is 55-80 μm .

SIMPLIFIED ASTATINE-211 DECAY SCHEME

Figure 1.1. Decay scheme of Astatine-211 (From ref. 71)

Astatine-211 is produced on a cyclotron by the reaction:

by bombardment with 28 MeV alpha particles. It has been studied for almost 20 years in various forms for radiotherapy (45,51,52,73).

Because of its relatively short half-life, ^{211}At like ^{212}Bi might be better suited for the treatment of diffuse disease as opposed to solid tumors. Howell *et al* (74) make the case for the use of long-lived radionuclides for radioimmunotherapy based on the linear-quadratic model of risk. However, a recent study in our laboratory has suggested that shorter-lived radionuclides might confer a therapeutic advantage over longer-lived isotopes (75).

Assuming the labeled MAb can be delivered to the tumor in a timely fashion, the problem remains to achieve a homogeneous distribution of activity within the tumor mass. In order to treat tumors with alpha emitters, a sufficient quantity of the drug must be localized close to tumor cells. However, studies have shown the tendency for radiolabeled MAbs to accumulate only around the periphery of the tumor without penetration to the core of the mass (76-78). Thus only a fraction of tumor cells would fall within the range of the alpha particle. Some of the reasons proposed for this heterogeneity include the presence of a necrotic core, inconsistent or lacking tumor vasculature, heterogeneity of antigen expression, convective forces within the tumor, increased interstitial fluid pressure and even a barrier due to the natural binding ability of the antibody.

Tumor size has been investigated as a barrier to MAb distribution due to the formation of central necrosis (79). Not only is there a lack of vessels in the necrotic region but also a

concurrent population of hypoxic cells which are more resistant to radiation (80). Here, alpha particles might have an advantage since their toxicity is less dependent on the presence of oxygen (81). In addition, tumor size could be related to the number of radioresistant cells present based on theories of genetic mutation (82). Howell *et al* have calculated the dose required for therapy taking into account the rapid growth rate of some tumors (83). Several authors have identified the disorganized structure of tumor vessels as a factor in perfusion (76,77,84,85), noting that vessels often do not penetrate to the tumor core. Some tumor vessels were found to be more "leaky" to large molecules at the periphery of tumors (84). Jain (76) and Cobb (77) report that due to convective forces and a lack of lymphatics to clear extracellular fluids, the interstitial pressure near the center of the tumor is much higher than at the periphery, retarding the flow of activity. This tendency increases as the tumor grows. Finally, Fujimori *et al* and van Osdol *et al* (86,87) have provided evidence that increased binding of antibody to antigen and a high binding affinity can actually reduce the percolation of MAbs into tumors resulting in a heterogeneous distribution of activity.

One potential method for overcoming the problem of poor perfusion is the use of hyperthermia. Studies have shown that the local application of heat can temporarily increase the blood flow in tumors and reduce interstitial fluid pressure (78,88-92). Furthermore, the environment of the tumor cells including hypoxia, low pH and nutritional deficiency accentuate the effect of heat (90,93). In fact, heat alone is known to be cytotoxic (85). When hyperthermia is used as the primary treatment, it leads to vascular collapse, which helps to destroy the tumor and at the same time, make it more susceptible to the further effects of heating (92). However, heating for prolonged periods of time, multiple heatings and the use

of temperatures above 42°C can lead to total vascular collapse and lack of flow (92,94). Thus, hyperthermia can be a very effective treatment on its own but must be used judiciously as an adjuvant to radiotherapy. Previous studies in our laboratory have shown that hyperthermia can increase the uptake of labeled MAbs by tumors(75,95). We have continued the use of hyperthermia in this study to evaluate the changes in distribution of activity at the microscopic level.

Quantitative Autoradiography

As discussed earlier, the challenge of internal dosimetry is to describe the biodistribution of activity in order to quantify the spatial distribution of energy emission events. For gamma emitters and high energy beta emitters, it is sufficient to measure the gross activity in an organ or tissue because of the long range of their emissions relative to the dimensions of the tissue. Most dosimetry for Nuclear Medicine has used gamma camera imaging or gross counting of tissues to quantify the source activities used in MIRD-type calculations of dose. Planar, SPECT, PET and CT imaging all have been used to measure organ activities (96-100). However, none have the resolution necessary to specify the cellular distribution of activity which is needed to estimate the dose to critical cells from short range emitters. Therefore, techniques are needed to evaluate the microscopic distribution of activity from radioimmunotherapy using alpha emitters.

Autoradiography is one such technique. In the method of autoradiography, tissue specimens containing radioactivity are exposed to a photographic emulsion. The interaction of radiation with silver halide grains in the emulsion causes the silver atoms to be reduced and when the intensity of the radiation exceeds a threshold and a sufficient number of silver atoms

are reduced, the cluster becomes a developable grain. This represents the formation of a latent image in the emulsion which then can be developed to create a visible pattern of silver. The degree of darkening in the film is proportional to the amount of radiation deposited in the emulsion and is thus a measure of the radioactivity present in the tissue sample. When the photographic medium is a piece of x-ray film, the technique is known as macroautoradiography and the information obtained is principally a gross distribution of radioactivity in the tissue. Alternatively, the tissue can be mounted on microscope slides, dipped in emulsion and studied by light or electron microscopy. In this way, individual grains can be identified and counted, providing quantification of radioactivity and information regarding the spatial distribution of the radionuclide relative to cellular structures. Autoradiography has existed since the days of Becquerel but did not come into routine use until the 1940's. The field was revolutionized by the work of Kety *et al*, who developed the early principles of quantitative autoradiography in the 1950's (101). The technique was expanded by Sokoloff and others with the advent of digital methods (102). Autoradiography has been widely used in the field of Nuclear Medicine, both qualitatively and quantitatively, for assessing the activity distribution in tissue (16,52,103-115).

Purpose

Previous studies in our laboratory have demonstrated the effectiveness of local hyperthermia in enhancing the uptake of MAb F(ab')₂ fragments in human glioma xenografts in mice. The purpose of this work was to evaluate the effect of hyperthermia on the distribution of a radiolabeled MAb using quantitative autoradiography. The goal was to assess the efficacy of hyperthermia in improving the distribution of activity, leading to a higher

therapeutic index for radioimmunotherapy using alpha emitters. Further, we sought to demonstrate that QAR is a useful technique for specifying the distribution of activity as a source term for dosimetry calculations, particularly in microdosimetry. Many studies using autoradiographic techniques with labeled MAbs have reported qualitative or semi-quantitative results but have stopped short of calculating doses (45,51,52,113). Other groups have attempted to model the dose to tumors at the cellular level using a combination of computer modeling and data obtained from biological systems (116). Still other models are based solely on mathematical formulations and usually involve the use of Monte Carlo codes (6,117). Our goal was to determine specific energy distributions directly from biological data.

CHAPTER II

ASSESSMENT OF ANTIBODY DISTRIBUTION BY QUANTITATIVE AUTORADIOGRAPHY

Background

In our laboratory, we have studied a method for enhancing the uptake and penetration of radiolabeled antibodies into xenografts of human gliomas in nude mice. This method involves the use of local hyperthermia by means of a water bath at temperatures above physiologic. In previous reports of our work, we showed that by raising the temperature of the tumor with a water bath at 42°C, we could increase the uptake of antibody into tumor by a factor of 2 to 3 (75,95). Earlier studies suggested that the mechanism for increased uptake was a non-specific increase in blood flow, blood volume and/or permeability (95). However, a later study indicated that increased retention of MAb was due to enhanced specific binding at elevated temperatures (75). Wong *et al* (118) have demonstrated a 50% increase in CEA expression at similar temperatures in LS174T tumors, suggesting that HT can in fact lead to increased binding of specific MAbs.

In addition, we have hypothesized that, corollary to the increased uptake was an enhanced homogeneity of distribution throughout the tumor. The results of our previous localization studies led us to perform a set of experiments employing quantitative autoradiography in order to validate this hypothesis. This chapter describes the experimental methods used in those experiments.

Materials and Methods

Human tumor xenograft model. The human glioma line D-54 MG, a subline of A-172 established by Giard et al (119) and characterized in our laboratory at Duke University (120), was used in these experiments. Eight-week-old male athymic mice (Balb/c, *nu/nu*) weighing 22-30 g were obtained from a colony maintained at the Cancer Center Isolation Facility of Duke University Medical Center. Tumors were established subcutaneously in the right hind leg of these mice by injecting 50 μ l of D-54 MG tumor homogenate from tumors grown in this strain and prepared as previously described (63). Tumor volumes were determined by the formula:

$$Volume(mm^3) = \frac{l \times s^2}{2}$$

where *l* is the length of the long axis of the tumor and *s* is the dimension of the short axis. When tumor volumes reached approximately 150-200 mm³, the animals were randomized by tumor volume into treatment groups.

Monoclonal antibodies. MAb Mel-14, a murine IgG 2a, is reactive with the tumor-associated chondroitin sulfate proteoglycan present in melanomas, gliomas and medulloblastomas (121). RPC-5 is an isotype matched control MAb which does not bind to any known antigen. Both MAbs were purified from athymic mouse ascites by Protein A-Sepharose affinity chromatography. F(ab')₂ fragments were prepared by digestion with pepsin and purity was assessed by high pressure liquid chromatography, as previously described (63).

Radioiodination. The stationary phase chloramide Iodogen (Pierce Chemical Co., Rockford, IL) was used to label both fragments in a variation of the original method of Fracker and Speck (122). Briefly, 100-350 μg of the desired fragment were reacted for 10 minutes at room temperature with 400-600 μCi (14.8-22.2 MBq) of Na^{125}I (Dupont-NEN, Boston, MA) in a glass vial coated with 10 μg of Iodogen. The radiolabeled fragments were separated from free iodide by passage through a Sephadex G-25 column. The specific activities were between 0.92 and 1.7 $\mu\text{Ci}/\mu\text{g}$ (34 -64 kBq/ μg). Radiochemical purity (i.e.- protein-associated activity), as determined by trichloroacetic acid (TCA) precipitability, ranged from 89% for the non-specific protein to 99% for the specific fragment.

Immunoreactivity assay. The binding characteristics of radiolabeled Mel-14 F(ab')₂ were determined *in vitro* using the D-54 MG homogenate immunoreactivity assay. Approximately 50 ng of radiolabeled Mel-14 F(ab')₂ were incubated in triplicate with 300 mg of D-54 MG homogenate or normal rat liver homogenate in 1 ml of phosphate buffered saline (PBS) containing 1% human serum albumin for 2 hours at 37°C. After washing the homogenates three times, the pooled supernatants and pellets were counted. Specific binding was calculated by subtracting the non-specific binding to liver from the total binding of Mel-14 F(ab')₂ to tumor homogenate.

Animal Studies/Hyperthermia. Animals were injected via a tail vein with either 5 $\mu\text{Ci}/2.9 \mu\text{g}$ (63.8 kBq/ μg) ^{125}I -labeled Mel-14 F(ab')₂ or 4 $\mu\text{Ci}/4.4 \mu\text{g}$ (33.6 kBq/ μg) ^{125}I -labeled RPC-5 F(ab')₂ in 100 μl PBS. The mice were restrained in 50 ml conical tubes which had several holes drilled in the sides for air circulation and a 1.5 cm hole cut in the bottom of the tube. The mice were placed into the tube with the tail and tumor-bearing leg positioned

through the hole in the bottom of the tube. The tails then were taped to the outside of the tubes to maintain the mice in position. A weight of approximately 4 g was attached to the tumor-bearing leg of each mouse using a loose-fitting pipe cleaner looped around the ankle. The tubes were placed upright in a Lucite support to allow immersion of the tumor-bearing leg into a circulating water bath. Mice were unanesthetized during heating to avoid potential complications from general anesthesia (95).

Tumors were heated either at 37°C or 42°C for 4 hours. These temperatures were selected based on previous optimization studies in our laboratory (75,95). Each mouse was injected i.p. with 2 ml lactated Ringer's solution before heating to prevent dehydration. Previous studies have demonstrated our ability to maintain core body temperature within a physiologic range (75,95). Therefore, core temperatures were not monitored in the current study. We have shown previously that tumor temperatures closely approximate water bath temperature and that equilibrium is achieved within 2-3 minutes after immersion (95). Thus, tumor temperatures were not monitored in these experiments to avoid histological artifacts and disruption of tumor parenchyma which could result in changes in tumor blood flow and antibody localization.

Groups of mice were killed by halothane overdose either immediately or at 4, 8 or 20 hours after heating. Their tumors were excised immediately and placed in liquid nitrogen for sectioning and autoradiography.

Histology and Autoradiography. The method of preparing tumors and standards for autoradiography was based largely on the work of Blasberg *et al* (115). Tumors were mounted on planchettes with M-1 embedding matrix (Lipshaw Corp., Detroit, MI) and frozen

in freon. Sections were cut 20 μm thick on a cryomicrotome at -20°C . Two sections were cut at each level, placed on 1 X 3 inch glass microscope slides and dried on a slide warmer at 65°C . Sections were cut at 200-600 μm intervals until the approximate midpoint of the tumor was reached. The slides were placed in cassettes along with the radioactive standards used for quantification. Slides from each group of animals were combined on one film so that 16 separate films were used to analyze the tumors in this experiment.

Standards were prepared by adding dilutions of a stock Na^{125}I solution to a 200 mg/ml solution of gelatin in water followed by vortexing and centrifugation to remove air bubbles. These solutions then were counted for radioactivity on a gamma counter. Approximately one milliliter of each standard was drawn into a 1 cc tuberculin syringe which was dipped into freon. The end was cut off of each syringe and a portion of the frozen solution was extruded onto a planchette, mounted with embedding medium, then frozen for sectioning. Standards also were cut at a thickness of 20 μm .

Single coated x-ray film (SB-5; Kodak, Rochester, NY) was used to obtain the autoradiographic images. The exposure time was approximately two weeks. After exposure to the film, the slides were removed from the cassettes and stained with hematoxylin and eosin (H&E) so that the histology of the tumors could be compared to the autoradiographic images to quantify the radioactivity that was actually associated with tumor cells.

Quantitative analysis of autoradiographic images. Digitized images of the x-ray films were created using a PC-based microdensitometer. The images were stored in a 480 X 512 matrix of pixels with 8 bits per pixel representing 256 gray levels. At the magnification that was used in these studies, the pixel size was approximately 90 X 90 μm . Due to the size

of the image files, data were stored on an optical disk (Information Storage, Inc., Colorado Springs, CO). In order to quantify the activity concentration in tissue sections, it first was necessary to create a standard curve which related measured optical density to concentration. The standards, which were prepared and mounted on slides as discussed above (Figure 2.1), were exposed to film along with tissue samples for two weeks. The images obtained then were digitized and using the In Situ/Grain image analysis program developed by Loats Associates, Inc. (Westminster, MD), a standard curve was created by measuring the optical densities of the digitized standards and relating these values to the known activity concentrations in the standards by a least squares fit of the data (Figure 2.2). The tumor sections then were analyzed by generating ROIs on the video display and measuring the optical densities in each section. A useful feature of this system is the ability to determine the number of pixels having a specific range of activity concentrations. This information was used to generate distributions of activity concentrations within tumors which then could be used to calculate the dose to tumors. Due to the time-intensive nature of this analysis, one slice was chosen from each tumor by visual inspection to be representative of the activity distribution within the whole tumor. In order to more accurately assess the dose to tumor cells alone, we used the histologic information from the H & E stains to subtract out regions of non-tumor activity.

A more detailed description of these analytical methods is provided in the appendices to this paper.

Figure 2.1

Diagram of QAR standards mounted on microscope slides. Each circle represents a 20μ thick cross-section of a frozen standard solution. These standards were exposed to film and digitized along with tissue sections to create a standard curve.

Figure 2.2

Typical standard curve (Film 3: M37-12) relating optical density to activity concentration;
 $A.C. (nCi/g) = -109.99 + 1315.53(OD)$

CHAPTER III

RESULTS AND DISCUSSION

A word is in order regarding the syntax used in the data. Each group of mice is defined by the antibody fragment with which they were injected, the temperature to which they were exposed and the length of time for which they were studied. The options then were: Mel-14 F(ab')₂ versus RPC-5 F(ab')₂; 37°C versus 42°C and; 4, 8, 12 or 24 hours post injection. In addition, the serial slices of tumor were assigned numbers indicative of their depth in the tumor mass, 1 being the first section at the periphery of the tumor with numbers increasing with depth in the mass. Therefore, a code was developed which is carried throughout the tables and figures of this paper as well as the discussion. The code is illustrated in the following example:

M37-4-2-90

where *M* represents Mel-14 F(ab')₂, 37 is the temperature at which the tumor was heated, 4 is the time post-injection at which the animal was killed, 2 is the number of the animal within the group and 90 is the number of the tumor section (i.e. - 1.8 mm from the surface of the tumor). Alternatively, the slice number might be dropped to represent a particular animal (M37-4-2) or the animal number and slice number could be dropped to represent a group of animals (M37-4).

Representative data are used in this section to describe the results of this study. A complete presentation of the data is provided in the appendices of this paper.

Results and Discussion

Antibody labeling and immunoreactivity. The labeling yield for Mel-14 F(ab')₂ was 90 % in this experiment based on the activity contained in the protein fractions from the purification column relative to the starting activity. The radiochemical purity as assessed by TCA precipitation was greater than 98 %. The labeling efficiency for RPC-5 F(ab')₂ based on the protein fractions from the Sephadex column purification was 56 %. However, the TCA precipitable activity in this preparation was only 89 %. These values are comparable to other studies in our laboratory using this labeling method (55,56,75,95).

The specific binding of Mel-14 F(ab')₂ determined by the D-54 MG homogenate assay was 57 % (mean of triplicates). This is somewhat higher than in previous studies with this MAb (55,56,95).

Quantitative analysis. Table 3.1 shows the activity concentrations of the fifteen standards that were used for QAR. The activities are decay corrected to the time at which the samples were first exposed to film. All fifteen standards were used on each film, however, Standards 1 and 2 did not contain sufficient activity to be detected on any of the sixteen films. Therefore, they were never used in the determination of any of the standard curves. In fact, due to variations in sensitivity between films, other standards sometimes were not imaged. Therefore, each film has a unique standard curve associated with its analysis. The use of individual standard curves for each film avoids many of the potential errors that can be encountered in quantifying radioactivity from autoradiographs (123). The standard curves for all films related to animals receiving the specific MAb were defined by linear functions as determined by the In Situ/Grain software. Standard curves associated with the non-specific

Table 3.1
QAR Standard Activity Concentrations⁺

Standard #	Act. Conc. (nCi/g)
1	*
2	*
3	14.25
4	22.31
5	30.26
6	36.60
7	54.11
8	70.50
9	155.20
10	249.10
11	318.30
12	399.00
13	761.30
14	1112.00
15	1719.00

Notes:

+ Concentrations shown are as of the date on which film exposures began.

* Activities in these standards were below the sensitivity of the film.
Therefore, they are never used in any standard curves.

MAB were characterized by polynomial functions of varying degree up to third order. The standard curve with the highest correlation coefficient was chosen for each film.

Figure 3.1 shows an example of a digitized autoradiograph from an animal at the 12 hour timepoint which was injected with Mel-14 F(ab')₂ and exposed to 37°C (M37-12-1-80). The grey scale shows the activity concentration per pixel in nCi/g with the white areas being the lowest activity and darker areas representing higher concentrations. The quantitative analysis performed by In Situ Grain is shown in Table 3.2. This table is a condensed version of the printout from the program. Column 2 shows the name of the tissue section. Columns 3, 4 and 5 present the total number of pixels in the ROI, the integrated optical density (OD) in the region, and the mean OD per pixel, respectively. Columns 6 and 7 represent the number of pixels after subtracting the film background and the corresponding integrated optical density. The percent of pixels found to contain a concentration of activity above background is shown in Column 8 followed by the mean net OD per pixel. The final column of Table 3.2 gives the calculated activity concentration of the section in nCi/g based on the standard curve of the film. The standard curve for Film 3 (M37-12) is given by:

$$\text{Activity concentration (nCi/g)} = -109.99 + 1315.53(\text{OD}).$$

The activity concentration for M37-12-1-80 based on the mean OD per pixel was 288.4 nCi/g. Pixel size was determined by digitizing the image of a clear plastic ruler placed on the lightbox. The average size of a pixel was approximately 90 X 90 μm. Given a slice thickness of 20 μm, the average mass of tissue per pixel would be 0.17 μg for unit density tissue.

Figure 3.1

Digitized autoradiograph of M37-12-1-80, a tumor from a mouse injected with $5\mu\text{Ci } ^{125}\text{I}$ -labeled Mel-14 F(ab')_2 (specific fragment). The animal was exposed to a 37°C water bath for 4 hours and killed 8 hours after heating.

Table A-3
Film 3: M37-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
1	std9	242	52.2	0.22	242	52.2	100.0	0.22	173.6
2	std10	242	80.3	0.33	242	80.3	100.0	0.33	326.4
3	std11	242	83.1	0.34	242	83.1	100.0	0.34	341.5
4	std12	242	91.3	0.38	242	91.3	100.0	0.38	386.3
5	std13	242	153.7	0.64	242	153.7	100.0	0.64	725.7
6	std14	242	238.4	0.99	242	238.4	100.0	0.99	1186.1
7	std15	242	326.3	1.35	242	326.3	100.0	1.35	1663.8
8	std6	242	20.0	0.08	177	15.6	73.1	0.09	5.9
9	std7	242	22.5	0.09	231	21.8	95.5	0.09	13.9
10	std8	242	29.8	0.12	242	29.8	100.0	0.12	51.9
11	1-1	543	52.3	0.10	440	45.5	81.0	0.10	26.1
12	1-20	1820	204.2	0.11	1255	167.5	69.0	0.13	65.6
13	1-30	4492	699.9	0.16	2316	568.1	51.6	0.25	212.7
14	1-40	5761	1093.6	0.19	3984	984.9	69.2	0.25	215.2
15	1-60	6288	1519.8	0.24	5872	1492.9	93.4	0.25	224.5
16	1-80	7447	2183.1	0.29	7140	2162.5	95.9	0.30	288.4
17	1-100	7405	2517.7	0.34	7400	2517.4	99.9	0.34	337.5
18	1-120	7667	2290.2	0.30	7622	2287.2	99.4	0.30	284.8
19	1-140	8260	2311.9	0.28	8103	2301.4	98.1	0.28	263.6
20	1-160	8881	2376.7	0.27	8656	2361.6	97.5	0.27	248.9
21	3-30	631	165.9	0.26	579	162.4	91.8	0.28	259.0
22	3-40	884	214.5	0.24	841	211.6	95.1	0.25	221.0
23	3-50	1972	433.6	0.22	1794	421.6	91.0	0.24	199.2

Table A-3
Film 3: M37-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	3-60	4330	938.0	0.22	3801	903.8	87.8	0.24	202.8
25	3-70	5017	1226.6	0.24	4850	1215.2	96.7	0.25	219.6
26	3-90	4580	1361.7	0.30	4548	1359.5	99.3	0.30	283.2
27	3-110	4742	1489.3	0.31	4731	1488.5	99.8	0.31	303.9

Intratumor variation of activity concentration. The mean activity concentration of all tumor sections was calculated for each tumor to examine the variation in activity concentration between tumor slices. The results of these calculations show that, along the axis of sectioning, there is a fairly homogeneous distribution of radioactivity within each tumor. Tables 3.3a and 3.3b give the mean slice activity concentration for each tumor studied along with the standard deviation (SD) and coefficient of variation (CV). We found that the majority of tumors (23/42) showed less than 15 % variation in activity concentration across sections while two-thirds (28/42) varied by less than 20%. This could be due to the fact that most tumors were sectioned perpendicular to their short axis and that the shorter diffusional distance resulted in a more homogeneous distribution of labeled MAb along this axis. However, one might expect this phenomenon to exhibit a dependence on time and treatment temperature. This temperature dependence was not seen in our analysis of intratumor variation in slice activity concentration. Nevertheless, these data support our approach of using one section to represent the whole tumor in most cases.

The complete data set for this part of the QAR analysis is presented in Appendix A.

