SmART: dosimetry and applications

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SmART

Dosimetry and Applications

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INTRODUCTION

CHAPTER

1

Introduction and outline of the thesis

Patrick V. Granton

THE EMPEROR OF ALL MALADIES

Cancer can arguably be considered the prevailing medical challenge of the 21st century. In the United States alone, 4 in 10 people will develop cancer over their lifetime ¹. As global life expectancies continue to rise through vaccinations and improved sanitary conditions, this number is expected to increase. In 2012, there were 33 million people living with cancer ². While it is true that other medical maladies like obesity or infectious diseases (e.g. AIDS, malaria), have a greater immediate impact on the total number of deaths worldwide, solutions or remedies to these challenges are principally not medical; they are societal issues or proliferate from a lack of access to education, medication, or other preventative resources.

The word *cancer* describes a complex condition involving unregulated cell growth that is characterized by the six hallmarks of cancer as illustrated in figure 1 ³. Cancer is frequently expressed and misunderstood as a single disease. This is a semantic discussion but one of significance because similar forms of cancer, for example, head and neck cancers exhibit identical signs and symptoms but have dramatically different survival outcomes following treatment. Figure 2 shows survival outcomes for head and neck patients when stratified based on the presence of the human papillomavirus (HPV) that is known to cause cancer, further reflecting the complexity of cancer and its potential treatments ⁴.

Figure 1. The six hallmarks of cancer demonstrating the characteristics of cancer. Image acquired from Cell with permissions from Elsevier.

Great medical achievements have helped to unravel cancer's complexity over the past century and we now know a great deal about its causes, which are diverse. Cancers can be caused by environmental factors, including substances that when exposed to directly cause cancer (i.e. carcinogens), for example; smoking, radiation (both man made or naturally occurring), asbestos, or they may heretical (e.g. breast cancer), or can be the result of an infection that directly alters your genetic code (i.e. oncovirus). Owing to its causal diversity, cancer, therefore can to a great extent, be prevented by a number of lifestyle choices and prevention must be a part of any public cancer reduction strategy⁵.

Figure 2. Kaplan-Meier survival curves for head-and-neck patients with (+) and without (-) the presence of the HPV. Image acquired from Cancer research with permissions from the American Association of Cancer Research

Cancer treatment has evolved with variable success. However the principal treatment strategies have remained relatively unchanged for the better part of the last century. The treatment of some cancers like Leukemia have achieved very favorable outcomes but others, like non-small cell lung cancer have ameliorated little over the past few decades⁶. Figure 3 shows median survivor times for disease sites that have seen little improvement in treatment by diagnosis year within the United Kingdom⁷. Cancer patients primarily undergo surgery, conformal radiation therapy, chemotherapy, or any combination of these treatment strategies to prolong life, often for only a few months and at a great cost of side effects. This is because treatment is often non-specific; what is toxic for the tumor is toxic to the rest of the healthy body. Treatment failure in cancer is death, for which the primary cause is relapse. All cancer cells must die if the patient is to survive long term. The balance the oncologist tries to

achieve is to give the most aggressive treatment strategy that just spares the patient's life but eradicates the tumor. Every cancer treatment strategy has its limitations and all oncological sub-disciplines are actively trying to push these limits or develop more specific treatment strategies, a limited number of research projects is actively trying to develop complete new drugs or strategies.

Figure 3. Median survival times for a select few of disease sites based on diagnosis year within the United Kingdom⁷.

RADIOTHERAPY IN CANCER TREATMENT

Radiation therapy or radiotherapy represents an important option in the treatment of solid cancers. Currently, 50 % of all cancer patients will undergo radiotherapy at some point in their treatment course; likely even more patients would benefit from radiotherapy given greater access^{8,9}. The word radiotherapy is the conjunction of radiation and therapy, indicating the use of ionization radiation for curative intent. Ionization radiation was discovered in 1895 by Wilhelm Conrad Röntgen who coined the term X-rays; shortly thereafter, the dichotomy of ionization radiation to be both harmful and beneficial was explored, though at that time poorly understood.