Area percent tables. As previously mentioned, an attractive feature of the In Situ Grain program is its ability to analyze the distribution of activity concentration within each section by means of the area percent table function which calculates the percent of pixels having an activity concentration within a specified range. Table 3.4 shows a modification of the data output from M37-12-1-80. The original output yields the spectrum of observed transmission values (TV) subdivided into ten ranges. Transmission values represent the intensity (256 gray levels) of transmitted light passing through the digitized images. For each

Table 3.3a
Intratumor Variation in Slice Activity Concentration
Mel-14 F(ab')₂

Tumor	Mean (nCi/g)	SD	CV
M37-4-1	182.4	19.6	0.107
M37-4-2	310.2	74.6	0.240
M37-4-3	263.2	37.7	0.143
M37-8-1	314.6	139.2	0.442
M37-8-2	125.9	11.1	0.088
M37-8-3	241.2	58.5	0.242
M37-12-1	216.7	98.2	0.453
M37-12-3	241.2	41.1	0.170
M37-24-1	205.6	23.7	0.115
M37-24-2	149.1	41.1	0.276
M37-24-3	137.2	15.6	0.114
M42-4-1	248.2	69.3	0.279
M42-4-2	135.5	51.3	0.378
M42-4-3	340.5	54.8	0.161
M42-8-1	323.6	37.2	0.115
M42-8-2	231.1	40.5	0.175
M42-8-3	238.7	103.0	0.432
M42-12-1	426.6	35.5	0.083
M42-12-3	186.8	27.7	0.148
M42-24-1	346.6	10.9	0.032
M42-24-2	365.7	86.5	0.236
M42-24-3	208.0	81.9	0.394

Table 3.3b
Intratumor Variation in Slice Activity Concentration
RPC-5 F(ab')₂

Tumor	Mean (nCi/g)	SD	CV
R37-4-1	68.0	8.6	0.127
R37-4-2	76.2	14.5	0.190
R37-4-3	59.1	4.6	0.077
R37-8-1	74.5	4.9	0.066
R37-8-2	71.4	14.0	0.196
R37-8-3	66.0	8.9	0.134
R37-12-1	71.0	3.6	0.050
R37-24-1	106.9	1.6	0.015
R37-24-3	104.5	0.8	0.0073
R42-4-1	97.8	11.5	0.117
R42-4-2	106.6	14.4	0.135
R42-4-3	80.1	7.4	0.092
R42-8-1	96.4	31.2	0.324
R42-8-2	60.5	4.5	0.075
R42-8-3	110.6	30.6	0.276
R42-12-1	69.1	19.1	0.276
R42-12-3	52.9	7.1	0.135
R42-24-1	81.0	4.8	0.059
R42-24-2	199.5	39.9	0.200
R42-24-3	80.1	3.4	0.043

Table 3.4
Area Percent Table
M37-12-1-80

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1318.70	3058.12	2188
2	21	41	0.80	1.09	936.46	1318.70	1128
3	41	61	0.62	0.80	709.47	936.46	823
4	61	81	0.50	0.62	547.45	709.47	628
5	81	101	0.40	0.50	421.38	547.45	484
6	101	121	0.33	0.40	318.16	421.38	370
7	121	141	0.26	0.33	230.76	318.16	274
8	141	161	0.20	0.26	154.98	230.76	193
9	161	181	0.15	0.20	88.08	154.98	122
10	181	210	0.09	0.15	3.17	88.08	46

$$nCi/g = -109.99 + 1315.53(OD)$$

A _i	# pixels	%	cum %	A _i x %	
2188	163	2.2642	2.264203	49.5501	
1128	209	2.90318	5.167384	32.7357	
823	307	4.26448	9.431866	35.095	
628	313	4.34783	13.77969	27.3243	
484	347	4.82011	18.59981	23.3493	
370	562	7.80664	26.40645	28.8663	
274	864	12.0017	38.40811	32.9395	
193	1308	18.1692	56.5773	35.0426	
122	1491	20.7112	77.28851	25.1697	
46	1635	22.7115	100	10.3622	
Total =	7199				
					Act. Conc. = 300.435 nCi/g

range of transmission values, the number of pixels falling within that range is given along with the percent of total pixels and the cumulative percent. In our analysis, we have converted transmission values into optical densities by the formula:

$$OD = -\log (TV/256).$$

This allowed us to obtain the corresponding activity concentrations from the standard curve. We then calculated the midpoint of the range to create the bottom half of Table 3.4 which shows the percent of pixels having an activity concentration equal to A_i . From these data, we were able to more precisely calculate the activity concentration in the section by taking the sum of the products of A_i and the percent of pixels in each range, which was found to be 300.4 nCi/g for M37-12-1-80. This analysis was carried out for every tumor.

Tumor uptakes. From these refined activity concentration calculations, we determined the percent of injected dose taken up per gram of tumor. These results are summarized in Table 3.5. It should be pointed out that, although it is included in this table, the data from Animal 2 of the R42-24 group are suspect, since this section had a very different appearance from other sections in that group. It seemed that perhaps due to a malfunction in the cryostat, the section was unintentionally cut at twice the expected thickness. This could explain the considerably higher activity seen in this sample. Therefore, this section probably should not be included in our analysis. However, since it was chosen as a representative sample from this tumor, the reader is provided with mean values both with and without this section included. Due to the small number of animals per group, which was accentuated by some animals dying prematurely, it was difficult if not impossible to perform

Table 3.5
Tumor Uptake as a Function of Time and Treatment Temperature

Tumor	Uptake (%ID/g)	Mean +/- SD	Tumor	Uptake (%ID/g)	Mean +/- SD
M37-4-1	11.06		M42-4-1	8.18	
M37-4-2	13.26	14.59 +/- 4.36	M42-4-2	14.22	12.21 +/- 3.49
M37-4-3	19.46		M42-4-3	14.24	
M37-8-1	10.24		M42-8-1	14.72	
M37-8-2	5.09	7.71 +/- 2.58	M42-8-2	8.68	10.32 +/- 3.86
M37-8-3	7.81		M42-8-3	7.55	
M37-12-1	11.60		M42-12-1	16.28	
M37-12-2	8.35	9.62 +/- 1.74	M42-12-2	Died	12.38
M37-12-3	8.91		M42-12-3	8.47	
M37-24-1	8.70		M42-24-1	14.32	
M37-24-2	6.68	6.83 +/- 1.80	M42-24-2	20.77	15.58 +/- 4.69
M37-24-3	5.11		M42-24-3	11.64	
R37-4-1	3.25		R42-4-1	4.91	
R37-4-2	4.45	3.68 +/- 0.67	R42-4-2	6.48	5.20 +/- 1.16
R37-4-3	3.33		R42-4-3	4.22	
R37-8-1	3.71		R42-8-1	4.84	
R37-8-2	4.68	4.19 +/- 0.49	R42-8-2	3.44	4.41 +/- 0.84
R37-8-3	4.18		R42-8-3	4.95	
R37-12-1	3.73		R42-12-1	2.84	
R37-12-2	Died	3.73	R42-12-2	Died	2.87
R37-12-3	Died		R42-12-3	2.90	
R37-24-1	6.35		R42-24-1	4.26	
R37-24-2	Died	5.88	R42-24-2	11.22	6.52 +/- 4.07
R37-24-3	5.41		R42-24-3	4.08	4.17 (n=2)

a rigorous statistical analysis of these data. However, standard deviations were calculated for each group containing three animals and an attempt was made to compare the effects of time and temperature on the uptake of the specific and non-specific MAb fragments. The results from Table 3.5 are plotted in Figure 3.2. Error bars are shown for each group having three mice.

Tumor uptake of Mel-14 F(ab')₂ was increased by heating at the 8, 12 and 24 hour timepoints, however, these differences only were significant at the 95% confidence level in the 24 hour group, where uptake increased from 6.83 ± 1.80 %ID/g to 15.58 ± 4.69 %ID/g ($p < 0.025$ by Student's *t* test for the difference of means). The significance of the difference at the 12 hour timepoint could not be assessed due to the paucity of data in the heated group. Uptake of the specific fragment immediately after heating for four hours decreased by a slight, but non-significant amount from 14.59 ± 4.36 %ID/g to 12.21 ± 3.49 %ID/g. The general magnitude of uptakes are consistent with other studies in our laboratory (75,95). No statistically significant differences were observed due to temperature in the groups receiving the non-specific fragment.

For every time point except perhaps the 24 hour groups at 37°C, the specific antibody fragment exhibited a two-fold increase in uptake over the control fragment. The differences for the 4 and 8 hour groups were significant at the 95% level. Significance could not be established at the other time points due to the small number of animals. However, except as noted at 24 hours, all other differences appeared to be significant. The time after heating at which tumors were studied did not have a major effect on uptake. The only group showing a time dependence of uptake was the group receiving specific MAb exposed to 37°C where

Figure 3.2
Tumor Uptake: Mel-14 F(ab)₂ vs. RPC-5 F(ab)₂
Effect of Time and Temperature

the 4 hour group had a significantly higher uptake than the 8, 12 or 24 hour groups. All other differences were not significant at the 95% confidence level.

Non-tumor activity corrections. In order to accurately assess the microscopic dose to tumor, it is necessary to quantify the activity which is intimately associated with individual tumor cells. Therefore, the next step in our analysis was to use the histological information from the H & E stained sections to identify tumor cells and non-malignant tissues within each section. Figures 3.3a-c show photomicrographs of tumor section M37-12-1-80 at increasing magnifications of 8, 25 and 100 times, respectively. The box outlines indicate the areas of magnification shown at the next higher power. After examining each section by light microscope, the observations were transposed to a photograph of the digitized autoradiograph. This is shown in Figure 3.4. Several non-tumor areas are identified including an area of muscle tissue (M), a region of hemorrhage (H), two bands of blood vessels (V) and a small area at the top of the section which was difficult to identify but clearly was not tumor (NT). It can be seen from Figure 3.4 that the areas of highest MAb uptake were associated with a concentration of blood vessels. The regions of non-tumor tissue exhibited very low uptake of radioactivity while the hemorrhage showed elevated levels of activity. The next step was to subtract out the areas of non-tumor activity to establish the distribution of activity concentration in tumor alone. This exposed a deficiency in the image analysis software. At first glance, it should have been straightforward to quantify the activity concentration in the non-tumor areas by drawing a ROI and applying the area percent function. However, in order to sample the section, first it is necessary to draw a ROI encompassing the whole slice. Unfortunately, one can only draw a single ROI during each analysis. Therefore, it was

Figure 3.3a

Photomicrograph of M37-12-1-80 stained by H and E at 8X magnification. The box shows the area of magnification in Figure 3.3b.

Figure 3.3b

Photomicrograph of M37-12-1-80 at 25X magnification. Box shows area of magnification in Figure 3.3c.

Figure 3.3c

Photomicrograph of M37-12-1-80 at 100X magnification.

Figure 3.4

QAR image of M37-12-1-80 showing the areas of non-tumor tissue identified by light microscopy: **M**, muscle; **V**, blood vessels; **H**, hemorrhage; **NT**, unspecified non-tumor tissue.

necessary to sample the non-tumor areas pixel-by-pixel and subtract each pixel from the overall distribution. This was an extremely time-consuming process which precluded analyzing every tumor from this experiment. Thus several representative study groups were analyzed to demonstrate the methodology: M37-4, M37-8, M37-12, M37-24 and M42-24.

The number of non-tumor pixels was subtracted from the total pixels identified in the original analysis and the resulting distribution was used to recalculate the slice activity concentration, as previously. Table 3.6a is a modification of Table 3.4 which incorporates the distribution of non-tumor pixels and adjusts the overall activity distribution, resulting in a revised calculated activity concentration of 311.5 nCi/g. Table 3.6b shows the corrected tumor uptakes as percent injected dose per gram of tumor for the above groups based on this analysis of non-tumor activity. A comparison of the adjusted means by Student's t test showed that no significant changes resulted to the uptake characteristics from subtracting out the non-tumor pixels ($P > 0.05$). The differences were in fact very subtle and for some tumors, no adjustments were required as for M37-8-1, M42-24-2 and M42-24-3. However, it should be noted that, in all cases, the calculated slice activity concentration increased even though activity in effect was being subtracted from the ROI. This seeming paradox can be explained by the fact that the distribution of pixels was shifted toward a higher concentration after non-specific uptake was excluded.

By comparing the histology of each section to its corresponding QAR image, it was seen that, for tumors exposed to the specific MAb, the areas of highest tumor uptake were associated with a concentration of blood vessels. Examples for many of the tumors studied are shown in Appendix D. Due to the limited resolution in this system, it was not possible

Table 3.6a
M37-12-1-80
Non-tumor Correction

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
2188	163	0	163	2.36335	2.36335	51.71	
1128	209	0	209	3.0303	5.39	34.1818	
823	307	0	307	4.45121	9.84486	36.6335	
628	313	0	313	4.53821	14.3831	28.4999	
484	347	0	347	5.03117	19.4142	24.3509	
370	562	0	562	8.14847	27.5627	30.1493	
274	864	0	864	12.5272	40.0899	34.3245	
193	1308	0	1308	18.9648	59.0547	36.602	
122	1491	22	1469	21.2991	80.3538	25.9849	
46	1635	280	1355	19.6462	100	9.03726	
Totals	7199	302	6897	Act. Conc. = 311.474 nCi/g			

Table 3.6b
Corrected Tumor Uptakes for Selected Study Groups

Tumor	Uptake (% ID/g)	Mean +/- SD
M37-4-1	11.33	
M37-4-2	13.53	15.29 +/- 5.08
M37-4-3	21.02	
M37-8-1*	10.24	
M37-8-2	4.69	7.73 +/- 2.81
M37-8-3	8.27	
M37-12-1	12.03	
M37-12-2	8.62	9.94 +/- 1.83
M37-12-3	9.16	
M37-24-1	9.29	
M37-24-2	6.75	6.92 +/- 2.29
M37-24-3	4.72	
M42-24-1	14.71	
M42-24-2*	20.77	15.71 +/- 4.65
M42-24-3*	11.64	
* Corrections were not made for these tumors as the sections were seen to contain virtually 100% tumor cells.		

to specify the precise location of labeled molecules, i.e. -confined to vessels, bound to tumor cell membranes or internalized within the tumor cells. However, our results suggest that, in the groups receiving specific MAb, the activity was largely associated with tumor cells since activity can be seen distal to blood vessels. This also can be appreciated in images of tumors receiving the non-specific MAb where foci of activity are seen in tumor cells only when blood vessels are present. In Figure 3.5a, radioactivity not only is seen in a region of tumor cells mixed with blood vessels but also in an area of non-tumor tissue. The specificity of uptake also can be seen in Figure 3.5b, where a "hole" exists in the middle of a region of uptake corresponding to tumor cells which are not vascularized.

Effect of HT on radiolabeled antibody distribution. In order to assess the homogeneity of MAb distribution within tumors, we fit the data from the area percent tables for each tumor to a function describing the cumulative fraction of pixels versus activity concentration in nCi/g. These functions were of the general form:

$$\% \text{pixels}_{\text{cum}} = a + b \log(A_i),$$

where A_i is the activity concentration of interest and a and b are coefficients determined by the curve-fitting software (Cricket Graph). Computations then were performed with these functions using theoretical activity concentrations ranging from 50 to 1000 nCi/g. These values (percentages) were subtracted from 100, yielding the percentage of pixels having an activity greater than the index activity. Summaries of these calculated cumulative frequencies are shown in Tables 3.7a and b and the data are plotted in Figures 3.6a-h, which compare 37°C and 42°C heating at each timepoint for both the specific and non-specific fragments.

Figure 3.5a

Digitized autoradiograph of a tumor section (R42-4-1-90) from a mouse receiving 4.4 μCi ^{125}I -labeled RPC-5 F(ab')₂ (non-specific MAb fragment). This animal was exposed to 42°C hyperthermia for 4 hours and killed immediately after heating. T+V, tumor cells mixed with blood vessels; NT, non-tumor tissue.

Figure 3.5b

Digitized autoradiograph of R42-8-1-70. This animal received non-specific MAb and was exposed to 42°C hyperthermia for 4 hours. It was killed 4 hours after the completion of treatment by HT. T, tumor with no vessels present; NT, unspecified non-tumor tissue; C, cyst.

Table 3.7a
Calculated Cumulative Frequencies
Mel-14 F(ab')₂, 37° vs. 42°

Act. Conc. (nCi/g)	%>Ai M37-4	%>Ai M37-8	%>Ai M37-12	%>Ai M37-24
50	76.52	57.98	60.92	56.32
100	59.58	43.60	47.29	40.99
150	49.68	35.19	39.32	32.02
200	42.65	29.22	33.66	25.66
250	37.20	24.59	29.27	20.73
300	32.74	20.81	25.69	16.69
350	28.98	17.62	22.65	13.29
400	25.71	14.85	20.03	10.33
450	22.84	12.40	17.71	7.73
500	20.26	10.22	15.64	5.40
550	17.93	8.24	13.77	3.29
600	15.81	6.44	12.06	1.37
650	13.85	4.78	10.48	-0.40
700	12.04	3.24	9.02	-2.04
750	10.36	1.81	7.67	-3.57
800	8.78	0.47	6.40	-5.00
850	7.30	-0.79	5.21	-6.34
900	5.90	-1.97	4.08	-7.60
950	4.58	-3.10	3.02	-8.80
1000	3.33	-4.16	2.01	-9.93
Act. Conc. (nCi/g)	%>Ai M42-4	%>Ai M42-8	%>Ai M42-12	%>Ai M42-24
50	64.56	83.28	67.13	98.90
100	50.28	62.14	52.93	77.35
150	41.92	49.77	44.62	64.75
200	36.00	41.00	38.73	55.81
250	31.40	34.19	34.16	48.87
300	27.65	28.63	30.42	43.21
350	24.47	23.93	27.26	38.41
400	21.72	19.85	24.52	34.26
450	19.29	16.26	22.11	30.60
500	17.12	13.05	19.95	27.33
550	15.16	10.14	18.00	24.36
600	13.37	7.49	16.22	21.66
650	11.72	5.05	14.58	19.17
700	10.19	2.79	13.06	16.87
750	8.77	0.68	11.64	14.72
800	7.44	-1.29	10.32	12.72
850	6.19	-3.14	9.08	10.83
900	5.02	-4.88	7.91	9.06
950	3.90	-6.53	6.80	7.38
1000	2.84	-8.09	5.75	5.78

Table 3.7b
Calculated Cumulative Frequencies
RPC-5 F(ab')₂, 37° vs. 42°

Act. Conc. (nCi/g)	%>Ai R37-4	%>Ai R37-8	%>Ai R37-12	%>Ai R37-24
50	31.50	47.39	46.68	15.91
100	13.45	22.19	11.57	7.84
150	2.89	7.46	-8.97	-3.01
200	-4.60	-3.00	-23.54	-10.71
250	-10.41	-11.11	-34.84	-16.68
300	-15.16	-17.74	-44.08	-21.56
350	-19.17	-23.34	-51.88	-25.69
400	-22.65	-28.19	-58.65	-29.26
450	-25.72	-32.47	-64.61	-32.42
500	-28.46	-36.30	-69.95	-35.24
550	-30.94	-39.77	-74.78	-37.79
600	-33.21	-42.93	-79.19	-40.12
650	-35.29	-45.84	-83.24	-42.26
700	-37.22	-48.53	-86.99	-44.24
750	-39.02	-51.04	-90.49	-46.09
800	-40.70	-53.39	-93.76	-47.82
850	-42.28	-55.59	-96.83	-49.44
900	-43.77	-57.67	-99.72	-50.97
950	-45.17	-59.63	-102.46	-52.41
1000	-46.51	-61.50	-105.06	-53.79
Act. Conc. (nCi/g)	%>Ai R42-4	%>Ai R42-8	%>Ai R42-12	%>Ai R42-24
50	49.16	55.13	17.89	32.71
100	29.23	25.70	8.06	19.17
150	17.58	8.48	2.31	11.25
200	9.31	-3.74	-1.78	5.63
250	2.89	-13.21	-4.94	1.27
300	-2.35	-20.96	-7.53	-2.29
350	-6.78	-27.50	-9.72	-5.30
400	-10.62	-33.17	-11.61	-7.91
450	-14.00	-38.17	-13.28	-10.21
500	-17.03	-42.65	-14.78	-12.27
550	-19.77	-46.70	-16.13	-14.13
600	-22.27	-50.39	-17.36	-15.83
650	-24.57	-53.79	-18.50	-17.39
700	-26.71	-56.94	-19.55	-18.84
750	-28.69	-59.87	-20.53	-20.19
800	-30.54	-62.61	-21.45	-21.45
850	-32.29	-65.18	-22.31	-22.63
900	-33.93	-67.61	-23.12	-23.75
950	-35.48	-69.90	-23.88	-24.81
1000	-36.96	-72.08	-24.61	-25.81

Figure 3.6a
Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
Mel-14 F(ab')₂, 4 hours

Figure 3.6b
Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
Mel-14 F(ab')₂, 8 hours

Figure 3.6c
Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
Mel-14 F(ab')₂, 12 hours

Figure 3.6d
 Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
 Mel-14 F(ab')₂, 24 hours

Figure 3.6e
Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
RCP-5 F(ab')₂, 4 hours

Figure 3.6f
Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
RCP-5 F(ab')₂, 8 hours

Figure 3.6g
Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
RPC-5 F(ab')₂, 12 hours

Figure 3.6h
Effect of 42°C Hyperthermia on the Integrated Frequency Distribution of Activity
RPC-5 F(ab')₂, 24 hours

The dotted line in Figure 3.6a shows a hypothetical distribution of pixels in which the activity concentration is perfectly homogeneous, i.e., each pixel contains the same amount of radioactivity. In this example, all pixels have a concentration of 600 nCi/g. For all combinations of MAb and timepoint, except the four hour groups receiving Mel-14 F(ab')₂, the distribution curves for the heated groups are higher than for the corresponding control groups. That is, the pixels in the heated tumors are skewed toward a higher concentration of activity. These differences were significant for the 8 and 24 hour groups receiving specific MAb and the 4 hour groups receiving the control fragment. These results are consistent with the analysis used to calculate tumor uptakes (see Table 3.5). We were not able to quantitatively assess the homogeneity of activity within these tumors using the current method of analysis as it was not possible to measure the shape of the curves relative to the "perfect" distribution. However, by visual inspection of the digitized images of tumor slices, it appears that radiolabeled Mel-14 F(ab')₂ has penetrated deeper into the tumor as a result of hyperthermia relative to controls, especially at later timepoints (Figures 3.7a-d and 3.8a-d). This also has contributed to a more even distribution of radioactivity within the tumor.

Dosimetry Calculations

Using QAR, we were able to calculate tumor activities from detailed distributions of radioactivity contained per pixel. In these studies, ¹²⁵I was used as an analog for ²¹¹At, an alpha emitter which we have studied extensively as a potential therapeutic radionuclide. This assumes that the biodistribution of the radioiodinated MAb would be the same as the astatinated version. Garg *et al* (53) previously have shown that, while the distribution of MAb C110 IgG was similar for both labels, the uptake of astatinated C110 F(ab')₂ was higher

Figure 3.7

A

B

C

D

Digitized autoradiographs of control tumors (37°C): A, M37-4-2-90; B, M37-8-3-94; C, M37-12-1-80; D, M37-24-1-83.

Figure 3.8

A

B

C

D

Digitized autoradiographs of tumors treated with HT at 42°C: A, M42-4-1-100; B, M42-8-1-110; C, M42-12-1-120; D, M42-24-1-70.

in all tissues than the iodinated fragment. Therefore, in order to perform an accurate dosimetric evaluation of radioastatinated MAb, one would either need to establish a method for normalizing iodine uptakes to astatine or perform these studies with ^{211}At . The latter option poses many technical challenges, however, for reasons discussed in the last chapter, it is the preferred method. The longer half-life of I-125 facilitated the QAR analysis, particularly in the preparation of standards. QAR with short half-life nuclides requires the preparation of new standards for each experiment. Since the preparation of standards is a lengthy procedure, the 7.2 hour half-life of ^{211}At could result in a loss of signal by the time samples are exposed to the photographic medium.

By assuming a uniform distribution of activity, we were able to calculate a dose to the tumor using the MIRD formalism (2). Cumulative activities were calculated for the specific antibody at 37 and 42°C using a trapezoid integration technique (124). The uptake data from the ^{125}I -labeled MAbs were converted from percent injected dose per gram to μCi per gram and corrected for the 7.2 hour physical half-life of ^{211}At . Activity remaining in tumor at 24 hours was assumed to decay with the physical half life only and the cumulative activity for this segment of the curve was given by:

$$\tilde{A} = 1.44 \times A \times T_{\text{eff}},$$

where T_{eff} in this case is 7.2 hours. These results are plotted in Figures 3.9a and b. Dose then could be calculated from the equation:

$$\bar{D} = 2.13 \sum n_i E_i \phi_i \times \tilde{A}.$$

The first term of the equation is the equilibrium dose constant which previously was

Figure 3.9a
Tumor Uptakes
 $^{211}\text{At-Mel-14 F(ab')}_2$, 37°C

Figure 3.9b
Tumor Uptakes
 $^{211}\text{At-Mel-14 F(ab')}_2$, 42°C

determined to be 14.445 g-rad/ μ Ci-hr for ^{211}At (72). Based on an injected activity of 100 μ Ci of ^{211}At -labeled Mel-14 F(ab')₂, the cumulative activity would be 89.5 μ Ci-hr/g and the dose to tumor would be approximately 1290 rads (12.9 Gy) for an animal not receiving local hyperthermia. Following hyperthermia at 42°C for four hours, the dose would increase to 1590 rads (15.9 Gy).

Using the methods of Crawford-Brown and Shyr (125) to calculate dose and hit probabilities for alpha particles, very similar results were obtained. Equation 17 from the above reference (shown below) gives the dose per unit emission density for a source in a volume:

$$\frac{D}{V} = \frac{E}{\rho \times 6.24 \times 10^9}$$

where D is the dose in gray, V is the emission density in alphas per cm^3 , E is the alpha energy in MeV and ρ is the density in g per cm^3 . For a mean alpha energy of 6.8 MeV in a unit density material, the dose per disintegration is 1.09×10^{-9} Gy/ α . By multiplying the number of disintegrations per unit cumulative activity by the cumulative activity, one can obtain the total number of disintegrations and from this, the total dose:

$$(1.332 \times 10^8 \text{ dis}/\mu\text{Ci-h}) \times (89.5 \mu\text{Ci-h}) = 1.2 \times 10^{10} \text{ dis}$$

$$(1.2 \times 10^{10} \text{ dis}) \times (1.09 \times 10^{-9} \text{ Gy/dis}) = 13.0 \text{ Gy (1300 rad)}.$$

For the 42°C group, the corresponding dose is 16.0 Gy or 1600 rad.

Further, by using Table 4 from the above reference, which provides the dose for a volumetric source based on hit probabilities, there is also close agreement with the MIRD approach. This table assumes an emission density of 1 alpha per μm^3 which is equivalent to

10^{12} alphas per cm^3 . From the above calculation of emission density in our study (1.2×10^{10} disintegrations), the fractional dose relative to that given in the table would be:

$$\frac{1.2 \times 10^{10} \text{ dis/g}}{1 \times 10^{12} \text{ dis/g}} = 0.012$$

Then, for a $5 \mu\text{m}$ target and an alpha range of $60 \mu\text{m}$, the dose would be 0.012 times 1106 Gy or 13.3 Gy for the unheated group and 16.2 Gy for the heated group.

The inherent resolution of the system was insufficient to allow a true microdosimetric analysis. At a size of $90 \times 90 \times 20 \mu\text{m}$, each pixel would contain about 20 cells. With the mean range of ^{211}At alpha particles at $\sim 60 \mu\text{m}$ (15), these particles would traverse several cells before depositing all of their energy. Our results offer a middle ground between MIRD-type dose averaging and microdosimetry. Using the cumulative frequency distributions of activity, we were able to describe the number of pixels which contained sufficient activity for cell killing as determined in a microdosimetric model by Humm (15). Using Monte Carlo techniques, Humm has calculated survival curves for tumor cells based on the stochastic nature of energy emission events from alpha particles deposited in tissue. He has predicted that, for ^{211}At deposited uniformly in tumor, a dose of 1.6 Gy would be required for 90% cell killing. Under the same conditions, it would require 3.2 Gy to effect 99% killing. Humm further concludes that, in order to achieve cell killing at the D_0 level, it would require 65 disintegrations of ^{211}At per cell. In our model, 99% cell killing would correspond to a cumulative activity of $22 \mu\text{Ci-hr/g}$ for unheated controls which would follow from an injection of $25 \mu\text{Ci}$, as shown below:

$$(3.2 \text{ Gy}/12.9 \text{ Gy}) \times 89.5 \text{ } \mu\text{Ci-h/g} = 22.20 \text{ } \mu\text{Ci-h/g}$$

$$(22.20 \text{ } \mu\text{Ci-h/g})/(89.5 \text{ } \mu\text{Ci-h/g}) = 0.248,$$

which is the fraction of a 100 μCi injection needed to achieve a dose of 3.2 Gy. The same dose for mice treated with hyperthermia would be achieved with an injected activity of 20 μCi . If one examines the activity concentration in tumor at 24 hours post injection, the tumor uptake corresponding to 22 $\mu\text{Ci-hrs}$ would be approximately 170 nCi/g for unheated controls (5×34 nCi/g, which is the uptake based on a 5 μCi injection) and 300 nCi/g for heated tumors (4×77 nCi/g). From our calculated cumulative frequency distributions (Figure 3.6d), it can be seen that only 30% of the pixels contained a specific activity greater than 170 nCi/g at 37°C while only 40% of pixels exceeded the required activity concentration at 42°C. Therefore, even though the mean absorbed dose calculated by the MIRD method was found to be well in excess of the dose predicted by Humm for 99% cell killing, an analysis of the microdistribution of activity in tumors found that only a fraction of tumor cells received a dose above this threshold.

CHAPTER IV

CONCLUSIONS

Studies with monoclonal antibodies directed to tumor-associated antigens have shown the potential of these molecules, when labeled with high energy radionuclides such as ^{131}I , ^{90}Y , ^{212}Bi and ^{211}At , to effect therapeutic responses in nude mice with xenografted tumors. However, similar efforts in human clinical trials have been hampered by much lower uptake of the labeled MAb into the tumor due to a number of possible factors including poor vasculature (especially at the center of tumors), low tumor blood flow, inadequate expression of antigen, tumor interstitial pressure and a so-called "binding-site barrier."

In the present study, we have evaluated the use of local hyperthermia to increase tumor uptake and improve the homogeneity of labeled MAb distribution in order, ultimately, to exploit the short range and radiotoxicity of alpha particles for radioimmunotherapy. We have used quantitative autoradiography to study the changes in distribution of radioactivity resulting from hyperthermia. In addition, we have considered the utility of QAR data as a source function for microdosimetric calculations. We report the following findings:

- **The current study employing QAR is suggestive of improved homogeneity of activity distribution.** In this work, and in previous studies in our laboratories, we have demonstrated the effectiveness of hyperthermia in increasing absolute tumor uptake. However, the current analysis did not allow us to develop a quantitative index of homogeneity. Future work examining the kurtosis of the antibody

distribution could aid in quantifying this important parameter (see Chapter V).

- **Tumor uptakes based on QAR data were in good agreement with previous studies in our laboratory.** The general magnitude of uptakes was comparable to that seen in studies by Cope *et al* (95) and Schuster *et al* (75) and a similar increase in uptake was observed following hyperthermia at 42°C. An attempt to validate QAR measurements by direct gamma counting of tumors within the same experiment was unsuccessful due to problems with histological preparation and film processing. Future studies should include this validation procedure, however, extreme care must be taken in the handling of specimens to avoid artifacts.
- **Although labeled Mel-14 F(ab')₂ was seen qualitatively to penetrate further into tumor masses following hyperthermia, only 30-40% of the tumor cells were exposed to sufficient radioactivity to result in a curative dose.** This is in spite of the fact that the average dose to the tumor was calculated to be 14-16 Gy (1400-1600 rad) based upon a 100μCi injected dose of astatinated MAb fragment which, if delivered to the whole tumor, certainly should be curative. Therefore, while hyperthermia might be a desirable adjuvant to RIT, it appears that other methods will need to be developed to enhance the targeting of radionuclides for therapy. Alternatively, the continued problem of MAb distribution might limit their utility to the treatment of micrometastases or intracompartmental therapy.
- **Our results showed a fairly homogeneous distribution of activity between slices along the short axis of tumors studied.** This is in contrast to the results of Griffith *et al* (103) who reported variations of 250 to 400% over distances of 500 to 1000

μm , respectively, representing the long axis of the tumor. However, these tumors were of a different type and were not treated with hyperthermia. Roberson *et al* have reported similarly heterogeneous distributions in three dimensions indicative of "hot spots" (126) using the same tumor model as Griffith but a different MAb. In reality, the variation between tumor sections would be highly dependent on the orientation of the tumor at the time of sectioning since each tumor possesses a unique architecture.

- **QAR was found to be a powerful technique for measuring the distribution of radioactivity in tissue.** The information obtained could be extremely valuable for treatment planning and the determination of dose or specific energy distribution for internal emitters. This method can be extended beyond medical treatment into the area of risk assessment for occupational or environmental exposure to particulate forms of radiation. However, the technique used in the current study suffered from an inherent lack of resolution which prevented us from performing a true microdosimetric analysis. The method would need to be modified using nuclear emulsions or plastic track etching techniques to achieve sufficient resolution for microdosimetry. QAR also is hindered by being extremely labor-intensive, from the preparation of tissue sections to the analysis of data. Furthermore, the method precludes the evaluation of the time course of activity within a tissue or even within the same animal. Techniques utilizing better image analysis software, faster computers and new technologies such as phosphor imaging could help to reduce the workload and yield a better understanding of the interactions of radiation in biological

matter, in general, and the factors influencing radioimmunotherapy, in particular.

The work presented in this paper represents the first step in assessing the specific energy distribution from internally deposited alpha emitters. The use of QAR in this study has demonstrated the potential impact of dose heterogeneity on the efficacy of radioimmunotherapy using MAbs labeled with short range emitters. The technique also was valuable in assessing the ability of hyperthermia to enhance uptake in tumors and to create a more homogeneous distribution of radioactivity. The current system had limitations which prevented us from fully describing the activity distribution and performing microdosimetric calculations, however, it illustrated the potential of this technique to provide essential information in the assessment of risk or benefit from internally deposited radionuclides. Further studies will be needed to improve the uptake and distribution of radiopharmaceuticals for the treatment of disease. These results are sufficiently encouraging to warrant continued investigation in the areas of combined modality cancer therapy and dose assessment using QAR data as a source term.

CHAPTER V

FUTURE DIRECTIONS

The current study has demonstrated the power of the technique of quantitative autoradiography to provide detailed information about the distribution of radionuclides within tissues. However, in order to assess the risk or benefit from internally deposited alpha emitters, it will be necessary to modify the system to be able to describe the spatial distribution of energy emission events at the cellular level. At the current level of resolution, we only were able to specify the activity distributed within a pixel containing a cluster of approximately 20 cells. We have demonstrated that, due to the short range of alphas in tissue and the heterogeneous distribution of labeled MAbs in tumors, widely differing results are obtained by microdosimetric techniques than would be predicted by gross counting of tumors and the calculation of a mean tumor dose. This chapter presents some suggestions for future work in the areas of dose assessment and radioimmunotherapy.

Dosimetry

Autoradiography. Since the range of alpha particles is only a few cell diameters, it is necessary to precisely locate each particle to determine which cells will be irradiated and to what extent. Film autoradiography offers the advantage of analyzing large sections if necessary and quantification of radioactivity can be accomplished rather easily by the use of standards on the same film. However, in order to analyze the distribution of alphas at the cellular level, it is necessary to examine the tissue sections by light or electron microscope.

This calls for the techniques of microautoradiography. Because of the differences in track structure between ^{125}I and ^{211}At , it will be necessary to conduct these experiments using ^{211}At . The difficulties in using short-lived radionuclides in autoradiography have been discussed previously. One of the most common techniques involves mounting the section on a microscope slide and directly dipping the slide into molten emulsion (127). As the particles travel through the emulsion, tracks of grains are produced which can be studied qualitatively and/or quantitatively after development. Quantification of tracks is rather difficult because they occur at numerous, random angles within the emulsion. With higher energy particles, the tracks are longer and it becomes harder to attribute the grains to a particular origin. However, statistical methods can be used to determine the origin of the track. Emulsion dipping techniques have been widely used in the field of health physics for the investigation of alpha particle distributions resulting from occupational, medical and environmental exposures both at the light microscopic (128-135) and electron microscopic levels (136,137). Studies already have been conducted using emulsion dipping methods with radiopharmaceuticals labeled with ^{211}At (52).

Other methods to be considered are the track etching techniques. A number of media have been examined including cellulose nitrate films (138,139) and a variety of plastics (140-143). The technique is similar for all of these materials. The tissue specimen is placed in direct contact with the medium and allowed to expose for a period of time. At the end of exposure, the section is removed and the detector is etched with concentrated sodium hydroxide revealing the ionization tracks. A major advantage of the track etching techniques is that the procedure does not need to be performed in a darkroom; the detector media are

not sensitive to light as are nuclear emulsions. They also provide very high resolution. The advantage of the plastics over cellulose nitrate films is that they are not adversely affected by moisture. Users of CR-39 claim that the detector is sensitive to a wider range of alpha energies than Lexan (142) while studies with Lexan indicate that it exhibits even higher resolution than CR-39(140). Further, Zamenhof *et al* state that their method using Lexan allows for detector etching without destruction of the tissue section ensuring precise registration between the sample and the autoradiograph. Finally, both plastics enjoy the advantage over other techniques of having extremely low backgrounds. This can result in sensitivities as low as 2 α -particles per cm² (141).

Another area for improvement in QAR lies in the image analysis system. Since the technique is so labor-intensive and even one digitized image produces a tremendous amount of data, it is imperative to have a powerful computer and software package that can process the data and provide a variety of analytical methods. It would be extremely useful to be able to sample several ROIs within a single image since the structure of tumors is highly variable and uptakes often are seen as "hot spots" scattered throughout the mass. More recent developments in the method of phosphor imaging could allow for significantly higher sample throughputs with very high resolution and sophisticated image analysis capabilities.

One of our goals in this study was to compare the distribution of labeled MAb fragments and assess whether hyperthermia created a more homogeneous uptake of activity. However, we were unable to do this in a quantitative fashion. Sung *et al* (114) have described a measure of the heterogeneity observed with QAR in a study of the distribution of labeled immunotoxins in solid tumors. This parameter is known as the kurtosis. In order

to employ this measure, it would be necessary to present activity distributions as the absolute number of pixels or percent of pixels versus specific activity instead of the *cumulative* percent of pixels. It appears that this would be a valuable parameter to study in future work. The authors also used an area-above-threshold analysis similar to our study.

Microdosimetry. With the advent of alpha particle radioimmunotherapy and the use of radionuclides in nuclear medicine which have short range, intermediate to high LET emissions, investigators have realized that the assumption of a homogeneous distribution of activity within organs is not tenable(6,14-19,21,76,86,87,103,106,114,116,117,144-147). This assumption is central to the use of standard dosimetry models, however, it may lead to large overestimates or underestimates of the dose to critical cells. The impact of heterogeneous activity distribution also has been examined in the context of environmental exposures to radionuclides (22-25). Therefore, it is essential to use the concepts of microdosimetry to fully describe the interactions of alpha particles with tissue. Microdosimetric theory draws on the stochastic nature of energy emission events to describe the deposition of energy in very small volumes of tissue (27).

Our studies have demonstrated that, with improvements in resolution, it is possible to use QAR data as the source term for microdosimetric calculations. After identifying the location of alpha sources in relation to cell nuclei, the next step in calculating dose is to integrate these data with a Monte Carlo code which simulates random emissions and energy deposition events. By simulating tens of thousands of emissions and summing the contribution from each event, the code can produce a statistically significant specific energy distribution. Humm has used Monte Carlo codes to model tumor dose with ^{211}At using both

a theoretical distribution of activity (15) and actual distributions from QAR data (109).

Several investigators have concluded that the traditional concepts of internal dosimetry are insufficient to describe and estimate the effects of high LET radiation in tissue (15,21-25,70). Crawford-Brown *et al* have studied the concept of hit probabilities as an index of risk as opposed to mean dose. These authors have examined hit probabilities as a function of alpha energy and source and target (cell nuclei) geometries. Energy deposition was determined by the difference in energy between entry and exit of the target based on the experimental values of Walsh for stopping power as a function of range in tissue (147). Due to the stochastic nature of alpha emissions, the hit probability density is a function of the random distribution of chord lengths or tracks through a cell based upon the range of the particle and its distance from the target (22). This work demonstrated that, for certain geometries, hit probability and dose are poorly correlated. If one assumes that "hits" - e.g., DNA double strand breaks - are the important phenomenon in determining risk (which could be any biological effect), then dose becomes a poor predictor of risk. Crawford-Brown *et al* have extended this work to develop models for cellular transformation and cancer risk which have shown promise in explaining prior epidemiological data on radiation carcinogenesis (24,149,150). Humm (15) has used the concept of hit probabilities to assess the specific activity of ^{211}At which would be needed to effect tumor cures. Humm and Cobb (14) have reported on the geometric enhancement of dose for ^{211}At when a radiolabeled MAb is located on the cell membrane as opposed to being uniformly distributed in the intracellular fluid (14). This effect was clearly seen in the current study where the average tumor dose overestimated the dose to the majority of cells, while underestimating the dose to a small population of cells

which were exposed to a high specific activity. Therefore, future work in dose assessment for high LET emitters should include an analysis of hit probability.

Radioimmunotherapy

This study showed that local hyperthermia could increase the uptake of radiolabeled MABs and contribute to a more homogeneous distribution of activity within tumors. However, this was not sufficient for us to be able to predict a higher therapeutic index for this modality. As described previously in this paper, the architecture of tumors is highly variable and their microenvironment is hostile to the transport of macromolecules. Therefore, new techniques or combinations of existing methods must be developed to overcome the barriers to radiopharmaceutical distribution in tumors.

Numerous methods previously have been explored for increasing distribution in tumors. Hyperthermia has been shown to increase the total uptake of labeled MABs in tumor (75,95,118,151-153). However, this effect is transitory in nature as the tumor vasculature collapses after prolonged exposure to heat (92). Our study has shown that improving uptake alone is not sufficient for therapy with alpha emitters if the distribution of activity is not made more homogeneous. Therefore, hyperthermia is probably limited in its usefulness, particularly in the time frame required for MABs labeled with short-lived radionuclides such as ^{211}At . With a half-life of 7.2 hours, astatine loses 90% of its activity within 24 hours. External beam irradiation has been effective in increasing MAB uptake (152,154) and the effect is more lasting than with hyperthermia. Further, radiation increases the permeability of tumors but not normal tissues, unlike hyperthermia. This method could prove to be of value in the future. Several different drugs have been investigated as enhancers of MAB delivery. One approach

has been to disrupt the blood-brain barrier for the treatment of brain tumors, however, this has been shown to increase the rate of delivery to normal tissues but not tumor (155). Other drugs have been recommended for altering the interstitial fluid pressure of tumors which is a major barrier to MAb delivery. Warfarin and heparin have been shown to reduce tumor interstitial pressure.² Other groups have used gamma-interferon to stimulate antigen expression as a means of increasing antibody binding and delivery (118). Waldman *et al* have studied the use of different types of antibodies to treat the same tumor, recommending an anti-tumor MAb if the tumor possesses a necrotic shell or an anti-vasculature MAb for tumors with a necrotic core.³ For tumors composed of all living tissue, one could use a combination of both MAbs. Radiosensitizers also have been examined as an adjuvant to RIT. 5-iododeoxyuridine has been shown to improve the treatment of human mammary tumors with ¹³¹I-labeled MAb (156).

Other approaches to MAb delivery have looked at different routes of administration suggesting that, by avoiding the systemic circulation, it is possible to deliver a higher dose to tumor cells. Biodistribution and dosimetry studies have shown that delivery by intratumoral (157), intrathecal (158-160), intraperitoneal (161,162) and intraarterial routes (163) have increased uptake, retention and dose to tumor. Preliminary studies in our laboratory have confirmed that delivery via intratumoral and intrathecal injection results in prolonged retention of labeled MAbs (unpublished results).

²B.D. Curti, NIH Clinical Staff Conference, "Monoclonal Antibody Therapy: Problems and Strategies for Overcoming Them," July 27, 1994.

³T.A. Waldmann, NIH Clinical Staff Conference, "Monoclonal Antibody Therapy: Problems and Strategies for Overcoming Them," July 27, 1994.

Because of the difficulties in delivering large molecules to tumors, some groups have investigated the use of smaller molecules as carriers of radionuclides for diagnosis and therapy. Yokota *et al* (113,164) have studied the biodistribution of a radioiodinated single-chain Fv fragment of an IgG MAb and found lower normal tissue uptake along with a more homogeneous distribution of radioactivity in tumor xenografts compared to larger immunoglobulin forms. Sung and co-workers examined the distribution of radiolabeled diphtheria toxin by QAR relative to a MAb-conjugated toxin and found a more even distribution of activity with the unconjugated form (114). The authors suggested that the higher uptake and more homogeneous distribution were due to the smaller molecular weight and lack of antigen binding of the free toxin. Other groups currently are investigating the use of labeled peptides as carriers (M.R. Zalutsky, personal communication). The use of small molecular weight molecules seems to hold greater promise for the future of radioimmunotherapy.

For situations in which delivery of radioactivity continues to be a problem, such as solid tumors, it might be necessary to rely upon longer range beta emitters to irradiate a sufficient number of tumor cells to effect cures. This will depend on the ability to strike a balance between the dose to tumor and non-specific irradiation of normal tissues. Alternatively, if the problem of delivery to tumors can be overcome, it still might be possible to use short range emitters for therapy. This might require the use of radionuclides with a longer half-life than ^{211}At in order to achieve the essential uptake and distribution of the radiopharmaceutical. The list of readily available alpha emitters is rather short as are their half-lives and some suffer from the presence of high energy gamma emissions in their decay

chain. Auger electron emitters such as ^{125}I have been shown to have the potential to deliver local doses to cells comparable to high LET alpha emitters when the nuclide is deposited in the DNA (16,26,165-167). Therefore, they could potentially fill an important role in radioimmunotherapy so long as the nuclide does not undergo substantial gamma emissions.

APPENDIX A

QUANTITATIVE ANALYSIS OF AUTORADIOGRAPHIC IMAGES

Quantification of the activity concentration present in the various tumors studied was carried out using the In Situ/Grain program developed by Loats Associates, Inc., Westminster, MD. The first step was to digitize the autoradiographs from each film. Each film represented a particular temperature/MAB/time combination. Thus, there were a total of 16 films prepared in this study. Each film was placed on a light box beneath a video camera and the camera height and focal length of the lens were adjusted to achieve the optimal image, i.e., the whole tumor slice was contained within the field of view as seen on a color monitor and the light intensity was judged to be most pleasing to the eye. Next, the gain on the densitometer was adjusted so that the transmission values (as seen on a computer-generated histogram) were spread across the full scale of the graph without any "clipping" of TV's at the higher end of the scale. Every tumor slice and standard was digitized in this manner and saved to individual .frm files which were stored on the optical disk drive. In addition, an image of a clear plastic ruler was digitized and later was used to determine the dimensions of pixels. The average size of pixels was found to be approximately $90 \times 90 \mu\text{m}$.

The next step was to create a standard curve relating OD per unit area to activity per unit mass (nCi/g). Each standard image file was loaded into a buffer for sampling and, using a circular sampling mode, a ROI was drawn around the image. This resulted in the display of a histogram showing the distribution of TV's for that image. By moving a cursor along

the histogram, one could manipulate the appearance of the image and effectively remove the background contribution from the film itself. The resulting transmission values were converted to optical densities and loaded into a table similar to those presented in this Appendix as integrated OD. After sampling each standard, a function was created to calculate the integrated OD per pixel (the columns labeled as "Mean"). Then their activities were entered into the table (see Table 3.1) and, using the calibration function, the program generated several potential fits for the standard curve ranging from a simple linear fit to a third order polynomial. The curve with the highest correlation coefficient was chosen and stored in a separate file for future sampling of the tumor sections.

Finally, the tumor slices were sampled by loading their images into the sampling buffer and subtracting film background as described above. The sample table containing the standards and standard curve (embedded within the "nCi/g" column) was loaded and ROI's were drawn around each tumor slice using a dark field edge technique which allowed for sampling of irregular surfaces. The raw data as well as the calculated activities were automatically entered into the sample table. The complete analysis of tumors from this experiment is presented in Tables A-1 through A-16. The tables shown here are a condensed version of the ASCII tables produced by the In Situ/Grain program. The tables in this paper were generated using Microsoft Excel. The results are discussed in Chapter 3.

Table A-1
Film 1: M37-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std6	242	17.3	0.07	97	8.2	40.1	0.08	78.0
2	std7	242	21.0	0.09	204	18.4	84.3	0.09	83.3
3	std8	242	24.0	0.10	233	23.4	96.3	0.10	93.0
4	std9	242	39.1	0.16	242	39.1	100.0	0.16	151.5
5	std10	242	47.8	0.20	242	47.8	100.0	0.20	185.9
6	std11	242	86.1	0.36	242	86.1	100.0	0.36	336.6
7	std12	242	98.2	0.41	242	98.2	100.0	0.41	384.2
8	std14	242	241.0	1.00	242	241.0	100.0	1.00	946.7
9	std15	242	466.8	1.93	242	466.8	100.0	1.93	1836.4
10	std13	242	169.4	0.70	242	169.4	100.0	0.70	664.6
11	1-10	4981	867.3	0.17	4216	819.9	84.6	0.19	182.9
12	1-20	5022	887.1	0.18	4641	863.8	92.4	0.19	174.9
13	1-60	4998	880.2	0.18	4745	863.5	94.9	0.18	170.9
14	1-70	6851	1393.0	0.20	5847	1332.3	85.3	0.23	214.7
15	1-110	5648	1037.8	0.18	5338	1018.1	94.5	0.19	179.3
16	1-130	4894	684.9	0.14	4185	643.7	85.5	0.15	144.1
17	1-140	5459	889.5	0.16	4264	820.1	78.1	0.19	180.8
18	1-160	4077	707.7	0.17	3149	652.5	77.2	0.21	195.0
19	1-170	4460	719.3	0.16	3560	665.1	79.8	0.19	175.6
20	1-180	3461	504.6	0.15	1896	413.9	54.8	0.22	205.6
21	2-1	3390	1685.5	0.50	3361	1683.5	99.1	0.50	474.9
22	2-20	3765	1461.5	0.39	3736	1459.5	99.2	0.39	369.9
23	2-30	5450	1857.4	0.34	5323	1848.8	97.7	0.35	328.6

Table A-1
Film 1: M37-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	2-40	5868	1996.1	0.34	5627	1980.1	95.9	0.35	332.9
25	2-60	6888	2209.4	0.32	6540	2186.9	94.9	0.33	316.2
26	2-90	7924	2233.0	0.28	7061	2177.6	89.1	0.31	291.4
27	2-100	5090	1520.3	0.30	4977	1512.7	97.8	0.30	287.2
28	2-110	5315	1213.0	0.23	4762	1176.9	89.6	0.25	233.1
29	2-130	3445	792.2	0.23	3030	765.4	88.0	0.25	238.3
30	2-140	3374	630.0	0.19	2322	565.1	68.8	0.24	229.5
31	3-1	1776	418.4	0.24	1507	401.2	84.9	0.27	251.3
32	3-10	2838	567.2	0.20	2442	541.6	86.0	0.22	208.9
33	3-20	3463	789.2	0.23	3145	768.5	90.8	0.24	230.4
34	3-30	4037	993.6	0.25	3628	967.1	89.9	0.27	251.6
35	3-40	5035	1091.7	0.22	4294	1045.8	85.3	0.24	229.6
36	3-50	5150	1209.4	0.23	4354	1159.6	84.5	0.27	251.3
37	3-80	4040	1138.1	0.28	3776	1121.0	93.5	0.30	280.5
38	3-100	4611	1286.9	0.28	4214	1261.2	91.4	0.30	282.8
39	3-110	3703	926.6	0.25	3266	899.0	88.2	0.28	259.9
40	3-120	3076	970.0	0.32	2928	960.1	95.2	0.33	310.1
41	3-130	2510	847.3	0.34	2334	835.7	93.0	0.36	338.8

Table A-2
Film 2: M37-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std5	242	21.0	0.09	152	14.2	62.8	0.09	14.5
2	std6	242	23.7	0.10	210	21.3	86.8	0.10	24.7
3	std7	242	24.8	0.10	226	23.5	93.4	0.10	28.1
4	std8	242	28.5	0.12	241	28.4	99.6	0.12	45.3
5	std9	242	54.6	0.23	242	54.6	100.0	0.23	180.7
6	std10	242	66.8	0.28	242	66.8	100.0	0.28	243.6
7	std11	242	87.4	0.36	242	87.4	100.0	0.36	350.2
8	std12	242	99.4	0.41	242	99.4	100.0	0.41	412.6
9	std13	242	174.4	0.72	242	174.4	100.0	0.72	801.2
10	std14	242	246.4	1.02	242	246.4	100.0	1.02	1173.9
11	std15	242	341.0	1.41	242	341.0	100.0	1.41	1663.9
12	1-32	1181	217.5	0.18	965	203.0	81.7	0.21	161.4
13	1-61	2713	1207.2	0.44	2311	1179.2	85.2	0.51	537.3
14	1-93	2422	715.0	0.30	2153	696.1	88.9	0.32	303.0
15	1-131	3218	1062.5	0.33	3117	1055.2	96.9	0.34	322.0
16	1-163	5587	1312.7	0.23	4402	1234.9	78.8	0.28	249.3
17	2-2	698	121.6	0.17	623	116.0	89.3	0.19	131.1
18	2-32	1267	197.6	0.16	1052	183.4	83.0	0.17	116.2
19	2-63	2083	325.0	0.16	1790	305.0	85.9	0.17	111.3
20	2-94	2417	398.0	0.16	2105	376.8	87.1	0.18	122.1
21	2-126	2921	500.3	0.17	2495	473.4	85.4	0.19	135.5
22	2-161	4217	748.4	0.18	3719	716.3	88.2	0.19	139.1
23	3-1	536	32.5	0.06	42	4.0	7.8	0.10	17.2