Radiation therapy works by localizing – to the best extent possible – ionizing radiation to cancerous tissue while minimizing radiation delivered to healthy tissue, preferentially inflicting or targeting biological damage to cancerous cells and arresting the disease in the process. Ionizing radiation is differentiated from other forms of radiation in that it is composed of particles that carry a sufficient amount of energy to liberate electrons bound to atoms, depositing energy to mass and, altering the chemical bonds. The

quantity that is used to measure the amount of ionizing radiation deposited per unit mass, or absorbed dose, is expressed in terms of Gray (1 Gy = 1 J/kg).

Radiation therapy principally acts directly or indirectly on cells by fatally damaging DNA – the genetic code that maintains and drives life. In direct action, radiation causes secondary ionized electrons to disrupt and break the molecular DNA chain directly. Indirect ionizing DNA damage is caused by transient creation of free radicals (i.e. ions, atoms or molecules with a net positive charge) that migrate with a probability of chemically interacting with a nearby DNA molecule, breaking chemical bonds in the process. For low linear energy transfer irradiation (X-ray, Gamma-rays), the presence of oxygen enhances indirect radiation damage and is one of the main reasons why hypoxic tumors (tumors with low amounts of oxygen <3 mmHg) resist cell damage to radiation¹⁰.

External beam photon radiotherapy makes use of fractionation, whereby dose is divided into discrete fractions (typically 2Gy) and delivered over a prolonged period of time (e.g. 5 weeks). The primary rationale for this radiation treatment regime vs. a single large dose of irradiation is to spare normal tissues with sublethal damage by allowing for a sufficient time to repair, and at the same time allow for a re-oxygenation effect to further sensitize tumors to radiation¹¹. However, the exact treatment protocol may vary widely between different tumor sites depending on severe acute reactions of some normal tissues (i.e. toxicity) or the behavior of tumors to repopulate and is an active area of scientific interest.

The past 30-odd years have seen a great deal of progress of developing technology that can better localize radiation to the desired targets. It was the pioneering work by inventor Sir Godfrey Hounsfield that introduced the CT scanner and allowed – for the first time – the ability to non-invasively visualize the gross tumor in 3-D, which ushered in the era of 3-D conformal radiotherapy (3-D CRT). In 3-D CRT, target definitions of individualized patients can be assigned and sensitive tissues identified, ergo treatment plans can be developed that conform to the target and avoid sensitive tissues as best as possible. In 3-D CRT forward-based treatment plan optimization, a number of parameters such as: beam size; number of beams; beam arrangements and weights (prescription intensities) have to be carefully chosen so that the resulting dose distribution is the best as reasonably achievable with the knowledge and devices available at the time of planning¹².

Recently, 3-D CRT is being replaced with intensity-modulated radiotherapy (IMRT) and volumetric modulated radiotherapy (VMAT), which allows an even greater control over the shape of the dose distribution using multi-leaf collimators (MLC)¹³. The possibilities to which field shapes can conform to the target make the decisions by experience radiotherapy technicians to define these field shapes intractable. Consequently, inverse planning is required whereby mathematical optimization algorithms such as simulated annealing propose optimum treatment plans that achieve specific treatment goals desired by the radiation oncologist. It suffices to say that the technical capability

to deliver the bulk of a desired dose distribution in patients can be met today, limited only by the physics that governs photons; and even this restriction is being exploited by a growing number of radiation therapy centers using light ion beams like proton or carbon beams to treat patients, which can deposit dose in an even more localized way

¹⁴.

Therefore, treatment plan optimization can only improve with greater knowledge of the target – the disease – and a greater understanding of normal tissue complication probability to achieve both patient and oncologist short-term (e.g. survival) and long-term goals (e.g. quality of life). The current standard of care for radiotherapy patients is ad hoc, standardized by national objectives, and principally tries only to satisfy survival mitigated only by the potential of a single adverse lethal event in the short term (i.e. < 5 years). Moreover, the gross tumor volume (GTV) is treated with a uniform prescription dose, which surmises that cancer cell resistance is uniform, which we know to be untrue ¹⁵⁻¹⁷. However, to implement changes to the existing standard of care, medical research must be conducted to test new treatments (e.g. novel drugs) or develop completely new insights to consider alternative treatment strategies.