Table A-2
Film 2: M37-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int OD	Area%	Mean	nCi/g
24	3-31	2486	463.9	0.19	1348	393.9	54.2	0.29	264.0
25	3-64	2379	703.2	0.30	1949	672.8	81.9	0.35	330.4
26	3-94	3109	647.4	0.21	2294	596.5	73.8	0.26	223.6
27	3-123	3980	735.4	0.18	2629	652.3	66.1	0.25	208.7
28	3-153	3874	686.5	0.18	2737	614.8	70.7	0.22	179.3

Table A-3
Film 3: M37-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std9	242	52.2	0.22	242	52.2	100.0	0.22	173.6
2	std10	242	80.3	0.33	242	80.3	100.0	0.33	326.4
3	std11	242	83.1	0.34	242	83.1	100.0	0.34	341.5
4	std12	242	91.3	0.38	242	91.3	100.0	0.38	386.3
5	std13	242	153.7	0.64	242	153.7	100.0	0.64	725.7
6	std14	242	238.4	0.99	242	238.4	100.0	0.99	1186.1
7	std15	242	326.3	1.35	242	326.3	100.0	1.35	1663.8
8	std6	242	20.0	0.08	177	15.6	73.1	0.09	5.9
9	std7	242	22.5	0.09	231	21.8	95.5	0.09	13.9
10	std8	242	29.8	0.12	242	29.8	100.0	0.12	51.9
11	1-1	543	52.3	0.10	440	45.5	81.0	0.10	26.1
12	1-20	1820	204.2	0.11	1255	167.5	69.0	0.13	65.6
13	1-30	4492	699.9	0.16	2316	568.1	51.6	0.25	212.7
14	1-40	5761	1093.6	0.19	3984	984.9	69.2	0.25	215.2
15	1-60	6288	1519.8	0.24	5872	1492.9	93.4	0.25	224.5
16	1-80	7447	2183.1	0.29	7140	2162.5	95.9	0.30	288.4
17	1-100	7405	2517.7	0.34	7400	2517.4	99.9	0.34	337.5
18	1-120	7667	2290.2	0.30	7622	2287.2	99.4	0.30	284.8
19	1-140	8260	2311.9	0.28	8103	2301.4	98.1	0.28	263.6
20	1-160	8881	2376.7	0.27	8656	2361.6	97.5	0.27	248.9
21	3-30	631	165.9	0.26	579	162.4	91.8	0.28	259.0
22	3-40	884	214.5	0.24	841	211.6	95.1	0.25	221.0
23	3-50	1972	433.6	0.22	1794	421.6	91.0	0.24	199.2

Table A-3
Film 3: M37-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	3-60	4330	938.0	0.22	3801	903.8	87.8	0.24	202.8
25	3-70	5017	1226.6	0.24	4850	1215.2	96.7	0.25	219.6
26	3-90	4580	1361.7	0.30	4548	1359.5	99.3	0.30	283.2
27	3-110	4742	1489.3	0.31	4731	1488.5	99.8	0.31	303.9

Table A-4
Film 4: M37-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std7	242	22.6	0.09	183	17.9	75.6	0.10	65.4
2	std8	242	27.6	0.11	237	27.1	97.9	0.11	80.4
3	std9	242	52.7	0.22	242	52.7	100.0	0.22	174.8
4	std10	242	72.6	0.30	242	72.6	100.0	0.30	249.8
5	std11	242	100.5	0.42	242	100.5	100.0	0.42	355.0
6	std12	242	111.0	0.46	242	111.0	100.0	0.46	394.6
7	std13	242	180.9	0.75	242	180.9	100.0	0.75	657.9
8	std15	242	470.1	1.94	242	470.1	100.0	1.94	1748.5
9	1-2	950	246.0	0.26	884	241.0	93.1	0.27	224.7
10	1-23	2416	644.1	0.27	2159	625.9	89.4	0.29	240.5
11	1-42	4467	1055.5	0.24	3674	999.1	82.2	0.27	224.1
12	1-62	6004	1261.3	0.21	4927	1184.3	82.1	0.24	195.3
13	1-83	6689	1499.1	0.22	6306	1470.6	94.3	0.23	188.7
14	1-104	6994	1497.3	0.21	6586	1467.6	94.2	0.22	179.3
15	1-123	6482	1457.6	0.22	6221	1438.5	96.0	0.23	186.9
16	2-1	1591	214.6	0.13	1095	180.4	68.8	0.16	126.3
17	2-10	1621	290.1	0.18	1196	261.0	73.8	0.22	175.1
18	2-20	1937	491.2	0.25	1559	466.8	80.5	0.30	249.1
19	2-30	1930	377.0	0.20	1869	372.4	96.8	0.20	157.8
20	2-80	2712	598.7	0.22	2511	584.2	92.6	0.23	188.2
21	2-90	3201	578.1	0.18	2590	537.0	80.9	0.21	165.1
22	2-120	4066	526.2	0.13	3120	459.3	76.7	0.15	110.3
23	2-130	4860	973.0	0.20	4238	928.1	87.2	0.22	175.8

Table A-4
Film 4: M37-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	2-160	6289	800.3	0.13	4098	649.7	65.2	0.16	120.6
25	2-170	5043	720.4	0.14	3666	621.0	72.7	0.17	130.5
26	2-180	6867	773.1	0.11	3795	567.0	55.3	0.15	112.3
27	2-190	6818	880.6	0.13	4842	740.2	71.0	0.15	115.4
28	2-210	6827	856.1	0.13	4727	705.1	69.2	0.15	112.1
29	3-1	1675	325.1	0.19	1501	312.1	89.6	0.21	165.7
30	3-10	2398	408.6	0.17	1788	368.4	74.6	0.21	164.0
31	3-20	2725	428.3	0.16	2383	404.3	87.4	0.17	130.8
32	3-30	3260	477.4	0.15	2811	445.6	86.2	0.16	120.6
33	3-40	3303	520.9	0.16	2962	495.7	89.7	0.17	128.6
34	3-50	4300	677.7	0.16	3678	635.5	85.5	0.17	133.6
35	3-60	4555	761.1	0.17	4078	725.9	89.5	0.18	138.4
36	3-90	5049	850.6	0.17	4779	830.0	94.7	0.17	134.4
37	3-110	5703	932.0	0.16	5127	889.9	89.9	0.17	134.3
38	3-120	5723	805.2	0.14	4483	717.1	78.3	0.16	121.9

Table A-5
Film 5: M42-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
1	std7	242	19.4	0.08	148	13.0	61.2	0.09	84.3
2	std8	242	20.6	0.08	185	16.7	76.4	0.09	86.7
3	std9	242	34.1	0.14	242	34.1	100.0	0.14	135.1
4	std10	242	49.9	0.21	242	49.9	100.0	0.21	197.0
5	std13	242	206.9	0.85	242	206.9	100.0	0.85	814.1
6	std14	242	277.6	1.15	242	277.6	100.0	1.15	1092.3
7	std15	242	435.2	1.80	242	435.2	100.0	1.80	1711.7
8	1-1	1704	473.8	0.28	1258	446.5	73.8	0.35	338.6
9	1-10	1788	774.5	0.43	1768	773.1	98.9	0.44	416.9
10	1-20	2868	970.7	0.34	2622	955.2	91.4	0.36	347.5
11	1-30	3317	969.6	0.29	3146	958.6	94.8	0.30	290.8
12	1-40	3201	861.9	0.27	2668	830.6	83.3	0.31	297.1
13	1-50	3997	858.3	0.21	3307	817.9	82.7	0.25	236.3
14	1-60	5308	967.4	0.18	4111	900.7	77.4	0.22	209.4
15	1-70	4796	1100.5	0.23	4366	1073.0	91.0	0.25	234.8
16	1-80	5937	1072.0	0.18	4876	1008.2	82.1	0.21	197.7
17	1-90	5742	1054.0	0.18	5118	1016.6	89.1	0.20	189.9
18	1-100	7163	1336.5	0.19	5866	1257.9	81.9	0.21	205.0
19	1-110	7967	1626.5	0.20	6764	1552.3	84.9	0.23	219.3
20	1-120	7914	1497.4	0.19	6721	1422.5	84.9	0.21	202.3
21	1-130	8088	1564.7	0.19	6913	1492.5	85.5	0.22	206.4
22	1-140	5747	1149.2	0.20	5627	1141.1	97.9	0.20	193.9
23	1-150	6104	1131.7	0.19	5714	1105.9	93.6	0.19	185.1

Table A-5
Film 5: M42-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	2-1	154	16.3	0.11	136	15.1	88.3	0.11	106.8
25	2-22	1253	79.3	0.06	250	24.7	20.0	0.10	95.1
26	2-41	2750	184.9	0.07	618	66.2	22.5	0.11	102.9
27	2-63	3180	256.9	0.08	1362	149.8	42.8	0.11	105.6
28	2-81	6539	962.5	0.15	3963	813.0	60.6	0.21	196.1
29	2-114	6849	1173.4	0.17	4893	1057.8	71.4	0.22	206.6
30	3-1	3284	895.7	0.27	3156	887.3	96.1	0.28	268.4
31	3-10	5764	1872.2	0.32	5529	1856.3	95.9	0.34	320.4
32	3-20	5930	2442.8	0.41	5867	2438.5	98.9	0.42	396.3
33	3-30	6548	1958.0	0.30	6326	1943.2	96.6	0.31	293.2
34	3-40	6915	2414.7	0.35	6641	2396.6	96.0	0.36	344.3
35	3-50	7293	2541.7	0.35	6842	2511.6	93.8	0.37	350.2
36	3-60	7968	3039.7	0.38	7782	3027.0	97.7	0.39	371.0
37	3-70	10401	3663.2	0.35	9432	3599.7	90.7	0.38	364.0
38	3-80	11603	4158.4	0.36	10349	4076.0	89.2	0.39	375.6
39	3-90	10207	3715.6	0.36	9130	3646.5	89.4	0.40	380.9
40	3-100	10404	4095.2	0.39	9645	4045.0	92.7	0.42	399.9
41	3-110	10462	4252.8	0.41	9904	4215.9	94.7	0.43	405.9
42	3-120	11996	3603.1	0.30	9591	3454.5	80.0	0.36	343.6
43	3-140	8708	2309.8	0.27	7917	2259.2	90.9	0.29	272.4
44	3-150	9743	1929.0	0.20	7790	1804.6	80.0	0.23	221.4

Table A-6
Film 6: M42-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std5	242	16.5	0.07	53	4.5	21.9	0.08	62.3
2	std6	242	17.3	0.07	85	7.2	35.1	0.08	62.5
3	std7	242	23.2	0.10	225	22.0	93.0	0.10	72.0
4	std8	242	23.6	0.10	236	23.2	97.5	0.10	72.3
5	std9	242	41.8	0.17	242	41.8	100.0	0.17	124.7
6	std10	242	58.9	0.24	242	58.9	100.0	0.24	174.5
7	std11	242	118.6	0.49	242	118.6	100.0	0.49	348.4
8	std12	242	148.7	0.61	242	148.7	100.0	0.61	435.7
9	std14	242	409.8	1.69	242	409.8	100.0	1.69	1195.5
10	std15	242	578.8	2.39	242	578.8	100.0	2.39	1687.3
11	std13	242	229.6	0.95	242	229.6	100.0	0.95	671.3
12	1-1	2919	1196.7	0.41	2766	1186.4	94.8	0.43	305.2
13	1-10	6078	2261.2	0.37	5307	2212.3	87.3	0.42	296.7
14	1-20	5790	2119.5	0.37	5452	2097.1	94.2	0.38	274.0
15	1-30	6497	2825.4	0.43	6409	2819.4	98.6	0.44	312.9
16	1-40	7957	3187.6	0.40	7547	3160.4	94.8	0.42	298.0
17	1-50	8052	3764.6	0.47	7996	3760.7	99.3	0.47	334.3
18	1-60	8597	3505.6	0.41	7977	3464.8	92.8	0.43	309.0
19	1-70	9287	4019.6	0.43	8788	3986.6	94.6	0.45	322.6
20	1-80	8690	3089.8	0.36	7621	3021.4	87.7	0.40	282.3
21	1-100	8633	3927.3	0.45	8433	3913.6	97.7	0.46	329.9
22	1-110	11139	5707.1	0.51	10884	5689.8	97.7	0.52	371.3
23	1-120	10637	5971.1	0.56	10629	5970.5	99.9	0.56	398.7

Table A-6
Film 6: M42-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
24	1-130	10237	5267.0	0.51	10035	5253.2	98.0	0.52	371.8
25	2-1	1056	226.3	0.21	883	215.8	83.6	0.24	175.2
26	2-50	7135	2758.0	0.39	6819	2737.0	95.6	0.40	285.8
27	2-70	6223	2130.3	0.34	6001	2115.3	96.4	0.35	251.4
28	2-90	7538	1892.2	0.25	6227	1807.8	82.6	0.29	207.6
29	2-100	7170	1723.4	0.24	6152	1656.9	85.8	0.27	192.8
30	2-110	9458	2919.5	0.31	8562	2861.0	90.5	0.33	238.5
31	2-130	8376	2979.6	0.36	7875	2946.3	94.0	0.37	266.6
32	3-1	357	35.9	0.10	285	31.3	79.8	0.11	80.4
33	3-35a	1350	376.9	0.28	1227	368.8	90.9	0.30	214.8
34	3-35b	472	76.2	0.16	401	71.5	85.0	0.18	128.7
35	3-62a	2258	608.8	0.27	1993	591.6	88.3	0.30	212.2
36	3-62b	971	236.6	0.24	932	234.0	96.0	0.25	179.9
37	3-91	4301	1200.9	0.28	4133	1189.7	96.1	0.29	205.9
38	3-124	6034	1851.8	0.31	5229	1801.1	86.7	0.34	245.7
39	3-152	5507	1433.8	0.26	5329	1421.8	96.8	0.27	191.0
40	3-182	6083	1761.9	0.29	5890	1749.1	96.8	0.30	212.3

Table A-7
Film 7: M42-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std6	242	17.6	0.07	107	8.8	44.2	0.08	38.6
2	std8	242	28.4	0.12	242	28.4	100.0	0.12	82.4
3	std11	242	76.3	0.32	242	76.3	100.0	0.32	329.4
4	std12	242	84.5	0.35	242	84.5	100.0	0.35	372.0
5	std13	242	155.3	0.64	242	155.3	100.0	0.64	737.4
6	std14	242	223.1	0.92	242	223.1	100.0	0.92	1087.4
7	std15	242	350.8	1.45	242	350.8	100.0	1.45	1746.4
8	std7	242	27.4	0.11	241	27.3	99.6	0.11	77.1
9	1-1	917	389.2	0.42	868	385.6	94.7	0.44	490.5
10	1-20	1642	565.7	0.34	1395	549.0	85.0	0.39	427.2
11	1-40	3996	1043.6	0.26	2579	948.6	64.5	0.37	395.0
12	1-60	5482	1753.5	0.32	4352	1674.5	79.4	0.38	416.2
13	1-80	8025	2238.2	0.28	5324	2069.9	66.3	0.39	421.3
14	1-100	6367	2155.8	0.34	5791	2114.1	91.0	0.37	391.6
15	1-120	6690	2435.0	0.36	6306	2406.4	94.3	0.38	412.3
16	1-140	8197	2608.1	0.32	6870	2516.8	83.8	0.37	393.2
17	1-160	7524	2955.1	0.39	6546	2883.4	87.0	0.44	485.9
18	1-170	5639	2132.6	0.38	5288	2106.4	93.8	0.40	433.2
19	3-1	4697	462.8	0.10	2003	291.7	42.6	0.15	117.5
20	3-10	5298	609.0	0.11	2081	405.9	39.3	0.20	179.3
21	3-20	7330	998.2	0.14	4021	790.2	54.9	0.20	181.1
22	3-30	9059	1190.0	0.13	4853	914.9	53.6	0.19	171.1
23	3-40	8243	1233.9	0.15	5207	1032.7	63.2	0.20	183.3

Table A-7
Film 7: M42-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	3-70	8243	1665.6	0.20	6967	1572.6	84.5	0.23	217.6
25	3-80	9752	1692.5	0.17	6761	1488.6	69.3	0.22	210.6
26	3-100	10589	1907.9	0.18	8075	1731.7	76.3	0.21	203.5
27	3-140	10502	1635.1	0.16	6760	1384.4	64.4	0.20	191.4
28	3-160	8317	1473.9	0.18	5905	1313.9	71.0	0.22	213.5
29	3-180	10199	1515.7	0.15	6265	1253.8	61.4	0.20	185.6

Table A-8
Film 8: M42-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std6	242	13.2	0.05	13	1.1	5.4	0.08	65.4
2	std7	242	20.0	0.08	168	15.0	69.4	0.09	71.6
3	std8	242	22.1	0.09	220	20.6	90.9	0.09	75.5
4	std9	242	42.4	0.18	242	42.4	100.0	0.18	152.2
5	std10	242	68.5	0.28	242	68.5	100.0	0.28	253.4
6	std11	242	87.7	0.36	242	87.7	100.0	0.36	328.1
7	std12	242	105.0	0.43	242	105.0	100.0	0.43	395.5
8	std13	242	191.9	0.79	242	191.9	100.0	0.79	732.7
9	std14	242	266.1	1.10	242	266.1	100.0	1.10	1021.2
10	std15	242	461.4	1.91	242	461.4	100.0	1.91	1779.5
11	1-1	4378	1504.3	0.34	3852	1471.0	88.0	0.38	346.5
12	1-20	7493	2434.9	0.32	6292	2361.9	84.0	0.38	340.3
13	1-50	8859	2596.0	0.29	6850	2479.9	77.3	0.36	327.8
14	1-70	9467	2980.9	0.31	7322	2856.1	77.3	0.39	354.1
15	1-90	6142	2388.6	0.39	6105	2386.1	99.4	0.39	354.9
16	1-100	5825	2272.8	0.39	5714	2265.5	98.1	0.40	360.2
17	1-120	4877	1671.0	0.34	4329	1636.0	88.8	0.38	342.7
18	2-1	2657	564.7	0.21	2382	547.2	89.6	0.23	203.4
19	2-10	4734	1089.1	0.23	3934	1041.6	83.1	0.26	236.4
20	2-20	5498	1713.7	0.31	4759	1672.7	86.6	0.35	317.9
21	2-30	6239	2254.1	0.36	5856	2230.6	93.9	0.38	345.5
22	2-60	5943	2414.2	0.41	5764	2402.8	97.0	0.42	379.3
23	2-70	7526	2843.2	0.38	6468	2784.5	85.9	0.43	392.2

Table A-8
Film 8: M42-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
24	2-90	6832	3828.4	0.56	6749	3822.9	98.8	0.57	519.9
25	2-100	7047	3547.3	0.50	6963	3541.8	98.8	0.51	465.6
26	2-110	6487	3287.4	0.51	6429	3283.6	99.1	0.51	467.6
27	2-120	5592	2453.9	0.44	5353	2438.6	95.7	0.46	415.7
28	2-130	5837	1923.6	0.33	4705	1860.0	80.6	0.40	359.1
29	2-140	4482	1627.4	0.36	3866	1589.4	86.3	0.41	373.9
30	2-150	2482	825.4	0.33	2316	815.0	93.3	0.35	318.3
31	2-160	3358	755.5	0.22	1880	676.3	56.0	0.36	325.6
32	3-1	1733	180.4	0.10	1028	143.8	59.3	0.14	119.0
33	3-10	2021	167.9	0.08	889	111.4	44.0	0.13	105.3
34	3-20	2300	229.5	0.10	1417	176.3	61.6	0.12	104.5
35	3-30	3024	355.9	0.12	2123	303.2	70.2	0.14	121.7
36	3-40	3375	415.4	0.12	2678	374.0	79.3	0.14	118.8
37	3-50	4567	760.3	0.17	4060	729.0	88.9	0.18	156.3
38	3-60	5058	980.8	0.19	4709	958.0	93.1	0.20	178.7
39	3-70	5641	1515.9	0.27	5482	1505.1	97.2	0.27	245.6
40	3-80	6003	1291.3	0.22	5045	1230.1	84.0	0.24	216.7
41	3-90	7228	2138.2	0.30	6456	2091.5	89.3	0.32	292.0
42	3-100	7612	2013.6	0.26	6604	1951.0	86.8	0.30	265.2
43	3-110	7814	2531.7	0.32	7473	2509.4	95.6	0.34	303.1
44	3-120	6966	2226.0	0.32	6544	2199.4	93.9	0.34	303.4
45	3-150	8060	2138.8	0.27	6654	2056.5	82.6	0.31	278.0
46	3-170	5917	1608.8	0.27	4395	1518.8	74.3	0.35	312.3

Table A-9
Film 9: R37-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std4	242	16.0	0.07	52	4.3	21.5	0.08	54.8
2	std5	242	18.5	0.08	130	11.2	53.7	0.09	57.8
3	std7	242	19.4	0.08	161	13.9	66.5	0.09	58.2
4	std8	242	20.5	0.08	199	17.5	82.2	0.09	60.3
5	std9	242	37.0	0.15	242	37.0	100.0	0.15	129.6
6	std10	242	54.6	0.23	242	54.6	100.0	0.23	209.1
7	std11	242	81.2	0.34	242	81.2	100.0	0.34	332.2
8	std12	242	89.3	0.37	242	89.3	100.0	0.37	370.4
9	std13	242	176.7	0.73	242	176.7	100.0	0.73	802.4
10	std14	242	233.0	0.96	242	233.0	100.0	0.96	1100.8
11	std15	242	340.9	1.41	242	340.9	100.0	1.41	1715.5
12	1-1	1104	69.3	0.06	248	23.2	22.5	0.09	66.1
13	1-34	3718	244.7	0.07	749	81.8	20.1	0.11	82.8
14	1-63	5229	341.2	0.07	1098	114.3	21.0	0.10	77.3
15	1-93	4548	235.7	0.05	343	31.6	7.5	0.09	64.6
16	1-123	6409	363.0	0.06	761	68.8	11.9	0.09	62.8
17	1-153	5676	316.7	0.06	615	53.9	10.8	0.09	59.8
18	1-184	6390	367.3	0.06	871	78.5	13.6	0.09	62.4
19	2-1	855	42.6	0.05	63	5.4	7.4	0.09	57.1
20	2-31	2290	154.7	0.07	785	78.9	34.3	0.10	73.5
21	2-61	2773	246.6	0.09	1589	175.5	57.3	0.11	84.1
22	2-91	3554	339.2	0.10	2280	264.8	64.2	0.12	90.2
23	3-2	655	37.4	0.06	69	5.7	10.5	0.08	54.3

Table A-9
Film 9: R37-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	3-31	1320	85.9	0.07	362	33.0	27.4	0.09	63.4
25	3-61	1435	89.0	0.06	314	27.4	21.9	0.09	59.5

Table A-10
Film 10: R37-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
1	std6	242	12.7	0.05	7	0.5	2.9	0.08	49.3
2	std7	242	17.9	0.07	112	9.5	46.3	0.08	56.5
3	std8	242	20.2	0.08	191	16.8	78.9	0.09	60.0
4	std9	242	38.6	0.16	242	38.6	100.0	0.16	143.2
5	std10	242	60.0	0.25	242	60.0	100.0	0.25	255.7
6	std11	242	71.2	0.29	242	71.2	100.0	0.29	318.4
7	std12	242	85.5	0.35	242	85.5	100.0	0.35	401.1
8	std13	242	143.0	0.59	242	143.0	100.0	0.59	759.7
9	std14	242	197.0	0.81	242	197.0	100.0	0.81	1112.1
10	std15	242	299.2	1.24	242	299.2	100.0	1.24	1719.1
11	1-1	2898	191.1	0.07	797	76.8	27.5	0.10	69.6
12	1-30	4460	326.8	0.07	1761	174.3	39.5	0.10	72.5
13	1-60	5906	474.2	0.08	2759	288.6	46.7	0.10	78.8
14	1-90	6882	491.3	0.07	2629	256.8	38.2	0.10	71.0
15	1-120	4626	434.6	0.09	3303	350.6	71.4	0.11	80.6
16	2-30	6210	308.2	0.05	203	17.8	3.3	0.09	59.8
17	2-60	7525	418.3	0.06	748	67.8	9.9	0.09	63.1
18	2-90	8148	478.6	0.06	1249	117.2	15.3	0.09	66.7
19	2-120	7689	591.5	0.08	3413	337.4	44.4	0.10	72.3
20	2-150	7694	749.1	0.10	4907	582.5	63.8	0.12	95.0
21	3-1	441	28.6	0.06	100	9.3	22.7	0.09	65.4
22	3-32	1305	66.5	0.05	57	5.3	4.4	0.09	66.6
23	3-61	2453	149.7	0.06	370	31.9	15.1	0.09	58.1

Table A-10
Film 10: R37-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	3-91	3726	225.0	0.06	733	66.0	19.7	0.09	62.5
25	3-121	4841	357.6	0.07	1889	188.9	39.0	0.10	73.7
26	3-153	5254	445.9	0.08	2705	294.7	51.5	0.11	83.8
27	3-182	5527	326.7	0.06	856	74.4	15.5	0.09	59.0
28	3-201	6456	378.0	0.06	1012	87.9	15.7	0.09	59.0

Table A-11
Film 11: R37-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std3	242	14.7	0.06	6	0.6	2.5	0.09	65.6
2	std4	242	18.5	0.08	58	5.4	24.0	0.09	65.9
3	std5	242	20.1	0.08	99	9.3	40.9	0.09	66.4
4	std7	242	25.6	0.11	228	24.4	94.2	0.11	78.4
5	std8	242	27.5	0.11	241	27.5	99.6	0.11	84.5
6	std9	242	48.7	0.20	242	48.7	100.0	0.20	163.0
7	std10	242	69.4	0.29	242	69.4	100.0	0.29	239.6
8	std11	242	88.1	0.36	242	88.1	100.0	0.36	309.2
9	std12	242	104.2	0.43	242	104.2	100.0	0.43	369.0
10	std13	242	179.2	0.74	242	179.2	100.0	0.74	647.5
11	std14	242	271.0	1.12	242	271.0	100.0	1.12	988.4
12	std15	242	497.0	2.05	242	497.0	100.0	2.05	1827.9
13	1-1	723	43.6	0.06	59	5.4	8.2	0.09	63.8
14	1-10	961	76.7	0.08	335	35.1	34.9	0.10	76.3
15	1-20	1594	117.6	0.07	436	44.6	27.4	0.10	73.9
16	1-30	2819	184.3	0.07	299	30.3	10.6	0.10	73.0
17	1-40	4091	263.7	0.06	309	29.8	7.6	0.10	68.8
18	1-50	3759	247.0	0.07	326	32.2	8.7	0.10	70.8
19	1-60	4890	324.1	0.07	491	48.6	10.0	0.10	71.0
20	1-80	5436	355.5	0.07	503	48.8	9.3	0.10	69.2
21	1-100	4988	348.8	0.07	732	73.3	14.7	0.10	72.0
22	1-120	5801	402.2	0.07	941	96.6	16.2	0.10	74.3
23	1-140	7271	539.1	0.07	1524	158.5	21.0	0.10	75.5

Table A-12
Film 12: R37-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std6	242	17.0	0.07	241	16.9	99.6	0.07	111.4
2	std7	242	17.6	0.07	242	17.6	100.0	0.07	117.4
3	std8	242	18.6	0.08	242	18.6	100.0	0.08	127.9
4	std9	242	24.8	0.10	242	24.8	100.0	0.10	188.3
5	std10	242	32.0	0.13	242	32.0	100.0	0.13	258.8
6	std11	242	38.9	0.16	242	38.9	100.0	0.16	326.2
7	std12	242	39.0	0.16	242	39.0	100.0	0.16	327.9
8	std13	242	60.5	0.25	242	60.5	100.0	0.25	538.6
9	std14	242	115.7	0.48	242	115.7	100.0	0.48	1079.7
10	std15	242	189.0	0.78	242	189.0	100.0	0.78	1798.9
11	1-40	1163	72.3	0.06	234	15.8	20.1	0.07	105.8
12	1-80	1849	115.1	0.06	395	26.7	21.4	0.07	105.7
13	1-100	2455	151.5	0.06	519	34.7	21.1	0.07	104.0
14	1-110	2367	148.8	0.06	701	47.6	29.6	0.07	106.4
15	1-140	2716	171.2	0.06	899	61.2	33.1	0.07	106.7
16	1-150	2698	165.7	0.06	578	39.4	21.4	0.07	106.8
17	1-160	3150	201.3	0.06	1253	86.1	39.8	0.07	108.2
18	1-170	2711	171.8	0.06	908	62.6	33.5	0.07	108.6
19	1-180	3596	229.9	0.06	1232	85.4	34.3	0.07	109.6
20	1-190	2611	163.2	0.06	771	52.8	29.5	0.07	107.6
21	3-30	1505	88.8	0.06	90	6.0	6.0	0.07	104.2
22	3-41	1929	117.9	0.06	308	20.8	16.0	0.07	105.1
23	3-61	1284	79.9	0.06	301	20.3	23.4	0.07	105.2

Table A-12
Film 12: R37-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int. OD	Area%	Mean	nCi/g
24	3-100	2858	171.3	0.06	213	14.2	7.5	0.07	103.6

Table A-13
Film 13: R42-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std3	242	10.5	0.04	0	0.0	0.0	0.00	0.0
2	std4	242	12.8	0.05	3	0.2	1.2	0.08	61.2
3	std5	242	13.4	0.06	5	0.4	2.1	0.08	59.4
4	std6	242	16.1	0.07	45	3.7	18.6	0.08	61.9
5	std7	242	21.9	0.09	217	20.2	89.7	0.09	71.1
6	std8	242	25.8	0.11	240	25.7	99.2	0.11	83.6
7	std9	242	48.4	0.20	242	48.4	100.0	0.20	167.2
8	std10	242	77.1	0.32	242	77.1	100.0	0.32	273.5
9	std11	242	87.2	0.36	242	87.2	100.0	0.36	310.9
10	std13	242	186.0	0.77	242	186.0	100.0	0.77	677.0
11	std14	242	253.6	1.05	242	253.6	100.0	1.05	927.5
12	std15	242	498.6	2.06	242	498.6	100.0	2.06	1835.5
13	1-1	2285	255.3	0.11	1860	228.2	81.4	0.12	97.7
14	1-10	3836	429.3	0.11	3149	386.9	82.1	0.12	97.9
15	1-20	4156	439.5	0.11	3311	389.2	79.7	0.12	93.1
16	1-30	3485	325.8	0.09	2324	258.8	66.7	0.11	87.6
17	1-40	5108	454.2	0.09	3340	358.0	65.4	0.11	83.8
18	1-50	5226	585.3	0.11	4107	519.2	78.6	0.13	101.1
19	1-60	7649	1119.0	0.15	6765	1066.8	88.4	0.16	129.1
20	1-70	6765	805.9	0.12	5953	753.6	88.0	0.13	101.2
21	1-80	8060	960.2	0.12	6409	857.3	79.5	0.13	107.7
22	1-90	8381	891.1	0.11	5976	741.3	71.3	0.12	98.9
23	1-100	9149	891.3	0.10	5050	651.9	55.2	0.13	103.5

Table A-13
Film 13: R42-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
24	1-110	7595	756.3	0.10	4723	575.5	62.2	0.12	97.0
25	1-120	8862	757.5	0.09	3943	473.9	44.5	0.12	95.5
26	1-130	10029	1059.1	0.11	6548	858.4	65.3	0.13	105.3
27	1-140	6754	503.4	0.07	2515	260.8	37.2	0.10	80.7
28	1-150	8123	703.9	0.09	4526	490.5	55.7	0.11	84.9
29	2-1	4314	530.4	0.12	3533	478.9	81.9	0.14	109.3
30	2-10	3915	566.9	0.14	3872	564.0	98.9	0.15	118.3
31	2-20	4561	487.4	0.11	3387	418.6	74.3	0.12	98.5
32	2-30	7356	844.7	0.11	5761	745.5	78.3	0.13	103.7
33	2-40	7758	976.1	0.13	6510	901.9	83.9	0.14	111.9
34	2-50	8360	1036.7	0.12	6855	946.1	82.0	0.14	111.5
35	2-60	8039	887.2	0.11	5660	746.8	70.4	0.13	106.0
36	2-70	9373	1373.0	0.15	8999	1348.6	96.0	0.15	122.1
37	2-80	8638	1126.2	0.13	7969	1081.6	92.3	0.14	109.4
38	2-90	14518	2112.5	0.15	11969	1962.5	82.4	0.16	134.7
39	2-100	11702	1553.8	0.13	10291	1469.5	87.9	0.14	115.8
40	2-110	12167	1614.3	0.13	10617	1519.3	87.3	0.14	116.0
41	2-120	11818	1250.4	0.11	9281	1093.9	78.5	0.12	93.4
42	2-130	8837	865.1	0.10	6866	735.2	77.7	0.11	83.7
43	2-140	11282	1060.4	0.09	8417	880.1	74.6	0.10	81.5
44	2-150	12544	1248.6	0.10	9511	1074.5	75.8	0.11	89.0
45	3-1	1488	95.5	0.06	318	29.0	21.4	0.09	69.5
46	3-10	3902	305.0	0.08	1930	185.2	49.5	0.10	73.8

Table A-13
Film 13: R42-4

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
47	3-20	4566	353.2	0.08	2216	207.1	48.5	0.09	71.5
48	3-30	6127	463.4	0.08	2855	264.6	46.6	0.09	70.8
49	3-50	6008	557.3	0.09	3945	433.6	65.7	0.11	86.3
50	3-60	6218	592.0	0.10	4235	471.7	68.1	0.11	87.6
51	3-70	6682	648.8	0.10	4749	526.5	71.1	0.11	87.1
52	3-80	7906	770.2	0.10	5813	637.6	73.5	0.11	86.1
53	3-110	10179	893.9	0.09	6136	664.1	60.3	0.11	84.8
54	3-130	9089	814.5	0.09	5667	602.4	62.4	0.11	83.0

Table A-14
Film 14: R42-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std3	242	16.0	0.07	8	0.7	3.3	0.09	50.6
2	std4	242	18.4	0.08	49	4.6	20.2	0.09	51.4
3	std5	242	19.9	0.08	90	8.5	37.2	0.09	51.6
4	std6	242	20.2	0.08	100	9.5	41.3	0.10	52.1
5	std7	242	28.8	0.12	242	28.8	100.0	0.12	76.2
6	std8	242	33.6	0.14	242	33.6	100.0	0.14	96.4
7	std9	242	56.2	0.23	242	56.2	100.0	0.23	190.8
8	std11	242	88.2	0.36	242	88.2	100.0	0.36	324.9
9	std12	242	98.5	0.41	242	98.5	100.0	0.41	368.0
10	std13	242	168.8	0.70	242	168.8	100.0	0.70	661.8
11	std14	242	231.4	0.96	242	231.4	100.0	0.96	924.0
12	std15	242	451.6	1.87	242	451.6	100.0	1.87	1845.1
13	1-1	895	80.4	0.09	475	52.5	53.1	0.11	67.7
14	1-20	1363	123.0	0.09	768	82.6	56.3	0.11	64.6
15	1-50	3186	425.6	0.13	2680	390.8	84.1	0.15	103.4
16	1-60	2540	443.7	0.17	2362	430.4	93.0	0.18	140.3
17	1-70	2988	435.6	0.15	2880	427.5	96.4	0.15	106.0
18	2-1	2195	143.1	0.07	340	35.1	15.5	0.10	60.2
19	2-10	3708	243.5	0.07	533	56.4	14.4	0.11	62.8
20	2-20	5102	358.8	0.07	940	98.9	18.4	0.11	62.3
21	2-30	4603	336.3	0.07	990	100.9	21.5	0.10	58.9
22	2-40	6936	441.5	0.06	533	51.9	7.7	0.10	54.3
23	2-50	6741	464.3	0.07	893	86.5	13.2	0.10	53.8

Table A-14
Film 14: R42-8

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
24	2-60	5560	396.3	0.07	828	81.0	14.9	0.10	54.8
25	2-70	7343	540.0	0.07	1622	163.1	22.1	0.10	57.6
26	2-80	6985	602.8	0.09	2986	322.4	42.7	0.11	65.1
27	2-90	7946	604.7	0.08	2015	209.6	25.4	0.10	61.1
28	2-110	8276	652.5	0.08	2590	270.1	31.3	0.10	61.3
29	2-120	9209	794.6	0.09	3919	428.7	42.6	0.11	66.5
30	2-130	7438	639.5	0.09	3059	338.4	41.1	0.11	67.8
31	3-1	1585	266.2	0.17	1104	235.0	69.7	0.21	171.3
32	3-10	1994	326.7	0.16	1627	300.3	81.6	0.18	142.6
33	3-20	2296	466.0	0.20	2185	457.4	95.2	0.21	167.7
34	3-30	2972	371.2	0.12	2344	324.4	78.9	0.14	95.9
35	3-40	3589	463.7	0.13	3162	432.3	88.1	0.14	94.2
36	3-50	4312	552.3	0.13	3492	496.2	81.0	0.14	99.6
37	3-60	5928	674.5	0.11	3735	546.7	63.0	0.15	103.9
38	3-70	4263	614.7	0.14	3728	576.1	87.5	0.15	112.2
39	3-80	5116	632.5	0.12	3859	545.3	75.4	0.14	98.8
40	3-90	5283	652.7	0.12	3816	549.7	72.2	0.14	101.6
41	3-100	5557	570.3	0.10	3052	400.2	54.9	0.13	88.5
42	3-120	4505	507.1	0.11	3087	405.9	68.5	0.13	88.9
43	3-130	4384	358.1	0.08	1444	167.4	32.9	0.12	73.1

Table A-15
Film 15: R42-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std5	242	17.4	0.07	31	2.9	12.8	0.09	37.6
2	std6	242	19.3	0.08	76	7.1	31.4	0.09	39.0
3	std7	242	24.9	0.10	230	23.9	95.0	0.10	52.8
4	std8	242	26.8	0.11	239	26.5	98.8	0.11	62.2
5	std9	242	45.8	0.19	242	45.8	100.0	0.19	170.2
6	std10	242	71.4	0.29	242	71.4	100.0	0.29	318.9
7	std12	242	86.7	0.36	242	86.7	100.0	0.36	409.6
8	std13	242	115.6	0.48	242	115.6	100.0	0.48	583.8
9	std14	242	224.4	0.93	242	224.4	100.0	0.93	1280.0
10	std15	242	276.3	1.14	242	276.3	100.0	1.14	1633.1
11	1-1	1107	141.9	0.13	856	124.4	77.3	0.15	109.4
12	1-10	2960	309.3	0.10	1973	245.0	66.7	0.12	80.4
13	1-20	3770	389.6	0.10	2474	302.3	65.6	0.12	77.7
14	1-40	5552	512.8	0.09	3141	340.2	56.6	0.11	58.8
15	1-60	8174	711.1	0.09	3619	390.2	44.3	0.11	58.1
16	1-80	8384	710.4	0.08	3574	379.3	42.6	0.11	55.8
17	1-100	10373	899.0	0.09	5002	529.7	48.2	0.11	55.4
18	1-120	9445	801.5	0.08	3996	429.1	42.3	0.11	57.5
19	3-1	1915	129.0	0.07	200	19.0	10.4	0.10	40.9
20	3-10	2741	220.5	0.08	994	97.5	36.3	0.10	44.8
21	3-40	5918	517.7	0.09	2995	305.5	50.6	0.10	50.1
22	3-50	7330	592.8	0.08	2589	261.9	35.3	0.10	49.0
23	3-60	6429	478.7	0.07	1192	120.5	18.5	0.10	48.9

Table A-15
Film 15: R42-12

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
24	3-70	6840	544.6	0.08	1966	202.9	28.7	0.10	51.7
25	3-80	7616	652.1	0.09	3088	328.5	40.5	0.11	56.1
26	3-90	8139	660.8	0.08	2481	266.6	30.5	0.11	57.6
27	3-100	8209	674.7	0.08	2747	296.0	33.5	0.11	58.0
28	3-110	9430	875.2	0.09	4932	557.4	52.3	0.11	65.2
29	3-130	10427	816.3	0.08	3130	341.8	30.0	0.11	59.9

Table A-16
Film 16: R42-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
1	std6	242	17.6	0.07	0	0.0	0.0	0.00	0.0
2	std7	242	23.0	0.09	40	4.6	16.5	0.11	75.6
3	std8	242	24.5	0.10	78	9.0	32.2	0.12	76.9
4	std9	242	40.7	0.17	241	40.6	99.6	0.17	134.0
5	std10	242	58.9	0.24	242	58.9	100.0	0.24	218.0
6	std11	242	88.2	0.36	242	88.2	100.0	0.36	358.6
7	std12	242	93.0	0.38	242	93.0	100.0	0.38	382.2
8	std13	242	169.7	0.70	242	169.7	100.0	0.70	766.2
9	std14	242	240.3	0.99	242	240.3	100.0	0.99	1107.8
10	std15	242	494.1	2.04	242	494.1	100.0	2.04	1719.2
11	1-1	506	43.5	0.09	74	8.5	14.6	0.11	76.3
12	1-21	1278	97.1	0.08	104	12.8	8.1	0.12	84.6
13	1-42	2146	163.7	0.08	219	26.1	10.2	0.12	80.8
14	1-62	3234	289.8	0.09	688	87.5	21.3	0.13	89.4
15	1-82	4199	362.8	0.09	746	91.0	17.8	0.12	83.8
16	1-102	3299	273.5	0.08	355	41.8	10.8	0.12	79.4
17	1-121	3279	248.1	0.08	136	15.5	4.1	0.11	74.9
18	1-141	3690	272.1	0.07	42	4.9	1.1	0.12	78.5
19	2-1	2069	530.3	0.26	1968	520.4	95.1	0.26	242.0
20	2-20	4939	865.0	0.18	3794	760.6	76.8	0.20	169.6
21	2-40	7578	1182.8	0.16	5322	983.1	70.2	0.18	152.1
22	2-60	6698	1129.7	0.17	5540	1023.0	82.7	0.18	152.0
23	2-80	9071	1754.5	0.19	7823	1639.2	86.2	0.21	179.7

Table A-16
Film 16: R42-24

Sample #	Slice	Tot. Pixels	Int. OD	Mean	Net Pixels	Int.OD	Area%	Mean	nCi/g
24	2-100	11085	2511.3	0.23	8874	2314.3	80.1	0.26	237.8
25	2-120	12279	2778.9	0.23	9896	2568.9	80.6	0.26	236.4
26	2-140	11985	2673.1	0.22	9916	2486.0	82.7	0.25	226.3
27	3-20	1880	152.9	0.08	68	7.7	3.6	0.11	74.6
28	3-40	4723	393.1	0.08	298	35.4	6.3	0.12	80.3
29	3-60	5088	393.7	0.08	96	11.5	1.9	0.12	81.2
30	3-80	6272	480.6	0.08	152	17.6	2.4	0.12	77.2
31	3-100	7917	622.7	0.08	425	50.2	5.4	0.12	79.6
32	3-120	9457	717.1	0.08	348	42.9	3.7	0.12	85.1
33	3-140	9935	793.2	0.08	587	70.9	5.9	0.12	82.6

APPENDIX B

AREA PERCENT TABLES

The Area Percent function of the In Situ/Grain program provides additional detail regarding the distribution of activity within images. This function partitions the total slice activity into ten equal ranges of transmission values and provides the number of pixels having an activity within each range. The output also shows the percent of total pixels in each range and the cumulative percent.

In order to use the Area Percent function, one must first load the desired image into the sample buffer and create a ROI as in the first part of the analysis described in Appendix A. When the ROI has been chosen, the operator then selects the Area Percent function which generates an ASCII table containing the information listed above. These tables were saved as separate files and stored to the optical disk.

The tables presented in this Appendix are an expanded version of the ASCII tables prepared using Excel. They provide additional data which we used to more precisely calculate the activity within tumor sections. Transmission values were converted to optical densities by the formula:

$$OD = -\log(I/I_0)$$

where I represents the TV for the upper and lower bounds of the range of TV's and I_0 is equal to 256 which is the TV corresponding to the maximum transmittal of light through the

film. The OD's then were converted to activities using the formula for the calibration curve determined previously. The mean activity for each range was calculated and appears in the tables as A_i . Total slice activity then was calculated by summing the products of the index activity and the fraction of total pixels for each range.

A representative section was chosen for each tumor and analyzed in the above fashion. The results of this analysis are shown in Tables B-1 through B-43 and are summarized and discussed in Chapter III.

Table B-1
M37-4-1-60

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1032.75	2293.21	1663
2	21	41	0.80	1.09	755.76	1032.75	894
3	41	61	0.62	0.80	591.27	755.76	674
4	61	81	0.50	0.62	473.87	591.27	533
5	81	101	0.40	0.50	382.51	473.87	428
6	101	121	0.33	0.40	307.71	382.51	345
7	121	141	0.26	0.33	244.38	307.71	276
8	141	161	0.20	0.26	189.47	244.38	217
9	161	181	0.15	0.20	140.99	189.47	165
10	181	210	0.09	0.15	79.46	140.99	110

$$nCi/g = -2.54 + 953.29(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1663	365	6.869942	6.869942	114.2459	
894	40	0.75287	7.622812	6.732592	
674	62	1.166949	8.789761	7.859602	
533	85	1.599849	10.38961	8.520361	
428	103	1.938641	12.32825	8.301096	
345	157	2.955016	15.28327	10.19812	
276	333	6.267645	21.55091	17.30167	
217	654	12.30943	33.86034	26.70211	
165	1316	24.76943	58.62978	40.92588	
110	2198	41.37022	100	45.6006	
Total =	5313				
				Activity = 286.3879 nCi/g	

Table B-2
M37-4-2-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1032.75	2293.21	1663
2	21	41	0.80	1.09	755.76	1032.75	894
3	41	61	0.62	0.80	591.27	755.76	674
4	61	81	0.50	0.62	473.87	591.27	533
5	81	101	0.40	0.50	382.51	473.87	428
6	101	121	0.33	0.40	307.71	382.51	345
7	121	141	0.26	0.33	244.38	307.71	276
8	141	161	0.20	0.26	189.47	244.38	217
9	161	181	0.15	0.20	140.99	189.47	165
10	181	210	0.09	0.15	79.46	140.99	110

$$\text{nCi/g} = -2.54 + 953.29(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
1663	445	5.97315	5.97315	99.3325	
894	201	2.69799	8.67114	24.1269	
674	234	3.14094	11.8121	21.1548	
533	300	4.02685	15.8389	21.4459	
428	444	5.95973	21.7987	25.5191	
345	689	9.24832	31.047	31.9171	
276	931	12.4966	43.5436	34.4966	
217	1136	15.2483	58.7919	33.0773	
165	940	12.6174	71.4094	20.8475	
110	2130	28.5906	100	31.5142	
Total =	7450				
				Activity = 343.432 nCi/g	

Table B-3
M37-4-3-50

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1032.75	2293.21	1663
2	21	41	0.80	1.09	755.76	1032.75	894
3	41	61	0.62	0.80	591.27	755.76	674
4	61	81	0.50	0.62	473.87	591.27	533
5	81	101	0.40	0.50	382.51	473.87	428
6	101	121	0.33	0.40	307.71	382.51	345
7	121	141	0.26	0.33	244.38	307.71	276
8	141	161	0.20	0.26	189.47	244.38	217
9	161	181	0.15	0.20	140.99	189.47	165
10	181	210	0.09	0.15	79.46	140.99	110

$$\text{nCi/g} = -2.54 + 953.29(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
1663	878	16.9106	16.9106	281.221	
894	145	2.79276	19.7034	24.9744	
674	177	3.40909	23.1125	22.9608	
533	234	4.50693	27.6194	24.0027	
428	261	5.02696	32.6464	21.525	
345	394	7.5886	40.235	26.1892	
276	574	11.0555	51.2904	30.5183	
217	569	10.9592	62.2496	23.7731	
165	690	13.2897	75.5393	21.9582	
110	1270	24.4607	100	26.962	
Total =	5192				
				Activity = 504.084 nCi/g	

Table B-4
M37-8-1-93

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	14	33	0.89	1.26	1013.05	1479.88	1246
2	33	52	0.69	0.89	765.47	1013.05	889
3	52	71	0.56	0.69	595.90	765.47	681
4	71	90	0.45	0.56	466.80	595.90	531
5	90	109	0.37	0.45	362.52	466.80	415
6	109	128	0.30	0.37	275.03	362.52	319
7	128	147	0.24	0.30	199.68	275.03	237
8	147	166	0.19	0.24	133.50	199.68	167
9	166	185	0.14	0.19	74.50	133.50	104
10	185	210	0.09	0.14	5.49	74.50	40

$$\text{nCi/g} = -102.35 + 1253.64(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
1246	21	0.82873	0.82873	10.3298	
889	96	3.78848	4.61721	33.6892	
681	141	5.56433	10.1815	37.8755	
531	201	7.93212	18.1137	42.1475	
415	257	10.1421	28.2557	42.0549	
319	283	11.1681	39.4238	35.6011	
237	278	10.9708	50.3946	26.0399	
167	240	9.47119	59.8658	15.7781	
104	221	8.72139	68.5872	9.07018	
40	796	31.4128	100	12.5632	
Total =	2534				
				Activity =	265.149 nCi/g

Table B-5
M37-8-2-126

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	30	47	0.74	0.93	820.51	1064.94	943
2	47	64	0.60	0.74	652.42	820.51	736
3	64	81	0.50	0.60	524.16	652.42	588
4	81	98	0.42	0.50	420.44	524.16	472
5	98	115	0.35	0.42	333.34	420.44	377
6	115	132	0.29	0.35	258.28	333.34	296
7	132	149	0.24	0.29	192.32	258.28	225
8	149	166	0.19	0.24	133.50	192.32	163
9	166	183	0.15	0.19	80.42	133.50	107
10	183	210	0.09	0.15	5.49	80.42	43

$$nCi/g = -102.35 + 1253.64(OD)$$

Ai	# pixels	%	cum %	Ai x %	
943	4	0.15582	0.15582	1.46899	
736	15	0.58434	0.74016	4.30344	
588	30	1.16868	1.90884	6.87522	
472	34	1.3245	3.23335	6.25562	
377	53	2.06467	5.29801	7.7815	
296	102	3.97351	9.27152	11.7541	
225	206	8.02493	17.2965	18.0803	
163	450	17.5302	34.8266	28.5586	
107	751	29.2559	64.0826	31.2916	
43	922	35.9174	100	15.4276	
Total =	2567				
			Activity = 131.797 nCi/g		

Table B-6
M37-8-3-94

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	12	31	0.92	1.33	1047.09	1563.81	1305
2	31	50	0.71	0.92	786.82	1047.09	917
3	50	69	0.57	0.71	611.46	786.82	699
4	69	88	0.46	0.57	479.03	611.46	545
5	88	107	0.38	0.46	372.60	479.03	426
6	107	126	0.31	0.38	283.61	372.60	328
7	126	145	0.25	0.31	207.14	283.61	245
8	145	164	0.19	0.25	140.10	207.14	174
9	164	183	0.15	0.19	80.42	140.10	110
10	183	210	0.09	0.15	5.49	80.42	43

$$\text{nCi/g} = -102.35 + 1253.64(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
1305	31	1.17025	1.17025	15.277	
917	54	2.03851	3.20876	18.6921	
699	91	3.43526	6.64402	24.0173	
545	135	5.09626	11.7403	27.7873	
426	149	5.62476	17.365	23.9512	
328	169	6.37977	23.7448	20.9322	
245	173	6.53077	30.2756	16.0247	
174	286	10.7965	41.0721	18.7448	
110	450	16.9875	58.0596	18.7301	
43	1111	41.9404	100	18.0146	
Total =	2649				
				Activity = 202.171 nCi/g	

Table B-7
M37-12-1-80

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1318.70	3058.12	2188
2	21	41	0.80	1.09	936.46	1318.70	1128
3	41	61	0.62	0.80	709.47	936.46	823
4	61	81	0.50	0.62	547.45	709.47	628
5	81	101	0.40	0.50	421.38	547.45	484
6	101	121	0.33	0.40	318.16	421.38	370
7	121	141	0.26	0.33	230.76	318.16	274
8	141	161	0.20	0.26	154.98	230.76	193
9	161	181	0.15	0.20	88.08	154.98	122
10	181	210	0.09	0.15	3.17	88.08	46

$$\text{nCi/g} = -109.99 + 1315.53(\text{OD})$$

A _i	# pixels	%	cum %	A _i x %	
2188	163	2.2642	2.2642	49.5501	
1128	209	2.90318	5.16738	32.7357	
823	307	4.26448	9.43187	35.095	
628	313	4.34783	13.7797	27.3243	
484	347	4.82011	18.5998	23.3493	
370	562	7.80664	26.4064	28.8663	
274	864	12.0017	38.4081	32.9395	
193	1308	18.1692	56.5773	35.0426	
122	1491	20.7112	77.2885	25.1697	
46	1635	22.7115	100	10.3622	
Total =	7199				
				Activity =	300.435 nCi/g