RADIATION RESEARCH

As a scientist, I would like to believe that current treatment regimes for site-specific cancers were derived from well-established randomized clinical trials or based on fundamental biological models, but that simply does not reflect the historical evolution of radiotherapy. A 2006 report by The Royal College of Radiologist quite succinctly put it, “radiotherapy evolved as an empirical art, not as an exact science” ¹⁸. Even today, while the linear quadratic model of cell survival has long been established, various mathematical models predicting tumor control probability (TCP) presented, and normal tissue complication probabilities estimated, they are rarely used. Recently Hoffmann *et. al.* stated that “currently, this compromise [between cure and toxicity] is ‘frozen’ in treatment protocols, which are based on consensus opinions about what is considered to be the best trade-off for a specific patient population.” ¹⁹

Current radiotherapy practice has however, benefited greatly from radiation research when applied with scientific rigor so that the results are irrefutable. The multi-center randomized trials of the CHART study (Continuous Hyperfractionated Accelerated Radiotherapy) demonstrated significant survival benefits of accelerated radiotherapy on overall total treatment in head and neck cancers and highlighted the importance of the repopulation effect on treated cells ²⁰. Other trials have demonstrated clear benefits of combined chemotherapy and radiotherapy, which have been adopted broadly as the new standard of care. ²¹

PRE-CLINICAL RESEARCH

Pre-clinical small animal models of disease are a means to evaluate new treatment strategies that would otherwise be unethical to investigate on patients and allow for studies with (1) large cohorts, (2) transgenic animals, (3) controlled experimental conditions and, (4) accelerated results due to the shorter lifespans. Pre-clinical small animal research has played a significant role in cancer; for example, before a new drug can enter clinical trials with patients it is often required by law that animal data has demonstrate the drug to be safe (i.e. non-toxic) and non-mutagenic (i.e. will not increase the frequency of genetic mutations). Tamoxifen, a wide-used and successful chemotherapy drug was extensively used in animals before it was applied to patients²². It was also in animal studies in 1911 that researchers demonstrated that cancer (i.e. rous sarcoma) can also be the direct result of a virus²³.

Despite the vast number of patients that undergo radiotherapy, the use of animals in radiation research has had less of an influencing role in clinical care. Animal data in radiotherapy has largely provided only background or supporting biological evidence for clinical observations; a 1984 article by Fowler *et al.* summarizes the available animal data at the time for the choice of fractionation, and quite convincingly demonstrates through a broad array of animal data that the dose per fraction is of principal importance in mitigating late term effects as seen clinically²⁴. Other examples of animal data used in radiation research are in TCP or NTCP modeling; often clinical data may be sparse (or even practically or ethically unattainable) and systematic prospective animals studies are employed²⁵. The volume effect – the change in the tolerance dose in partial volume irradiation to normal tissues – is one more recent example in specific organs like the spine²⁶ to evaluate the robustness of models like the Lyman-Kutcher-Burman (LKB) NTCP model on serial or parallel organs. However, despite the fact that animal studies have “produced important proof-of-principle insights [these data] have had relatively little impact on clinical NTCP modeling so far”²⁷.

One potential explanation for the lack of translation of animal data to clinical usable parameters may be due to the poor dose conformity of earlier animal irradiation studies and the lack of dedicated devices to perform clinically similar treatments. In the past, tumor-growth delay assays were hindered by high doses to normal tissues²⁸. Moreover, these erstwhile studies crudely estimated and poorly reported the delivered dose to animal specimens. Certainly, the tools at our current disposal to calculate the delivered dose to small specimens like mice or rats has drastically improved over the last 20 years. The clinical utility of the results of these earlier animal studies may be limited due to the radiation dose distributions being highly dissimilar to current radiotherapy practice.

The adaptation of conformal radiation therapy followed by IMRT has created a large technology gap between clinical and pre-clinical radiation studies, which has persisted for the past 40 years. In contrast, non-invasive medical imaging technologies (e.g. CT, PET, MR) have been quite swiftly adopted and commercialized for small animal use²⁹.

Conventionally these technologies add the adjective *micro* to denote and distinguish it from clinical technologies that fundamentally use the same physical process to probe materials or tissues³⁰.