Table B-8
M37-12-2-120

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1318.70	3058.12	2188
2	21	41	0.80	1.09	936.46	1318.70	1128
3	41	61	0.62	0.80	709.47	936.46	823
4	61	81	0.50	0.62	547.45	709.47	628
5	81	101	0.40	0.50	421.38	547.45	484
6	101	121	0.33	0.40	318.16	421.38	370
7	121	141	0.26	0.33	230.76	318.16	274
8	141	161	0.20	0.26	154.98	230.76	193
9	161	181	0.15	0.20	88.08	154.98	122
10	181	210	0.09	0.15	3.17	88.08	46

$$nCi/g = -109.99 + 1315.53(OD)$$

Ai	# pixels	%	cum %	Ai x %	
2188	57	0.75277	0.75277	16.4738	
1128	59	0.77919	1.53196	8.78595	
823	92	1.215	2.74696	9.99901	
628	181	2.39039	5.13735	15.0226	
484	351	4.6355	9.77285	22.455	
370	673	8.88801	18.6609	32.8648	
274	1027	13.5631	32.224	37.225	
193	1392	18.3835	50.6075	35.4559	
122	1536	20.2853	70.8928	24.6521	
46	2204	29.1072	100	13.2803	
Total =	7572				
				Activity = 216.215 nCi/g	

Table B-9
M37-12-3-110

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1318.70	3058.12	2188
2	21	41	0.80	1.09	936.46	1318.70	1128
3	41	61	0.62	0.80	709.47	936.46	823
4	61	81	0.50	0.62	547.45	709.47	628
5	81	101	0.40	0.50	421.38	547.45	484
6	101	121	0.33	0.40	318.16	421.38	370
7	121	141	0.26	0.33	230.76	318.16	274
8	141	161	0.20	0.26	154.98	230.76	193
9	161	181	0.15	0.20	88.08	154.98	122
10	181	210	0.09	0.15	3.17	88.08	46

$$\text{nCi/g} = -109.99 + 1315.53(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
2188	93	1.34763	1.34763	29.4917	
1128	163	2.36198	3.70961	26.6332	
823	152	2.20258	5.91219	18.1264	
628	193	2.7967	8.70888	17.5761	
484	256	3.70961	12.4185	17.9699	
370	461	6.68019	19.0987	24.7011	
274	894	12.9546	32.0533	35.555	
193	1019	14.766	46.8193	28.4788	
122	715	10.3608	57.1801	12.5912	
46	2955	42.8199	100	19.5367	
Total =	6901				
				Activity =	230.66 nCi/g

Table B-10
M37-24-1-83

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	966.96	2173.51	1570
2	21	41	0.80	1.09	701.81	966.96	834
3	41	61	0.62	0.80	544.36	701.81	623
4	61	81	0.50	0.62	431.98	544.36	488
5	81	101	0.40	0.50	344.52	431.98	388
6	101	121	0.33	0.40	272.92	344.52	309
7	121	141	0.26	0.33	212.30	272.92	243
8	141	161	0.20	0.26	159.73	212.30	186
9	161	181	0.15	0.20	113.33	159.73	137
10	181	210	0.09	0.15	54.44	113.33	84

$$\text{nCi/g} = -24.06 + 912.52(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
1570	176	2.42826	2.42826	38.1292	
834	81	1.11755	3.54581	9.32464	
623	151	2.08333	5.62914	12.9809	
488	335	4.62196	10.2511	22.5629	
388	368	5.07726	15.3284	19.7125	
309	438	6.04305	21.3714	18.6563	
243	547	7.54691	28.9183	18.3098	
186	1000	13.7969	42.7152	25.6647	
137	1628	22.4614	65.1766	30.667	
84	2524	34.8234	100	29.2109	
Total =	7248				
			Activity = 225.219 nCi/g		

Table B-11
M37-24-2-130

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	25	43	0.77	1.01	682.93	897.86	790
2	43	61	0.62	0.77	544.36	682.93	614
3	61	79	0.51	0.62	441.88	544.36	493
4	79	97	0.42	0.51	360.54	441.88	401
5	97	115	0.35	0.42	293.08	360.54	327
6	115	133	0.28	0.35	235.45	293.08	264
7	133	151	0.23	0.28	185.15	235.45	210
8	151	169	0.18	0.23	140.52	185.15	163
9	169	187	0.14	0.18	100.41	140.52	120
10	187	210	0.09	0.14	54.44	100.41	77

$$nCi/g = -24.06 + 912.52(OD)$$

Ai	# pixels	%	cum %	Ai x %	
790	12	0.2741	0.2741	2.16646	
614	37	0.84513	1.11923	5.18614	
493	80	1.82732	2.94655	9.01089	
401	158	3.60895	6.5555	14.4795	
327	237	5.41343	11.9689	17.6916	
264	391	8.93102	20.9	23.6015	
210	610	13.9333	34.8333	29.3015	
163	635	14.5043	49.3376	23.6176	
120	887	20.2604	69.598	24.4059	
77	1331	30.402	100	23.5375	
Total =	4378				
			Activity = 172.999 nCi/g		

Table B-12
M37-24-3-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	7	27	0.98	1.56	867.36	1402.34	1135
2	27	47	0.74	0.98	647.68	867.36	758
3	47	67	0.58	0.74	507.18	647.68	577
4	67	87	0.47	0.58	403.66	507.18	455
5	87	107	0.38	0.47	321.65	403.66	363
6	107	127	0.30	0.38	253.74	321.65	288
7	127	147	0.24	0.30	195.79	253.74	225
8	147	167	0.19	0.24	145.23	195.79	171
9	167	187	0.14	0.19	100.41	145.23	123
10	187	210	0.09	0.14	54.44	100.41	77

$$nCi/g = -24.06 + 912.52(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1135	28	0.54623	0.54623	6.19894	
758	51	0.99493	1.54116	7.53679	
577	40	0.78034	2.3215	4.5059	
455	53	1.03394	3.35544	4.70876	
363	107	2.0874	5.44284	7.57006	
288	114	2.22396	7.6668	6.3983	
225	179	3.492	11.1588	7.84881	
171	443	8.64222	19.801	14.7359	
123	1216	23.7222	43.5232	29.1356	
77	2895	56.4768	100	43.7248	
Total =	5126				
			Activity = 132.364 nCi/g		

Table B-13
M42-4-1-100

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	22	1.07	2.41	1014.81	2291.72	1653
2	22	43	0.77	1.07	737.96	1014.81	876
3	43	64	0.60	0.77	573.68	737.96	656
4	64	85	0.48	0.60	456.45	573.68	515
5	85	106	0.38	0.48	365.25	456.45	411
6	106	127	0.30	0.38	290.58	365.25	328
7	127	148	0.24	0.30	227.36	290.58	259
8	148	169	0.18	0.24	172.55	227.36	200
9	169	190	0.13	0.18	124.17	172.55	148
10	190	215	0.08	0.13	73.10	124.17	99

$$nCi/g = 1 + 951.2(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1653	69	1.17467	1.17467	19.4203	
876	119	2.02588	3.20054	17.7545	
656	116	1.9748	5.17535	12.9512	
515	150	2.55363	7.72898	13.1529	
411	183	3.11542	10.8444	12.7997	
328	288	4.90296	15.7474	16.0775	
259	470	8.00136	23.7487	20.7213	
200	719	12.2404	35.9891	24.4757	
148	1343	22.8635	58.8526	33.9202	
99	2417	41.1474	100	40.5857	
Total =	5874				
			Activity = 211.859 nCi/g		

Table B-14
M42-4-2-114

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	22	1.07	2.41	1014.81	2291.72	1653
2	22	43	0.77	1.07	737.96	1014.81	876
3	43	64	0.60	0.77	573.68	737.96	656
4	64	85	0.48	0.60	456.45	573.68	515
5	85	106	0.38	0.48	365.25	456.45	411
6	106	127	0.30	0.38	290.58	365.25	328
7	127	148	0.24	0.30	227.36	290.58	259
8	148	169	0.18	0.24	172.55	227.36	200
9	169	190	0.13	0.18	124.17	172.55	148
10	190	215	0.08	0.13	73.10	124.17	99

$$nCi/g = 1 + 951.2(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1653	736	11.3266	11.3266	187.258	
876	134	2.06217	13.3887	18.0726	
656	198	3.04709	16.4358	19.9835	
515	220	3.38566	19.8215	17.4384	
411	214	3.29332	23.1148	13.5306	
328	249	3.83195	26.9468	12.5655	
259	351	5.40166	32.3484	13.9888	
200	635	9.77224	42.1207	19.5404	
148	1149	17.6824	59.803	26.2335	
99	2612	40.197	100	39.6483	
Total =	6498				
				Activity = 368.259 nCi/g	

Table B-15
M42-4-3-110

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	22	1.07	2.41	1014.81	2291.72	1653
2	22	43	0.77	1.07	737.96	1014.81	876
3	43	64	0.60	0.77	573.68	737.96	656
4	64	85	0.48	0.60	456.45	573.68	515
5	85	106	0.38	0.48	365.25	456.45	411
6	106	127	0.30	0.38	290.58	365.25	328
7	127	148	0.24	0.30	227.36	290.58	259
8	148	169	0.18	0.24	172.55	227.36	200
9	169	190	0.13	0.18	124.17	172.55	148
10	190	215	0.08	0.13	73.10	124.17	99

$$nCi/g = 1 + 951.2(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1653	796	6.78371	6.78371	112.152	
876	797	6.79223	13.5759	59.526	
656	778	6.63031	20.2062	43.4829	
515	643	5.4798	25.686	28.2247	
411	591	5.03665	30.7227	20.6931	
328	518	4.41452	35.1372	14.4758	
259	735	6.26385	41.4011	16.2216	
200	1100	9.37447	50.7755	18.745	
148	1566	13.3458	64.1214	19.7998	
99	4210	35.8786	100	35.3889	
Total =	11734				
				Activity =	368.71 nCi/g

Table B-16
M42-8-1-110

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	767.84	1698.81	1233
2	21	41	0.80	1.09	563.25	767.84	666
3	41	61	0.62	0.80	441.76	563.25	503
4	61	81	0.50	0.62	355.05	441.76	398
5	81	101	0.40	0.50	287.57	355.05	321
6	101	121	0.33	0.40	232.32	287.57	260
7	121	141	0.26	0.33	185.55	232.32	209
8	141	161	0.20	0.26	144.99	185.55	165
9	161	181	0.15	0.20	109.18	144.99	127
10	181	210	0.09	0.15	63.74	109.18	86

$$nCi/g = 3.17 + 704.1(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1233	1117	10.0758	10.0758	124.267	
666	1109	10.0036	20.0794	66.5784	
503	1056	9.52553	29.6049	47.8663	
398	1105	9.96753	39.5724	39.711	
321	1041	9.39022	48.9627	30.1716	
260	868	7.8297	56.7924	20.353	
209	752	6.78333	63.5757	14.1727	
165	621	5.60166	69.1773	9.25765	
127	639	5.76403	74.9414	7.3251	
86	2778	25.0586	100	21.6654	
Total =	11086				
				Activity = 381.368 nCi/g	

Table B-17
M42-8-2-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	767.84	1698.81	1233
2	21	41	0.80	1.09	563.25	767.84	666
3	41	61	0.62	0.80	441.76	563.25	503
4	61	81	0.50	0.62	355.05	441.76	398
5	81	101	0.40	0.50	287.57	355.05	321
6	101	121	0.33	0.40	232.32	287.57	260
7	121	141	0.26	0.33	185.55	232.32	209
8	141	161	0.20	0.26	144.99	185.55	165
9	161	181	0.15	0.20	109.18	144.99	127
10	181	210	0.09	0.15	63.74	109.18	86

$$nCi/g = 3.17 + 704.1(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1233	117	2.01031	2.01031	24.7936	
666	292	5.01718	7.02749	33.3915	
503	250	4.29553	11.323	21.5853	
398	254	4.36426	15.6873	17.3874	
321	268	4.60481	20.2921	14.7956	
260	375	6.4433	26.7354	16.7491	
209	641	11.0137	37.7491	23.0115	
165	870	14.9485	52.6976	24.7047	
127	1057	18.1615	70.8591	23.0802	
86	1696	29.1409	100	25.1948	
Total =	5820				
				Activity =	224.694 nCi/g

Table B-18
M42-8-3-182

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	11	30	0.93	1.37	658.77	965.57	812
2	30	49	0.72	0.93	508.74	658.77	584
3	49	68	0.58	0.72	408.54	508.74	459
4	68	87	0.47	0.58	333.20	408.54	371
5	87	106	0.38	0.47	272.79	333.20	303
6	106	125	0.31	0.38	222.38	272.79	248
7	125	144	0.25	0.31	179.11	222.38	201
8	144	163	0.20	0.25	141.21	179.11	160
9	163	182	0.15	0.20	107.50	141.21	124
10	182	210	0.09	0.15	63.74	107.50	86

$$\text{nCi/g} = 3.17 + 704.1(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
812	13	0.18813	0.18813	1.52796	
584	157	2.27207	2.4602	13.2634	
459	298	4.31259	6.77279	19.7794	
371	476	6.88857	13.6614	25.5476	
303	653	9.45007	23.1114	28.6333	
248	686	9.92764	33.0391	24.5794	
201	749	10.8394	43.8784	21.7593	
160	801	11.5919	55.4703	18.5655	
124	657	9.50796	64.9783	11.8234	
86	2420	35.0217	100	29.9843	
Total =	6910				
				Activity = 195.464 nCi/g	

Table B-19
M42-12-1-120

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	21	1.09	2.41	1292.28	2944.06	2118
2	21	41	0.80	1.09	929.29	1292.28	1111
3	41	61	0.62	0.80	713.74	929.29	822
4	61	81	0.50	0.62	559.89	713.74	637
5	81	101	0.40	0.50	440.17	559.89	500
6	101	121	0.33	0.40	342.14	440.17	391
7	121	141	0.26	0.33	259.15	342.14	301
8	141	161	0.20	0.26	187.19	259.15	223
9	161	181	0.15	0.20	123.66	187.19	155
10	181	210	0.09	0.15	43.03	123.66	83

$$\text{nCi/g} = -64.43 + 1249.25(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
2118	163	2.52831	2.52831	53.5539	
1111	596	9.24461	11.7729	102.688	
822	626	9.70994	21.4829	79.7688	
637	477	7.39879	28.8817	47.1166	
500	364	5.64604	34.5277	28.2317	
391	333	5.16519	39.6929	20.2039	
301	440	6.82488	46.5178	20.5189	
223	714	11.0749	57.5927	24.7158	
155	851	13.1999	70.7926	20.5157	
83	1883	29.2074	100	24.3429	
Total =	6447				
				Activity = 421.656 nCi/g	

Table B-20
M42-12-3-70

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	4	24	1.03	1.81	1219.83	2191.94	1706
2	24	44	0.76	1.03	890.98	1219.83	1055
3	44	64	0.60	0.76	687.69	890.98	789
4	64	84	0.48	0.60	540.16	687.69	614
5	84	104	0.39	0.48	424.28	540.16	482
6	104	124	0.31	0.39	328.86	424.28	377
7	124	144	0.25	0.31	247.73	328.86	288
8	144	164	0.19	0.25	177.17	247.73	212
9	164	184	0.14	0.19	114.74	177.17	146
10	184	210	0.09	0.14	43.03	114.74	79

$$\text{nCi/g} = -64.43 + 1249.25(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
1706	63	0.88633	0.88633	15.1197	
1055	166	2.3354	3.22172	24.648	
789	172	2.41981	5.64153	19.1004	
614	218	3.06697	8.7085	18.8289	
482	279	3.92515	12.6337	18.9279	
377	352	4.95217	17.5858	18.6484	
288	447	6.28869	23.8745	18.1299	
212	731	10.2842	34.1587	21.8487	
146	1302	18.3174	52.4761	26.7352	
79	3378	47.5239	100	37.4896	
Total =	7108				
					Activity = 219.477 nCi/g

Table B-21
M42-24-1-70

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	22	1.07	2.41	989.34	2251.17	1620
2	22	43	0.77	1.07	715.76	989.34	853
3	43	64	0.60	0.77	553.42	715.76	635
4	64	85	0.48	0.60	437.58	553.42	495
5	85	106	0.38	0.48	347.45	437.58	393
6	106	127	0.30	0.38	273.66	347.45	311
7	127	148	0.24	0.30	211.19	273.66	242
8	148	169	0.18	0.24	157.03	211.19	184
9	169	190	0.13	0.18	109.21	157.03	133
10	190	215	0.08	0.13	58.75	109.21	84

$$nCi/g = -12.5 + 939.97(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1620	185	2.54365	2.54365	41.2137	
853	714	9.81713	12.3608	83.6959	
635	794	10.9171	23.2779	69.2788	
495	717	9.85838	33.1363	48.8481	
393	591	8.12595	41.2622	31.8953	
311	610	8.38719	49.6494	26.0467	
242	594	8.16719	57.8166	19.7995	
184	672	9.23965	67.0562	17.0111	
133	798	10.9721	78.0283	14.606	
84	1598	21.9717	100	18.4523	
Total =	7273				
				Activity = 370.847 nCi/g	

Table B-22
M42-24-2-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	22	1.07	2.41	989.34	2251.17	1620
2	22	43	0.77	1.07	715.76	989.34	853
3	43	64	0.60	0.77	553.42	715.76	635
4	64	85	0.48	0.60	437.58	553.42	495
5	85	106	0.38	0.48	347.45	437.58	393
6	106	127	0.30	0.38	273.66	347.45	311
7	127	148	0.24	0.30	211.19	273.66	242
8	148	169	0.18	0.24	157.03	211.19	184
9	169	190	0.13	0.18	109.21	157.03	133
10	190	215	0.08	0.13	58.75	109.21	84

$$\text{nCi/g} = -12.5 + 939.97(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
1620	591	8.49504	8.49504	137.641	
853	1150	16.5301	25.0252	140.927	
635	1052	15.1215	40.1466	95.9593	
495	785	11.2836	51.4302	55.91	
393	591	8.49504	59.9253	33.3441	
311	554	7.9632	67.8885	24.73	
242	575	8.26506	76.1535	20.0367	
184	457	6.56892	82.7224	12.094	
133	398	5.72086	88.4433	7.6156	
84	804	11.5567	100	9.70558	
Total =	6957				
				Activity = 537.964 nCi/g	

Table B-23
M42-24-3-120

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	7	27	0.98	1.56	905.73	1456.81	1181
2	27	47	0.74	0.98	679.45	905.73	793
3	47	67	0.58	0.74	534.72	679.45	607
4	67	87	0.47	0.58	428.08	534.72	481
5	87	107	0.38	0.47	343.61	428.08	386
6	107	127	0.30	0.38	273.66	343.61	309
7	127	147	0.24	0.30	213.96	273.66	244
8	147	167	0.19	0.24	161.89	213.96	188
9	167	187	0.14	0.19	115.71	161.89	139
10	187	215	0.08	0.14	58.75	115.71	87

$$nCi/g = -12.5 + 939.97(OD)$$

Ai	# pixels	%	cum %	Ai x %	
1181	147	2.17939	2.17939	25.7445	
793	490	7.26464	9.44403	57.579	
607	566	8.3914	17.8354	50.9429	
481	483	7.16086	24.9963	34.4724	
386	438	6.4937	31.49	25.0558	
309	515	7.63529	39.1253	23.5653	
244	603	8.93996	48.0652	21.7965	
188	667	9.88881	57.954	18.5834	
139	934	13.8473	71.8013	19.2198	
87	1902	28.1987	100	24.5979	
Total =	6745				
				Activity = 301.558 nCi/g	

Table B-24
R37-4-1-123

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	123	132	0.29	0.32	278.25	312.76	296
2	132	141	0.26	0.29	246.25	278.25	262
3	141	150	0.23	0.26	216.45	246.25	231
4	150	159	0.21	0.23	188.58	216.45	203
5	159	168	0.18	0.21	162.41	188.58	175
6	168	177	0.16	0.18	137.76	162.41	150
7	177	186	0.14	0.16	114.47	137.76	126
8	186	195	0.12	0.14	92.40	114.47	103
9	195	204	0.10	0.12	71.45	92.40	82
10	204	215	0.08	0.10	47.19	71.45	59

$$\text{nCi/g} = -32.34 + 1038.3(\text{OD}) + 143.83(\text{OD})^2$$

Ai	# pixels	%	cum %	Ai x %	
296	1	0.13514	0.13514	0.39933	
262	0	0	0.13514	0	
231	0	0	0.13514	0	
203	1	0.13514	0.27027	0.27367	
175	0	0	0.27027	0	
150	2	0.27027	0.54054	0.40563	
126	12	1.62162	2.16216	2.04509	
103	41	5.54054	7.7027	5.73087	
82	120	16.2162	23.9189	13.2849	
59	563	76.0811	100	45.1299	
Total =	740				
			Activity = 67.2694 nCi/g		

Table B-25
R37-4-2-91

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	120	129	0.30	0.33	289.45	324.90	307
2	129	138	0.27	0.30	256.66	289.45	273
3	138	147	0.24	0.27	226.16	256.66	241
4	147	156	0.22	0.24	197.67	226.16	212
5	156	165	0.19	0.22	170.96	197.67	184
6	165	174	0.17	0.19	145.82	170.96	158
7	174	183	0.15	0.17	122.09	145.82	134
8	183	192	0.12	0.15	99.63	122.09	111
9	192	201	0.11	0.12	78.31	99.63	89
10	201	215	0.08	0.11	47.19	78.31	63

$$\text{nCi/g} = -32.34 + 1038.3(\text{OD}) + 143.83(\text{OD})^2$$

Ai	# pixels	%	cum %	Ai x %	
307	5	0.22292	0.22292	0.68474	
273	6	0.2675	0.49041	0.73042	
241	13	0.57958	1.07	1.39915	
212	19	0.84708	1.91708	1.79508	
184	57	2.54124	4.45831	4.68383	
158	134	5.97414	10.4325	9.46225	
134	207	9.22871	19.6612	12.3622	
111	264	11.77	31.4311	13.0481	
89	420	18.7249	50.156	16.6599	
63	1118	49.844	100	31.2784	
Total =	2243				
			Activity = 92.104 nCi/g		

Table B-26
R37-4-3-31

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	113	123	0.32	0.36	312.76	354.57	334
2	123	133	0.28	0.32	274.57	312.76	294
3	133	143	0.25	0.28	239.45	274.57	257
4	143	153	0.22	0.25	206.96	239.45	223
5	153	163	0.20	0.22	176.75	206.96	192
6	163	173	0.17	0.20	148.54	176.75	163
7	173	183	0.15	0.17	122.09	148.54	135
8	183	193	0.12	0.15	97.21	122.09	110
9	193	203	0.10	0.12	73.72	97.21	85
10	203	215	0.08	0.10	47.19	73.72	60

$$\text{nCi/g} = -32.34 + 1038.3(\text{OD}) + 143.83(\text{OD})^2$$

Ai	# pixels	%	cum %	Ai x %	
334	1	0.25575	0.25575	0.85336	
294	0	0	0.25575	0	
257	0	0	0.25575	0	
223	1	0.25575	0.51151	0.57085	
192	0	0	0.51151	0	
163	2	0.51151	1.02302	0.83194	
135	1	0.25575	1.27877	0.34607	
110	16	4.09207	5.37084	4.48687	
85	72	18.4143	23.7852	15.7377	
60	298	76.2148	100	46.0769	
Total =	391				
				Activity = 68.9037 nCi/g	

Table B-27
R37-8-1-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	100	111	0.36	0.41	414.80	480.80	448
2	111	122	0.32	0.36	356.53	414.80	386
3	122	133	0.28	0.32	304.70	356.53	331
4	133	144	0.25	0.28	258.28	304.70	281
5	144	155	0.22	0.25	216.49	258.28	237
6	155	166	0.19	0.22	178.66	216.49	198
7	166	177	0.16	0.19	144.30	178.66	161
8	177	188	0.13	0.16	112.96	144.30	129
9	188	199	0.11	0.13	84.30	112.96	99
10	199	215	0.08	0.11	46.77	84.30	66

$$\text{nCi/g} = -31.3 + 966.0(\text{OD}) + 875.4(\text{OD})^2 - 413.79(\text{OD})^3$$

Ai	# pixels	%	cum %	Ai x %	
448	2	0.07943	0.07943	0.35568	
386	1	0.03971	0.11914	0.15316	
331	0	0	0.11914	0	
281	4	0.15886	0.278	0.44717	
237	6	0.23828	0.51628	0.56565	
198	5	0.19857	0.71485	0.39232	
161	24	0.95314	1.66799	1.53914	
129	134	5.32168	6.98967	6.84536	
99	415	16.4813	23.471	16.2559	
66	1927	76.529	100	50.1551	
Total =	2518				
				Activity = 76.7095	nCi/g

Table B-28
R37-8-2-150

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	124	133	0.28	0.31	304.70	346.66	326
2	133	142	0.26	0.28	266.36	304.70	286
3	142	151	0.23	0.26	231.19	266.36	249
4	151	160	0.20	0.23	198.83	231.19	215
5	160	169	0.18	0.20	168.97	198.83	184
6	169	178	0.16	0.18	141.33	168.97	155
7	178	187	0.14	0.16	115.70	141.33	129
8	187	196	0.12	0.14	91.87	115.70	104
9	196	205	0.10	0.12	69.68	91.87	81
10	205	215	0.08	0.10	46.77	69.68	58

$$\text{nCi/g} = -31.3 + 966.0(\text{OD}) + 875.4(\text{OD})^2 - 413.79(\text{OD})^3$$

Ai	# pixels	%	cum %	Ai x %	
326	2	0.04156	0.04156	0.13536	
286	8	0.16625	0.20781	0.4747	
249	41	0.85204	1.05985	2.11967	
215	110	2.28595	3.3458	4.91512	
184	206	4.28096	7.62677	7.87275	
155	334	6.94098	14.5677	10.7689	
129	573	11.9077	26.4755	15.3031	
104	786	16.3342	42.8096	16.9523	
81	1069	22.2153	65.0249	17.9449	
58	1683	34.9751	100	20.3655	
Total =	4812				
				Activity =	96.8523 nCi/g

Table B-29
R37-8-3-153

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	114	124	0.31	0.35	346.66	398.20	372
2	124	134	0.28	0.31	300.27	346.66	323
3	134	144	0.25	0.28	258.28	300.27	279
4	144	154	0.22	0.25	220.11	258.28	239
5	154	164	0.19	0.22	185.27	220.11	203
6	164	174	0.17	0.19	153.35	185.27	169
7	174	184	0.14	0.17	124.03	153.35	139
8	184	194	0.12	0.14	97.02	124.03	111
9	194	204	0.10	0.12	72.07	97.02	85
10	204	215	0.08	0.10	46.77	72.07	59

$$nCi/g = -31.3 + 966.0(OD) + 875.4(OD)^2 - 413.79(OD)^3$$

Ai	# pixels	%	cum %	Ai x %	
372	4	0.14981	0.14981	0.55795	
323	5	0.18727	0.33708	0.60575	
279	3	0.11236	0.44944	0.3138	
239	7	0.26217	0.71161	0.62711	
203	19	0.71161	1.42322	1.44237	
169	74	2.77154	4.19476	4.69254	
139	231	8.65169	12.8464	11.9993	
111	436	16.3296	29.176	18.0483	
85	659	24.6816	53.8577	20.8672	
59	1232	46.1423	100	27.4193	
Total =	2670				
				Activity = 86.5736 nCi/g	

Table B-30
R37-12-1-140

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	139	146	0.24	0.27	201.24	220.42	211
2	146	153	0.22	0.24	182.96	201.24	192
3	153	160	0.20	0.22	165.50	182.96	174
4	160	167	0.19	0.20	148.78	165.50	157
5	167	174	0.17	0.19	132.75	148.78	141
6	174	181	0.15	0.17	117.35	132.75	125
7	181	188	0.13	0.15	102.54	117.35	110
8	188	195	0.12	0.13	88.27	102.54	95
9	195	202	0.10	0.12	74.50	88.27	81
10	202	210	0.09	0.10	59.34	74.50	67

$$\text{nCi/g} = -17.98 + 898.86(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
211	4	0.27082	0.27082	0.57097	
192	4	0.27082	0.54164	0.52024	
174	1	0.0677	0.60934	0.11796	
157	2	0.13541	0.74475	0.21278	
141	3	0.20311	0.94787	0.28591	
125	25	1.69262	2.64049	2.11666	
110	68	4.60393	7.24441	5.06189	
95	161	10.9005	18.1449	10.3997	
81	322	21.8009	39.9458	17.743	
67	887	60.0542	100	40.1891	
Total =	1477				
				Activity = 77.2182 nCi/g	

Table B-31
R37-24-1-140

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	181	184	0.14	0.15	285.53	302.47	294
2	184	187	0.14	0.14	268.86	285.53	277
3	187	190	0.13	0.14	252.45	268.86	261
4	190	193	0.12	0.13	236.30	252.45	244
5	193	196	0.12	0.12	220.40	236.30	228
6	196	199	0.11	0.12	204.74	220.40	213
7	199	202	0.10	0.11	189.32	204.74	197
8	202	205	0.10	0.10	174.12	189.32	182
9	205	208	0.09	0.10	159.15	174.12	167
10	208	220	0.07	0.09	101.33	159.15	130

$$nCi/g = -54.89 + 2373.54(OD)$$

Ai	# pixels	%	cum %	Ai x %	
294	1	0.15432	0.15432	0.4537	
277	0	0	0.15432	0	
261	1	0.15432	0.30864	0.40224	
244	0	0	0.30864	0	
228	1	0.15432	0.46296	0.3524	
213	3	0.46296	0.92593	0.98414	
197	0	0	0.92593	0	
182	1	0.15432	1.08025	0.28044	
167	1	0.15432	1.23457	0.25715	
130	640	98.7654	100	128.631	
Total =	648				
			Activity = 131.361 nCi/g		

Table B-32
R37-24-3-61

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	207	208	0.09	0.09	159.15	164.12	162
2	208	209	0.09	0.09	154.20	159.15	157
3	209	210	0.09	0.09	149.28	154.20	152
4	210	211	0.08	0.09	144.39	149.28	147
5	211	212	0.08	0.08	139.51	144.39	142
6	212	213	0.08	0.08	134.66	139.51	137
7	213	214	0.08	0.08	129.83	134.66	132
8	214	215	0.08	0.08	125.03	129.83	127
9	215	216	0.07	0.08	120.24	125.03	123
10	216	220	0.07	0.07	101.33	120.24	111

$$\text{nCi/g} = -54.89 + 2373.54(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
162	1	0.3861	0.3861	0.62406	
157	0	0	0.3861	0	
152	0	0	0.3861	0	
147	0	0	0.3861	0	
142	0	0	0.3861	0	
137	2	0.7722	1.1583	1.05859	
132	2	0.7722	1.9305	1.02122	
127	3	1.1583	3.0888	1.47603	
123	8	3.0888	6.17761	3.78799	
111	243	93.8224	100	103.943	
Total =	259				
				Activity = 111.911 nCi/g	

Table B-33
R42-4-1-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	101	112	0.36	0.40	309.67	349.93	330
2	112	123	0.32	0.36	273.18	309.67	291
3	123	134	0.28	0.32	239.82	273.18	257
4	134	145	0.25	0.28	209.09	239.82	224
5	145	156	0.22	0.25	180.62	209.09	195
6	156	167	0.19	0.22	154.08	180.62	167
7	167	178	0.16	0.19	129.23	154.08	142
8	178	189	0.13	0.16	105.88	129.23	118
9	189	200	0.11	0.13	83.85	105.88	95
10	200	215	0.08	0.11	55.68	83.85	70

$$\text{nCi/g} = -12.3 + 896.8(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
330	4	0.0693	0.0693	0.22855	
291	9	0.15593	0.22523	0.45441	
257	40	0.693	0.91823	1.77756	
224	96	1.6632	2.58143	3.7332	
195	192	3.3264	5.90783	6.48167	
167	421	7.29383	13.2017	12.206	
142	526	9.11296	22.3146	12.909	
118	728	12.6126	34.9272	14.8269	
95	821	14.2238	49.1511	13.4931	
70	2935	50.8489	100	35.4734	
Total =	5772				
				Activity =	101.584 nCi/g

Table B-34
R42-4-2-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	35	52	0.69	0.86	608.50	762.69	686
2	52	69	0.57	0.69	498.33	608.50	553
3	69	86	0.47	0.57	412.55	498.33	455
4	86	103	0.40	0.47	342.30	412.55	377
5	103	120	0.33	0.40	282.80	342.30	313
6	120	137	0.27	0.33	231.20	282.80	257
7	137	154	0.22	0.27	185.64	231.20	208
8	154	171	0.18	0.22	144.86	185.64	165
9	171	188	0.13	0.18	107.94	144.86	126
10	188	215	0.08	0.13	55.68	107.94	82

$$\text{nCi/g} = -12.3 + 896.8(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
686	9	0.07555	0.07555	0.51799	
553	19	0.1595	0.23506	0.88271	
455	37	0.31061	0.54567	1.41465	
377	171	1.43553	1.9812	5.41803	
313	384	3.22364	5.20484	10.0754	
257	690	5.79248	10.9973	14.8866	
208	979	8.2186	19.2159	17.1292	
165	1304	10.9469	30.1629	18.0898	
126	2279	19.132	49.2948	24.1831	
82	6040	50.7052	100	41.4828	
Total =	11912				
			Activity = 134.08 nCi/g		

Table B-35
R42-4-3-80

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	114	124	0.31	0.35	270.03	302.78	286
2	124	134	0.28	0.31	239.82	270.03	255
3	134	144	0.25	0.28	211.79	239.82	226
4	144	154	0.22	0.25	185.64	211.79	199
5	154	164	0.19	0.22	161.14	185.64	173
6	164	174	0.17	0.19	138.09	161.14	150
7	174	184	0.14	0.17	116.32	138.09	127
8	184	194	0.12	0.14	95.71	116.32	106
9	194	204	0.10	0.12	76.13	95.71	86
10	204	215	0.08	0.10	55.68	76.13	66

$$\text{nCi/g} = -12.3 + 896.8(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
286	2	0.03527	0.03527	0.10102	
255	3	0.05291	0.08818	0.13488	
226	4	0.07055	0.15873	0.1593	
199	38	0.67019	0.82892	1.33178	
173	100	1.76367	2.59259	3.05801	
150	230	4.05644	6.64903	6.06889	
127	399	7.03704	13.6861	8.95132	
106	751	13.2451	26.9312	14.0418	
86	1520	26.8078	53.739	23.0335	
66	2623	46.261	100	30.4888	
Total =	5670				
			Activity = 87.3694 nCi/g		

Table B-36
R42-8-1-70

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	124	132	0.29	0.31	247.01	274.50	261
2	132	140	0.26	0.29	221.14	247.01	234
3	140	148	0.24	0.26	196.70	221.14	209
4	148	156	0.22	0.24	173.55	196.70	185
5	156	164	0.19	0.22	151.56	173.55	163
6	164	172	0.17	0.19	130.62	151.56	141
7	172	180	0.15	0.17	110.63	130.62	121
8	180	188	0.13	0.15	91.51	110.63	101
9	188	196	0.12	0.13	73.19	91.51	82
10	196	210	0.09	0.12	42.85	73.19	58

$$nCi/g = -44.24 + 1012.45(OD)$$

Ai	# pixels	%	cum %	Ai x %	
261	3	0.09119	0.09119	0.23777	
234	9	0.27356	0.36474	0.64032	
209	17	0.51672	0.88146	1.07952	
185	72	2.18845	3.06991	4.05142	
163	181	5.50152	8.57143	8.9432	
141	345	10.4863	19.0578	14.7954	
121	549	16.6869	35.7447	20.1289	
101	681	20.6991	56.4438	20.9209	
82	559	16.9909	73.4347	13.9919	
58	874	26.5653	100	15.4132	
Total =	3290				
			Activity = 100.203 nCi/g		

Table B-37
R42-8-2-130

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	122	130	0.29	0.32	253.72	281.65	268
2	130	138	0.27	0.29	227.46	253.72	241
3	138	146	0.24	0.27	202.68	227.46	215
4	146	154	0.22	0.24	179.23	202.68	191
5	154	162	0.20	0.22	156.96	179.23	168
6	162	170	0.18	0.20	135.76	156.96	146
7	170	178	0.16	0.18	115.54	135.76	126
8	178	186	0.14	0.16	96.21	115.54	106
9	186	194	0.12	0.14	77.70	96.21	87
10	194	210	0.09	0.12	42.85	77.70	60

$$nCi/g = -44.24 + 1012.45(OD)$$

Ai	# pixels	%	cum %	Ai x %	
268	1	0.03283	0.03283	0.08788	
241	3	0.09849	0.13132	0.23696	
215	4	0.13132	0.26264	0.28243	
191	10	0.3283	0.59094	0.62691	
168	33	1.08339	1.67433	1.8211	
146	36	1.18188	2.8562	1.72982	
126	79	2.59357	5.44977	3.25894	
106	187	6.1392	11.589	6.50015	
87	375	12.3112	23.9002	10.7053	
60	2318	76.0998	100	45.8689	
Total =	3046				
			Activity = 71.1184 nCi/g		

Table B-38
R42-8-3-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	102	112	0.36	0.40	319.25	360.38	340
2	112	122	0.32	0.36	281.65	319.25	300
3	122	132	0.29	0.32	247.01	281.65	264
4	132	142	0.26	0.29	214.90	247.01	231
5	142	152	0.23	0.26	184.98	214.90	200
6	152	162	0.20	0.23	156.96	184.98	171
7	162	172	0.17	0.20	130.62	156.96	144
8	172	182	0.15	0.17	105.77	130.62	118
9	182	192	0.12	0.15	82.25	105.77	94
10	192	210	0.09	0.12	42.85	82.25	63

$$\text{nCi/g} = -44.24 + 1012.45(\text{OD})$$

Ai	# pixels	%	cum %	Ai x %	
340	3	0.07796	0.07796	0.26493	
300	12	0.31185	0.38981	0.93695	
264	50	1.29938	1.68919	3.43461	
231	103	2.67672	4.3659	6.18195	
200	193	5.01559	9.3815	10.028	
171	248	6.44491	15.8264	11.0187	
144	309	8.03015	23.8565	11.5466	
118	425	11.0447	34.9012	13.0546	
94	640	16.632	51.5333	15.6364	
63	1865	48.4667	100	30.3174	
Total =	3848				
			Activity = 102.42 nCi/g		

Table B-39
R42-12-1-100

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	105	115	0.35	0.39	394.11	451.19	423
2	115	125	0.31	0.35	342.22	394.11	368
3	125	135	0.28	0.31	294.68	342.22	318
4	135	145	0.25	0.28	250.85	294.68	273
5	145	155	0.22	0.25	210.20	250.85	231
6	155	165	0.19	0.22	172.33	210.20	191
7	165	175	0.17	0.19	136.90	172.33	155
8	175	185	0.14	0.17	103.62	136.90	120
9	185	195	0.12	0.14	72.26	103.62	88
10	195	210	0.09	0.12	28.38	72.26	50

$$\text{nCi/g} = -87.33 + 1331.98(\text{OD}) + 153.36(\text{OD})^2$$

Ai	# pixels	%	cum %	Ai x %	
423	1	0.01879	0.01879	0.07943	
368	1	0.01879	0.03759	0.06919	
318	0	0	0.03759	0	
273	0	0	0.03759	0	
231	1	0.01879	0.05638	0.04332	
191	2	0.03759	0.09397	0.07189	
155	23	0.43225	0.52622	0.66833	
120	215	4.04059	4.56681	4.85928	
88	700	13.1554	17.7222	11.569	
50	4378	82.2778	100	41.4032	
Total =	5321				
				Activity = 58.7637 nCi/g	

Table B-40
R42-12-3-90

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	119	128	0.30	0.33	327.53	372.79	350
2	128	137	0.27	0.30	285.63	327.53	307
3	137	146	0.24	0.27	246.64	285.63	266
4	146	155	0.22	0.24	210.20	246.64	228
5	155	164	0.19	0.22	176.01	210.20	193
6	164	173	0.17	0.19	143.81	176.01	160
7	173	182	0.15	0.17	113.39	143.81	129
8	182	191	0.13	0.15	84.59	113.39	99
9	191	200	0.11	0.13	57.23	84.59	71
10	200	210	0.09	0.11	28.38	57.23	43

$$\text{nCi/g} = -87.33 + 1331.98(\text{OD}) + 153.36(\text{OD})^2$$

Ai	# pixels	%	cum %	Ai x %	
350	2	0.07189	0.07189	0.25173	
307	3	0.10784	0.17973	0.33061	
266	9	0.32351	0.50324	0.86098	
228	24	0.86269	1.36592	1.97058	
193	44	1.5816	2.94752	3.05412	
160	48	1.72538	4.6729	2.75899	
129	84	3.01941	7.69231	3.88298	
99	184	6.61395	14.3063	6.54723	
71	368	13.2279	27.5341	9.38006	
43	2016	72.4659	100	31.0215	
Total =	2782				
			Activity = 60.0588 nCi/g		

Table B-41
R42-24-1-82

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	125	132	0.29	0.31	268.72	296.20	282
2	132	139	0.27	0.29	242.88	268.72	256
3	139	146	0.24	0.27	218.52	242.88	231
4	146	153	0.22	0.24	195.49	218.52	207
5	153	160	0.20	0.22	173.69	195.49	185
6	160	167	0.19	0.20	153.01	173.69	163
7	167	174	0.17	0.19	133.35	153.01	143
8	174	181	0.15	0.17	114.64	133.35	124
9	181	188	0.13	0.15	96.81	114.64	106
10	188	200	0.11	0.13	68.08	96.81	82

$$\text{nCi/g} = -41.54 + 982.32(\text{OD}) + 399.05(\text{OD})^2 - 224.21(\text{OD})^3$$

Ai	# pixels	%	cum %	Ai x %	
282	2	0.2139	0.2139	0.6042	
256	0	0	0.2139	0	
231	0	0	0.2139	0	
207	2	0.2139	0.42781	0.44279	
185	2	0.2139	0.64171	0.39485	
163	4	0.42781	1.06952	0.69882	
143	6	0.64171	1.71123	0.91879	
124	41	4.38503	6.09626	5.43722	
106	94	10.0535	16.1497	10.6288	
82	784	83.8503	100	69.1304	
Total =	935				
					Activity = 88.2559 nCi/g

Table B-42
R42-24-2-100

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	1	20	1.11	2.41	1230.97	1506.95	1369
2	20	39	0.82	1.11	905.31	1230.97	1068
3	39	58	0.64	0.82	697.68	905.31	801
4	58	77	0.52	0.64	547.77	697.68	623
5	77	96	0.43	0.52	431.98	547.77	490
6	96	115	0.35	0.43	338.65	431.98	385
7	115	134	0.28	0.35	261.18	338.65	300
8	134	153	0.22	0.28	195.49	261.18	228
9	153	172	0.17	0.22	138.87	195.49	167
10	172	200	0.11	0.17	68.08	138.87	103

$$\text{nCi/g} = -41.54 + 982.32(\text{OD}) + 399.05(\text{OD})^2 - 224.21(\text{OD})^3$$

Ai	# pixels	%	cum %	Ai x %	
1369	206	2.26797	2.26797	31.0476	
1068	159	1.75052	4.0185	18.698	
801	147	1.61841	5.6369	12.9715	
623	147	1.61841	7.25531	10.0782	
490	203	2.23494	9.49026	10.9484	
385	395	4.34878	13.839	16.7563	
300	694	7.64065	21.4797	22.9154	
228	1095	12.0555	33.5352	27.5273	
167	1777	19.564	53.0992	32.7071	
103	4260	46.9008	100	48.5309	
Total =	9083				
			Activity = 232.181 nCi/g		

Table B-43
R42-24-3-100

#	Transmission Value		Optical Density		nCi/g		
	Lower	Upper	Lower	Upper	Lower	Upper	Mean
1	127	134	0.28	0.30	261.18	288.17	275
2	134	141	0.26	0.28	235.78	261.18	248
3	141	148	0.24	0.26	211.81	235.78	224
4	148	155	0.22	0.24	189.14	211.81	200
5	155	162	0.20	0.22	167.67	189.14	178
6	162	169	0.18	0.20	147.29	167.67	157
7	169	176	0.16	0.18	127.91	147.29	138
8	176	183	0.15	0.16	109.46	127.91	119
9	183	190	0.13	0.15	91.86	109.46	101
10	190	200	0.11	0.13	68.08	91.86	80

$$\text{nCi/g} = -41.54 + 982.32(\text{OD}) + 399.05(\text{OD})^2 - 224.21(\text{OD})^3$$

Ai	# pixels	%	cum %	Ai x %	
275	1	0.15898	0.15898	0.43669	
248	1	0.15898	0.31797	0.39504	
224	1	0.15898	0.47695	0.35579	
200	1	0.15898	0.63593	0.31872	
178	2	0.31797	0.9539	0.56727	
157	0	0	0.9539	0	
138	5	0.79491	1.74881	1.0938	
119	13	2.06677	3.81558	2.45294	
101	57	9.062	12.8776	9.12178	
80	548	87.1224	100	69.6744	
Total =	629				
			Activity = 84.4164 nCi/g		

APPENDIX C

CUMULATIVE FREQUENCY TABLES

In order to evaluate the homogeneity of Mab distribution, we fit the data from each area percent table to a function describing the cumulative frequency of pixels versus specific activity in nCi/g. Using Cricket Graph statistical software, curves were fit to these data which were of the general form:

$$\% \text{pixels}_{\text{cum}} = a + b \log(A_i),$$

where A_i is the index activity and a and b are coefficients determined by the software. Using these formulae, we calculated the cumulative percent of pixels for theoretical activities ranging from 50 to 1000 nCi/g. These percentages then were subtracted from 100, yielding the percent of pixels having an activity concentration greater than the index activity. Tables C-1 through C-8 show the results of these calculations for each tumor along with the mean and standard deviation for each temperature/MAb/time combination. These results are summarized and discussed in Chapter III.

Table C-1
Calculated Cumulative Frequency (4 hr)
Mel-14 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	M37-4-1 c.f.	M37-4-2 c.f.	M37-4-3 c.f.	Mean M37-4	S.D. M37-4	%>Ai M37-4
50	44.94	16.55	8.96	23.48	18.97	76.52
100	57.60	35.76	27.89	40.42	15.39	59.58
150	65.01	47.00	38.97	50.32	13.34	49.68
200	70.26	54.97	46.82	57.35	11.90	42.65
250	74.34	61.15	52.92	62.80	10.81	37.20
300	77.67	66.21	57.90	67.26	9.93	32.74
350	80.49	70.48	62.11	71.02	9.20	28.98
400	82.93	74.18	65.75	74.29	8.59	25.71
450	85.08	77.45	68.97	77.16	8.06	22.84
500	87.00	80.37	71.85	79.74	7.60	20.26
550	88.74	83.01	74.45	82.07	7.19	17.93
600	90.33	85.42	76.83	84.19	6.84	15.81
650	91.79	87.64	79.01	86.15	6.52	13.85
700	93.15	89.69	81.04	87.96	6.24	12.04
750	94.41	91.60	82.92	89.64	5.99	10.36
800	95.59	93.39	84.68	91.22	5.77	8.78
850	96.69	95.07	86.34	92.70	5.57	7.30
900	97.74	96.66	87.90	94.10	5.39	5.90
950	98.73	98.16	89.38	95.42	5.24	4.58
1000	99.66	99.58	90.78	96.67	5.11	3.33
Activity (nCi/g)	M42-4-1 c.f.	M42-4-2 c.f.	M42-4-3 c.f.	Mean M42-4	S.D. M42-4	%>Ai M42-4
50	44.64	36.87	24.82	35.44	9.99	64.56
100	58.11	50.22	40.84	49.72	8.65	50.28
150	65.99	58.03	50.21	58.08	7.89	41.92
200	71.59	63.57	56.85	64.00	7.38	36.00
250	75.92	67.86	62.01	68.60	6.99	31.40
300	79.47	71.37	66.22	72.35	6.68	27.65
350	82.46	74.34	69.78	75.53	6.42	24.47
400	85.06	76.91	72.87	78.28	6.21	21.72
450	87.35	79.18	75.59	80.71	6.03	19.29
500	89.40	81.21	78.03	82.88	5.87	17.12
550	91.25	83.05	80.23	84.84	5.73	15.16
600	92.94	84.72	82.24	86.63	5.60	13.37
650	94.50	86.26	84.09	88.28	5.49	11.72
700	95.94	87.69	85.80	89.81	5.39	10.19
750	97.28	89.02	87.39	91.23	5.30	8.77
800	98.53	90.26	88.88	92.56	5.22	7.44
850	99.71	91.43	90.29	93.81	5.14	6.19
900	100.82	92.53	91.61	94.98	5.07	5.02
950	101.87	93.57	92.86	96.10	5.01	3.90
1000	102.87	94.56	94.04	97.16	4.95	2.84

Table C-2
Calculated Cumulative Frequency (8 hr)
Mel-14 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	M37-8-1 c.f.	M37-8-2 c.f.	M37-8-3 c.f.	Mean M37-8	S.D. M37-8	%>Ai M37-8
50	28.75	50.83	46.49	42.02	11.70	57.98
100	45.11	64.95	59.14	56.40	10.20	43.60
150	54.68	73.21	66.55	64.81	9.39	35.19
200	61.47	79.06	71.80	70.78	8.84	29.22
250	66.73	83.61	75.87	75.41	8.45	24.59
300	71.04	87.32	79.20	79.19	8.14	20.81
350	74.67	90.46	82.02	82.38	7.90	17.62
400	77.83	93.18	84.45	85.15	7.70	14.85
450	80.61	95.58	86.60	87.60	7.54	12.40
500	83.09	97.73	88.53	89.78	7.40	10.22
550	85.34	99.67	90.27	91.76	7.28	8.24
600	87.40	101.44	91.86	93.56	7.18	6.44
650	89.29	103.07	93.32	95.22	7.09	4.78
700	91.03	104.58	94.67	96.76	7.01	3.24
750	92.66	105.99	95.93	98.19	6.94	1.81
800	94.19	107.30	97.11	99.53	6.88	0.47
850	95.62	108.53	98.22	100.79	6.83	-0.79
900	96.97	109.70	99.26	101.97	6.79	-1.97
950	98.24	110.80	100.25	103.10	6.75	-3.10
1000	99.45	111.85	101.18	104.16	6.71	-4.16
Activity (nCi/g)	M42-8-1 c.f.	M42-8-2 c.f.	M42-8-3 c.f.	Mean M42-8	S.D. M42-8	%>Ai M42-8
50	2.42	27.54	20.20	16.72	12.92	83.28
100	24.39	46.24	42.96	37.86	11.78	62.14
150	37.24	57.17	56.27	50.23	11.25	49.77
200	46.36	64.93	65.72	59.00	10.95	41.00
250	53.44	70.94	73.05	65.81	10.77	34.19
300	59.22	75.86	79.03	71.37	10.64	28.63
350	64.10	80.02	84.09	76.07	10.56	23.93
400	68.34	83.62	88.48	80.15	10.51	19.85
450	72.07	86.79	92.35	83.74	10.48	16.26
500	75.41	89.64	95.81	86.95	10.46	13.05
550	78.43	92.21	98.94	89.86	10.45	10.14
600	81.19	94.55	101.79	92.51	10.45	7.49
650	83.73	96.71	104.42	94.95	10.46	5.05
700	86.08	98.71	106.86	97.21	10.47	2.79
750	88.27	100.57	109.12	99.32	10.48	0.68
800	90.31	102.31	111.24	101.29	10.50	-1.29
850	92.23	103.94	113.23	103.14	10.52	-3.14
900	94.04	105.49	115.11	104.88	10.54	-4.88
950	95.76	106.94	116.88	106.53	10.57	-6.53
1000	97.39	108.33	118.57	108.09	10.59	-8.09