Small animal models of disease are also becoming more complex, specific, and similar to the pathology of human disease. Historically, and still in wide-use today, xenograft transplantation of tumor tissues extracted from patients or developed in cell culture and placed subcutaneously in the flank, hind limb, or back of immunosuppressed rats or mice were, and are the most common pre-clinical cancer disease models used in research. These models allow tumors to grow with relative ease, require little surgery, and do not require sophisticated methods to measure tumor growth in response to treatments³¹. However, because these models are based on single cell solutions or have been derived from small regions of patient tumors, they do not replicate the tumor micro-environment (stromal, laminin, collagen matrix, etc...) and may not be a representative model of therapy response or tumor progression in patients. Therefore, significant effort has been placed into developing orthotopic models of disease (i.e. placed in the same region from whence it came) that are generally consider more clinically relevant³². Recent publications using orthotopic tumor models include disease site of the lung³³ and brain³⁴.

Researchers also have now at their disposal a host of genetically engineered mouse models (GEMM) of disease³⁵. These models were developed through transgenesis, whereby foreign genes are introduced into a living organism to express or inhibit (knockout mice) specific properties transcribed the introduced gene. Through selective breeding of the desired phenotype, a large population of the desired murine genetic background can be established. Some GEMM allow for the spontaneous development of cancer that represents a more natural evolution of tumor development (e.g. angiogenesis more closely integrated with the host), though these models are also not without drawbacks³⁶. Nonetheless, the results of new drug efficacy studies using these spontaneous GEMM are seeing promising outcomes that merit consideration for clinical studies³⁷.

Like patient care, pre-clinical targeted therapies in orthotopic xenografts or GEMM of cancer, require non-invasive imaging for diagnosis or drug efficacy evaluation; and increasingly, radiation oncology centers are relying on multi-modal imaging for target identification like magnetic resonance imaging (MRI), or positron emission tomography (PET). It is also theorized that by boosting dose to regions of high molecular activity indicating regions of high molecular activity (e.g. FDG) or proliferation (e.g. FLT), for example, that better survival outcomes will result³⁸. Currently, there are a number of clinical trials underway investigating this hypothesis^{39,40}, however, it is likely that the results of these trials will not immediately reveal the true impact of dose-redistribution given the many limitations that exist in patient studies (e.g. co-morbidity). Colleagues and I argue that pre-clinical studies would help augment the data for determining the efficacy of dose-boosting.

Hitherto, radiation oncologists have had only a limited means to try and ameliorate treatment outcomes other than through the use of clinical trials or the introduction of new drugs – a de facto “shoot first and ask questions later” approach. By scaling down radiation technology for use in small animals, it is foreseen that direct and relevant clinical questions can be addressed and the knowledge gained translated back to patient care. Particularly, clinically relevant questions include: dose-boosting, the volume effect of normal tissue toxicity, and radiation adjuvant drug efficacy studies of faithful orthotopic or transgenic tumor models.

PRECISION IMAGE-GUIDED SMALL ANIMAL RADIATION

To redress the deficiency of poor conformity in the delivery of externally applied radiation beams in pre-clinical research a number of research institutions have developed their own bespoke image-guided micro-irradiation (micro-IR) devices, or have purchased one of two commercially available ones⁴¹. The following chapter is a review of the historical efforts and present implementations of image-guided small animal irradiator devices and so for brevity this section is purposely concise. There are however, peripheral challenges to achieving downscaled radiation therapy treatments in small animals that are not addressed in this review, in particular, these can be broadly categorized into challenges in target delivery; small field dosimetry, device modeling for dose calculations, tissue assignment, treatment planning and verification; these aspects form the basis of this thesis and body of work.