Table C-3
Calculated Cumulative Frequency (12 hr)
Mel-14 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	M37-12-1 c.f.	M37-12-2 c.f.	M37-12-3 c.f.	Mean M37-12	S.D. M37-12	%>Ai M37-12
50	30.83	39.57	46.83	39.08	8.01	60.92
100	45.94	53.61	58.58	52.71	6.37	47.29
150	54.77	61.82	65.46	60.68	5.43	39.32
200	61.04	67.65	70.34	66.34	4.78	33.66
250	65.90	72.17	74.12	70.73	4.29	29.27
300	69.87	75.86	77.21	74.31	3.91	25.69
350	73.23	78.98	79.83	77.35	3.59	22.65
400	76.14	81.68	82.09	79.97	3.32	20.03
450	78.71	84.07	84.09	82.29	3.10	17.71
500	81.00	86.20	85.87	84.36	2.91	15.64
550	83.08	88.13	87.49	86.23	2.75	13.77
600	84.97	89.90	88.97	87.94	2.61	12.06
650	86.72	91.52	90.32	89.52	2.50	10.48
700	88.33	93.02	91.58	90.98	2.40	9.02
750	89.84	94.41	92.75	92.33	2.32	7.67
800	91.24	95.72	93.84	93.60	2.25	6.40
850	92.56	96.95	94.87	94.79	2.19	5.21
900	93.81	98.11	95.84	95.92	2.15	4.08
950	94.99	99.20	96.76	96.98	2.12	3.02
1000	96.10	100.24	97.63	97.99	2.09	2.01
Activity (nCi/g)	M42-12-1 c.f.		M42-12-3 c.f.	Mean M42-12	S.D. M42-12	%>Ai M42-12
50	17.43		48.31	32.87	21.83	67.13
100	33.94		60.19	47.07	18.56	52.93
150	43.61		67.15	55.38	16.65	44.62
200	50.46		72.08	61.27	15.29	38.73
250	55.78		75.91	65.84	14.23	34.16
300	60.12		79.04	69.58	13.37	30.42
350	63.80		81.68	72.74	12.65	27.26
400	66.98		83.97	75.48	12.02	24.52
450	69.79		85.99	77.89	11.46	22.11
500	72.30		87.80	80.05	10.96	19.95
550	74.57		89.43	82.00	10.51	18.00
600	76.64		90.93	83.78	10.10	16.22
650	78.55		92.30	85.42	9.72	14.58
700	80.31		93.57	86.94	9.37	13.06
750	81.96		94.75	88.36	9.05	11.64
800	83.50		95.86	89.68	8.74	10.32
850	84.94		96.90	90.92	8.46	9.08
900	86.30		97.88	92.09	8.19	7.91
950	87.59		98.81	93.20	7.93	6.80
1000	88.81		99.69	94.25	7.69	5.75

Table C-4
Calculated Cumulative Frequency (24 hr)
Mel-14 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	M37-24-1 c.f.	M37-24-2 c.f.	M37-24-3 c.f.	Mean M37-24	S.D. M37-24	%>Ai M37-24
50	37.99	27.58	65.47	43.68	19.58	56.32
100	53.19	48.92	74.93	59.01	13.95	40.99
150	62.08	61.39	80.46	67.98	10.81	32.02
200	68.39	70.25	84.38	74.34	8.75	25.66
250	73.28	77.11	87.43	79.27	7.32	20.73
300	77.28	82.72	89.91	83.31	6.34	16.69
350	80.66	87.47	92.02	86.71	5.71	13.29
400	83.59	91.58	93.84	89.67	5.38	10.33
450	86.17	95.20	95.44	92.27	5.28	7.73
500	88.48	98.44	96.88	94.60	5.36	5.40
550	90.57	101.38	98.18	96.71	5.55	3.29
600	92.48	104.05	99.37	98.63	5.82	1.37
650	94.24	106.52	100.46	100.40	6.14	-0.40
700	95.86	108.80	101.47	102.04	6.49	-2.04
750	97.37	110.92	102.41	103.57	6.85	-3.57
800	98.79	112.91	103.29	105.00	7.21	-5.00
850	100.12	114.77	104.12	106.34	7.57	-6.34
900	101.37	116.53	104.90	107.60	7.93	-7.60
950	102.56	118.20	105.64	108.80	8.28	-8.80
1000	103.68	119.77	106.34	109.93	8.63	-9.93
Activity (nCi/g)	M42-24-1 c.f.	M42-24-2 c.f.	M42-24-3 c.f.	Mean M42-24	S.D. M42-24	%>Ai M42-24
50	5.69	-15.75	13.36	1.10	15.09	98.90
100	26.34	7.74	33.86	22.65	13.44	77.35
150	38.41	21.48	45.85	35.25	12.49	64.75
200	46.98	31.23	54.36	44.19	11.81	55.81
250	53.63	38.80	60.96	51.13	11.29	48.87
300	59.06	44.97	66.35	56.79	10.87	43.21
350	63.65	50.20	70.91	61.59	10.51	38.41
400	67.63	54.72	74.86	65.74	10.20	34.26
450	71.14	58.72	78.34	69.40	9.93	30.60
500	74.27	62.29	81.46	72.67	9.69	27.33
550	77.11	65.52	84.28	75.64	9.47	24.36
600	79.70	68.47	86.85	78.34	9.27	21.66
650	82.09	71.18	89.22	80.83	9.09	19.17
700	84.30	73.69	91.41	83.13	8.92	16.87
750	86.35	76.03	93.45	85.28	8.76	14.72
800	88.27	78.22	95.36	87.28	8.62	12.72
850	90.08	80.27	97.15	89.17	8.48	10.83
900	91.78	82.21	98.84	90.94	8.35	9.06
950	93.39	84.04	100.44	92.62	8.23	7.38
1000	94.92	85.78	101.96	94.22	8.11	5.78

Table C-5
Calculated Cumulative Frequency (4 hr)
RPC-5 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	R37-4-1 c.f.	R37-4-2 c.f.	R37-4-3 c.f.	Mean R37-4	S.D. R37-4	%>Ai R37-4
50	76.03	52.22	77.24	68.50	14.10	31.50
100	93.36	73.62	92.66	86.55	11.20	13.45
150	103.50	86.13	101.68	97.11	9.54	2.89
200	110.69	95.01	108.09	104.60	8.40	-4.60
250	116.27	101.90	113.05	110.41	7.54	-10.41
300	120.83	107.53	117.11	115.16	6.86	-15.16
350	124.69	112.29	120.54	119.17	6.31	-19.17
400	128.03	116.41	123.51	122.65	5.86	-22.65
450	130.97	120.04	126.13	125.72	5.47	-25.72
500	133.60	123.30	128.48	128.46	5.15	-28.46
550	135.99	126.24	130.60	130.94	4.88	-30.94
600	138.16	128.92	132.53	133.21	4.66	-33.21
650	140.17	131.40	134.31	135.29	4.47	-35.29
700	142.02	133.68	135.96	137.22	4.31	-37.22
750	143.74	135.81	137.50	139.02	4.18	-39.02
800	145.36	137.80	138.93	140.70	4.07	-40.70
850	146.87	139.68	140.28	142.28	3.99	-42.28
900	148.30	141.44	141.55	143.77	3.93	-43.77
950	149.66	143.11	142.76	145.17	3.88	-45.17
1000	150.94	144.69	143.90	146.51	3.86	-46.51
Activity (nCi/g)	R42-4-1 c.f.	R42-4-2 c.f.	R42-4-3 c.f.	Mean R42-4	S.D. R42-4	%>Ai R42-4
50	47.51	50.43	54.60	50.84	3.56	49.16
100	69.89	66.02	76.40	70.77	5.24	29.23
150	82.97	75.15	89.15	82.42	7.02	17.58
200	92.26	81.62	98.20	90.69	8.40	9.31
250	99.46	86.64	105.22	97.11	9.51	2.89
300	105.35	90.74	110.95	102.35	10.43	-2.35
350	110.33	94.21	115.80	106.78	11.22	-6.78
400	114.64	97.22	120.00	110.62	11.91	-10.62
450	118.44	99.87	123.71	114.00	12.52	-14.00
500	121.84	102.24	127.02	117.03	13.07	-17.03
550	124.92	104.38	130.02	119.77	13.57	-19.77
600	127.73	106.34	132.76	122.27	14.03	-22.27
650	130.31	108.14	135.27	124.57	14.45	-24.57
700	132.70	109.81	137.60	126.71	14.84	-26.71
750	134.93	111.36	139.77	128.69	15.20	-28.69
800	137.01	112.81	141.80	130.54	15.54	-30.54
850	138.97	114.18	143.71	132.29	15.86	-32.29
900	140.81	115.46	145.51	133.93	16.16	-33.93
950	142.56	116.68	147.21	135.48	16.45	-35.48
1000	144.22	117.84	148.82	136.96	16.72	-36.96

Table C-6
Calculated Cumulative Frequency (8 hr)
RPC-5 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	R37-8-1 c.f.	R37-8-2 c.f.	R37-8-3 c.f.	Mean R37-8	S.D. R37-8	%>Ai R37-8
50	74.76	41.75	41.34	52.61	19.18	47.39
100	89.20	67.30	76.92	77.81	10.98	22.19
150	97.65	82.24	97.74	92.54	8.92	7.46
200	103.65	92.85	112.50	103.00	9.84	-3.00
250	108.30	101.07	123.96	111.11	11.70	-11.11
300	112.10	107.79	133.32	117.74	13.67	-17.74
350	115.31	113.48	141.23	123.34	15.52	-23.34
400	118.09	118.40	148.09	128.19	17.23	-28.19
450	120.55	122.74	154.14	132.47	18.79	-32.47
500	122.74	126.62	159.55	136.30	20.22	-36.30
550	124.73	130.14	164.44	139.77	21.54	-39.77
600	126.54	133.34	168.91	142.93	22.75	-42.93
650	128.21	136.29	173.01	145.84	23.88	-45.84
700	129.75	139.03	176.82	148.53	24.93	-48.53
750	131.19	141.57	180.36	151.04	25.92	-51.04
800	132.54	143.95	183.67	153.39	26.84	-53.39
850	133.80	146.18	186.79	155.59	27.72	-55.59
900	134.99	148.29	189.72	157.67	28.55	-57.67
950	136.12	150.28	192.50	159.63	29.33	-59.63
1000	137.19	152.17	195.13	161.50	30.08	-61.50
Activity (nCi/g)	R42-8-1 c.f.	R42-8-2 c.f.	R42-8-3 c.f.	Mean R42-8	S.D. R42-8	%>Ai R42-8
50	13.17	74.44	46.99	44.87	30.69	55.13
100	63.82	90.26	68.83	74.30	14.04	25.70
150	93.45	99.51	81.60	91.52	9.11	8.48
200	114.47	106.08	90.66	103.74	12.08	-3.74
250	130.78	111.17	97.69	113.21	16.64	-13.21
300	144.10	115.33	103.43	120.96	20.91	-20.96
350	155.36	118.85	108.29	127.50	24.70	-27.50
400	165.12	121.90	112.50	133.17	28.07	-33.17
450	173.73	124.59	116.21	138.17	31.08	-38.17
500	181.43	126.99	119.53	142.65	33.79	-42.65
550	188.39	129.17	122.53	146.70	36.26	-46.70
600	194.75	131.15	125.27	150.39	38.53	-50.39
650	200.60	132.98	127.79	153.79	40.62	-53.79
700	206.02	134.67	130.12	156.94	42.56	-56.94
750	211.06	136.24	132.30	159.87	44.38	-59.87
800	215.77	137.72	134.33	162.61	46.07	-62.61
850	220.20	139.10	136.24	165.18	47.67	-65.18
900	224.38	140.40	138.04	167.61	49.18	-67.61
950	228.33	141.64	139.74	169.90	50.61	-69.90
1000	232.08	142.81	141.36	172.08	51.96	-72.08

Table C-7
Calculated Cumulative Frequency (12 hr)
RPC-5 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	R37-12-1 c.f.	%>Ai R37-12			
50	53.32	46.68			
100	88.43	11.57			
150	108.97	-8.97			
200	123.54	-23.54			
250	134.84	-34.84			
300	144.08	-44.08			
350	151.88	-51.88			
400	158.65	-58.65			
450	164.61	-64.61			
500	169.95	-69.95			
550	174.78	-74.78			
600	179.19	-79.19			
650	183.24	-83.24			
700	186.99	-86.99			
750	190.49	-90.49			
800	193.76	-93.76			
850	196.83	-96.83			
900	199.72	-99.72			
950	202.46	-102.46			
1000	205.06	-105.06			
Activity (nCi/g)	R42-12-1 c.f.	R42-12-3 c.f.	Mean R42-12	S.D. R42-12	%>Ai R42-12
50	83.85	80.36	82.11	2.47	17.89
100	95.11	88.77	91.94	4.49	8.06
150	101.70	93.69	97.69	5.67	2.31
200	106.37	97.18	101.78	6.50	-1.78
250	110.00	99.89	104.94	7.15	-4.94
300	112.96	102.10	107.53	7.68	-7.53
350	115.46	103.97	109.72	8.13	-9.72
400	117.63	105.59	111.61	8.52	-11.61
450	119.55	107.02	113.28	8.86	-13.28
500	121.26	108.30	114.78	9.16	-14.78
550	122.81	109.45	116.13	9.44	-16.13
600	124.22	110.51	117.36	9.69	-17.36
650	125.52	111.48	118.50	9.93	-18.50
700	126.72	112.38	119.55	10.14	-19.55
750	127.84	113.22	120.53	10.34	-20.53
800	128.89	114.00	121.45	10.53	-21.45
850	129.88	114.74	122.31	10.71	-22.31
900	130.81	115.43	123.12	10.87	-23.12
950	131.68	116.08	123.88	11.03	-23.88
1000	132.52	116.71	124.61	11.18	-24.61

Table C-8
Calculated Cumulative Frequency (24 hr)
RPC-5 F(ab')₂, 37° vs. 42°

Activity (nCi/g)	R37-24-1 c.f.		R37-24-3 c.f.	Mean R37-24	S.D. R37-24	%>Ai R37-24
50	97.00		71.19	84.09	18.25	15.91
100	93.36		90.96	92.16	1.69	7.84
150	103.50		102.53	103.01	0.69	-3.01
200	110.69		110.73	110.71	0.03	-10.71
250	116.27		117.10	116.68	0.58	-16.68
300	120.83		122.30	121.56	1.04	-21.56
350	124.69		126.69	125.69	1.42	-25.69
400	128.03		130.50	129.26	1.75	-29.26
450	130.97		133.86	132.42	2.04	-32.42
500	133.60		136.87	135.24	2.31	-35.24
550	135.99		139.59	137.79	2.54	-37.79
600	138.16		142.07	140.12	2.76	-40.12
650	140.17		144.35	142.26	2.96	-42.26
700	142.02		146.46	144.24	3.14	-44.24
750	143.74		148.43	146.09	3.31	-46.09
800	145.36		150.27	147.82	3.48	-47.82
850	146.87		152.00	149.44	3.63	-49.44
900	148.30		153.63	150.97	3.77	-50.97
950	149.66		155.17	152.41	3.90	-52.41
1000	150.94		156.64	153.79	4.03	-53.79
Activity (nCi/g)	R42-24-1 c.f.	R42-24-2 c.f.	R42-24-3 c.f.	Mean R42-24	S.D. R42-24	%>Ai R42-24
50	74.96	45.41	81.50	67.29	19.23	32.71
100	90.74	58.31	93.43	80.83	19.55	19.17
150	99.98	65.86	100.41	88.75	19.82	11.25
200	106.53	71.22	105.37	94.37	20.06	5.63
250	111.61	75.37	109.21	98.73	20.26	1.27
300	115.76	78.76	112.35	102.29	20.45	-2.29
350	119.27	81.63	115.00	105.30	20.61	-5.30
400	122.31	84.12	117.30	107.91	20.75	-7.91
450	124.99	86.31	119.33	110.21	20.89	-10.21
500	127.39	88.27	121.14	112.27	21.01	-12.27
550	129.56	90.05	122.78	114.13	21.13	-14.13
600	131.54	91.67	124.28	115.83	21.24	-15.83
650	133.36	93.16	125.66	117.39	21.34	-17.39
700	135.05	94.54	126.93	118.84	21.44	-18.84
750	136.62	95.82	128.12	120.19	21.53	-20.19
800	138.09	97.02	129.23	121.45	21.61	-21.45
850	139.47	98.15	130.28	122.63	21.69	-22.63
900	140.77	99.22	131.26	123.75	21.77	-23.75
950	142.01	100.22	132.19	124.81	21.85	-24.81
1000	143.17	101.18	133.08	125.81	21.92	-25.81

APPENDIX D

CORRECTION FOR NON-TUMOR ACTIVITY

In an attempt to more accurately measure the microscopic dose to tumor cells, we used histologic information from the sections which were stained by hematoxylin and eosin. Each section was studied under light microscope to determine the morphology of the tumor. Areas of non-malignant cells were identified and these observations were transferred to a Polaroid photograph of the corresponding digital image. The next step was to use these drawings to re-sample the digitized autoradiographs and subtract the pixels containing activity which was associated with non-tumor tissue.

Due to limitations in the image analysis software, it was not possible to sample multiple areas within a single section concurrently. Therefore, it was necessary to count individual pixels and subtract them from the appropriate range of TV's. The sampling was carried out as before except that, instead of using the dark field edge technique, a point was chosen as the sample mode. The point was precisely the size of an individual pixel. This resulted in as many as 864 separate samples being taken from one tumor section. This method was very cumbersome and time-consuming and limited the total number of sections that could be analyzed. The results are shown in Tables D-1 through D-12 as another modification to the Area Percent tables. A column has been added to the Excel spreadsheet for the count of non-tumor pixels, facilitating a simple recalculation of the activity in the section.

Table D-1
Non-tumor Correction
M37-4-1-60

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
1663	365	0	365	7.22343	7.22343	120.126	
894	40	0	40	0.79161	8.01504	7.07698	
674	62	0	62	1.22699	9.24203	8.26994	
533	85	0	85	1.68217	10.9242	8.96596	
428	103	2	101	1.99881	12.923	8.55492	
345	157	7	150	2.96853	15.8915	10.2414	
276	333	21	312	6.17455	22.0661	17.0418	
217	654	19	635	12.5668	34.6329	27.2699	
165	1316	29	1287	25.47	60.1029	42.0255	
110	2198	182	2016	39.8971	100	43.8868	
Totals	5313	260	5053		Activity = 293.459 nCi/g		

Table D-2
Non-tumor Correction
M37-4-2-90

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
1663	445	0	445	6.1926	6.1926	102.983	
894	201	0	201	2.79711	8.9897	25.0061	
674	234	0	234	3.25633	12.246	21.9477	
533	300	0	300	4.17478	16.4208	22.2516	
428	444	3	441	6.13693	22.5578	26.2661	
345	689	4	685	9.53242	32.0902	32.8869	
276	931	39	892	12.413	44.5032	34.2599	
217	1136	13	1123	15.6276	60.1308	33.9119	
165	940	19	921	12.8166	72.9474	21.1474	
110	2130	186	1944	27.0526	100	29.7579	
Totals	7450	264	7186		Activity = 350.418 nCi/g		

Table D-3
Non-tumor Correction
M37-4-3-50

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
1663	878	0	878	18.8817	18.8817	314.003	
894	145	0	145	3.11828	22.00	27.8774	
674	177	0	177	3.80645	25.8065	25.6555	
533	234	0	234	5.03226	30.8387	26.8219	
428	261	0	261	5.6129	36.4516	24.0232	
345	394	1	393	8.45161	44.9032	29.1581	
276	574	25	549	11.8065	56.7097	32.5858	
217	569	82	487	10.4731	67.1828	22.7267	
165	690	221	469	10.086	77.2688	16.6419	
110	1270	213	1057	22.7312	100	25.0043	
Totals	5192	542	4650		Activity = 544.498 nCi/g		

Table D-4
Non-tumor Correction
M37-8-2-126

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
943	4	3	1	0.04156	0.04156	0.39194	
736	15	15	0	0	0.04	0	
588	30	13	17	0.70657	0.74813	4.15461	
472	34	14	20	0.83126	1.57938	3.92352	
377	53	13	40	1.66251	3.2419	6.26766	
296	102	16	86	3.5744	6.81629	10.5802	
225	206	8	198	8.22943	15.0457	18.5162	
163	450	19	431	17.9135	32.9593	29.1991	
107	751	14	737	30.6318	63.591	32.776	
43	922	46	876	36.409	100	15.6559	
Totals	2567	161	2406		Activity = 121.465 nCi/g		

Table D-5
Non-tumor Correction
M37-8-3-94

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
1305	31	0	31	1.25863	1.25863	16.4251	
917	54	0	54	2.19245	3.45	20.1048	
699	91	0	91	3.69468	7.14576	25.8258	
545	135	0	135	5.48112	12.6269	29.8721	
426	149	0	149	6.04953	18.6764	25.771	
328	169	0	169	6.86155	25.538	22.5059	
245	173	0	173	7.02395	32.5619	17.2087	
174	286	0	286	11.6119	44.1738	20.2046	
110	450	0	450	18.2704	62.4442	20.0974	
43	1111	186	925	37.5558	100	16.149	
Totals	2649	186	2463		Activity = 214.164 nCi/g		

Table D-6
Non-tumor Correction
M37-12-1-80

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
2188	163	0	163	2.36335	2.36335	51.71	
1128	209	0	209	3.0303	5.39	34.1818	
823	307	0	307	4.45121	9.84486	36.6335	
628	313	0	313	4.53821	14.3831	28.4999	
484	347	0	347	5.03117	19.4142	24.3509	
370	562	0	562	8.14847	27.5627	30.1493	
274	864	0	864	12.5272	40.0899	34.3245	
193	1308	0	1308	18.9648	59.0547	36.602	
122	1491	22	1469	21.2991	80.3538	25.9849	
46	1635	280	1355	19.6462	100	9.03726	
Totals	7199	302	6897		Activity = 311.474 nCi/g		

Table D-7
Non-tumor Correction
M37-12-2-120

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
2188	57	0	57	0.79222	0.79222	17.3337	
1128	59	0	59	0.82001	1.61	9.24976	
823	92	0	92	1.27867	2.8909	10.5234	
628	181	0	181	2.51564	5.40653	15.7982	
484	351	0	351	4.87839	10.2849	23.6114	
370	673	1	672	9.33982	19.6247	34.5573	
274	1027	40	987	13.7179	33.3426	37.5869	
193	1392	25	1367	18.9993	52.3419	36.6687	
122	1536	19	1517	21.0841	73.426	25.7226	
46	2204	292	1912	26.574	100	12.224	
Totals	7572	377	7195		Activity = 223.276 nCi/g		

Table D-8
Non-tumor Correction
M37-12-3-110

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
2188	93	0	93	1.45063	1.45063	31.7398	
1128	163	0	163	2.54251	3.99	28.6795	
823	152	3	149	2.32413	6.31727	19.1276	
628	193	8	185	2.88567	9.20293	18.122	
484	256	15	241	3.75916	12.9621	18.1944	
370	461	28	433	6.75402	19.7161	24.9899	
274	894	49	845	13.1805	32.8966	36.1145	
193	1019	66	953	14.8651	47.7617	28.6896	
122	715	71	644	10.0452	57.8069	12.2552	
46	2955	250	2705	42.1931	100	19.4088	
Totals	6901	490	6411		Activity = 237.321 nCi/g		

Table D-9
Non-tumor Correction
M37-24-1-83

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
1570	176	0	176	2.75689	2.75689	43.2832	
834	81	0	81	1.2688	4.03	10.5818	
623	151	3	148	2.3183	6.34398	14.443	
488	335	6	329	5.15351	11.4975	25.1491	
388	368	17	351	5.49812	16.9956	21.3327	
309	438	14	424	6.6416	23.6372	20.5226	
243	547	21	526	8.23935	31.8766	20.0216	
186	1000	39	961	15.0533	46.9298	27.9991	
137	1628	84	1544	24.1855	71.1153	33.1341	
84	2524	680	1844	28.8847	100	24.2632	
Totals	7248	864	6384		Activity = 240.73 nCi/g		

Table D-10
Non-tumor Correction
M37-24-2-130

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
790	12	0	12	0.28986	0.28986	2.28986	
614	37	0	37	0.89372	1.18	5.48744	
493	80	0	80	1.93237	3.11594	9.52657	
401	158	1	157	3.79227	6.90821	15.207	
327	237	4	233	5.62802	12.5362	18.4036	
264	391	14	377	9.10628	21.6425	24.0406	
210	610	29	581	14.0338	35.6763	29.471	
163	635	52	583	14.0821	49.7585	22.9539	
120	887	32	855	20.6522	70.4106	24.7826	
77	1331	106	1225	29.5894	100	22.7838	
Totals	4378	238	4140		Activity = 174.946 nCi/g		

Table D-11
Non-tumor Correction
M37-24-3-90

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
1135	28	18	10	0.2158	0.2158	2.44929	
758	51	19	32	0.69055	0.91	5.23435	
577	40	12	28	0.60423	1.51057	3.4864	
455	53	12	41	0.88476	2.39534	4.02568	
363	107	31	76	1.64005	4.03539	5.95339	
288	114	34	80	1.72637	5.76176	4.97195	
225	179	42	137	2.95641	8.71817	6.65192	
171	443	56	387	8.35132	17.0695	14.2808	
123	1216	67	1149	24.795	41.8645	30.4978	
77	2895	201	2694	58.1355	100	44.7644	
Totals	5126	492	4634		Activity = 122.316 nCi/g		

Table D-12
Non-tumor Correction
M42-24-1-70

Ai	# pixels	non-tumor	Adj. #	%	Cum. %	Ai x %	
1620	185	11	174	2.58314	2.58314	41.8468	
853	714	33	681	10.1099	12.69	86.2371	
635	794	30	764	11.342	24.035	72.022	
495	717	25	692	10.2732	34.3082	50.8521	
393	591	15	576	8.55107	42.8593	33.6057	
311	610	16	594	8.81829	51.6776	27.4249	
242	594	9	585	8.68468	60.3622	21.0169	
184	672	40	632	9.38242	69.7447	17.2637	
133	798	70	728	10.8076	80.5523	14.3741	
84	1598	288	1310	19.4477	100	16.3361	
Totals	7273	537	6736		Activity = 380.979 nCi/g		

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