ORGANIZATION OF THIS THESIS

In this **Chapter 1**, a general introduction is given on the background and importance of radiation therapy and of preclinical radiation research. In particular, an emphasis is placed on being able to replicate, as best as possible, clinical radiation therapy practices in small animal studies such that the resulting research can be translational; this is made possible by new commercially available small animal image-guided precision irradiators. **Chapter 2** is a thorough review of the efforts of others to performal conformal irradiation studies using the commercially available devices or bespoke ones created in-house. Advice is given on the desired requirements that these devices should possess in order to best replicate the equivalent clinical counterpart. Lastly, in this chapter a discussion of the treatment planning efforts are outlined. **Chapter 3** is a multi-center study, discussing and evaluating the commissioning procedure of these small animal micro-irradiators. Aspects of the commissioning procedure that are more or less sensitive to the final dose estimation are communicated. In **Chapter 4** significant efforts were placed in trying to model a small animal irradiation device using Monte Carlo package to generate 3-D dose calculations in animals and predict the dose behind the animals as means to verify if a treatment plan was delivered as planned.

Chapter 5 was a further exploration of some of the deviations that were observed for the smallest fields by developing an analytical model to more rapidly generate fluence distributions and explore the influence of the focal spot intensity distribution. **Chapter 6** is the software development of a dedicated small animal treatment planning software – SmART-Plan, which is the culmination of all the expertise of the previous work put into a single package. This is now a commercial product. **Chapter 7** details a large cohort study of radiation-induced lung fibrosis in mice that required precision targeting, repeated CT imaging, and conformal irradiation – highlighting all the attractive features of these new image-guided precision small animal irradiators. In addition we were able to demonstrate the high throughput performance of these machines. **Chapter 8** summarizes the previous findings from the earlier chapters and outlines the remaining challenges to be addressed in future research.

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CHAPTER

2

Small Animal Radiotherapy Research Platforms

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ABSTRACT

The advances in conformal radiation therapy and advancements in pre-clinical radiotherapy research have recently stimulated the development of precise micro-irradiators for small animals such as mice and rats. These devices are often kilovolt x-ray radiation sources combined with high resolution CT imaging equipment for image guidance, as the latter allows precise and accurate beam positioning. This is similar as in modern human radiotherapy practice. These devices are considered a major step forward compared to the current standard of animal experimentation in cancer radiobiology research. The availability of this novel equipment enables a wide variety of pre-clinical experiments on e.g. the synergy of radiation with other therapies, complex radiation schemes, sub-target boost studies, hypofractionated radiotherapy, contrast-enhanced radiotherapy, and studies of relative biological effectiveness, to name just a few examples.

In this review we discuss the required irradiation and imaging capabilities of small animal radiation research platforms. We describe the need for improved small animal radiotherapy research, and highlight the pioneering efforts, some of which led recently to commercially available prototypes. From this, it will be clear that much further development is still needed, on both the irradiation side and imaging side. We discuss at length the need for improved treatment planning tools for small animal platforms, and the current lack of a standard therein. Finally, we mention some recent experimental work using the early animal radiation research platforms, and the potential they offer for advancing radiobiology research.

INTRODUCTION

Cancer is a disease with high mortality and morbidity, which represents a significant burden on any country's health care system. In developed nations, about half of all cancer patients undergo radiotherapy¹⁰ at some stage of their care. This is usually administered in the form of external-beam radiotherapy with high-energy photon beams. Until the mid-nineties radiotherapy often consisted of the application of fairly large radiation fields which targeted the tumour but also deposited a significant radiation dose in the surrounding healthy tissues. This restricted the achievable Tumour Control Probability (TCP) because of limitations imposed by the Normal Tissue Complication Probability (NTCP) to avoid debilitating side effects. In the last fifteen years large efforts were made to redress this balance by designing novel beam delivery techniques which allow modulation of the spatio-temporal aspects of radiation dose. Radiation beams can now be made to tailor the dose to the tumour while sparing healthy tissue to a high degree.

This technological evolution, from simple, large radiation beams to highly sophisticated conformal beams was largely enabled by physicists and engineers. These advances were never thoroughly validated on animal models, nor were any prospective randomized human trials held to evaluate the theorized treatment benefits of the technological advances, since these kinds of trials are generally considered unethical due to the perceived violation of the principle of clinical equipoise¹¹. Furthermore, a new problem posed by the modern tightly-conforming techniques is the risk of accidentally missing the target for several possible reasons, with ill-understood consequences. Therefore, in parallel with the development of novel beam delivery techniques, a variety of imaging techniques were developed and implemented to ensure the accuracy of the dose positioning. These methods are mostly based on x-ray imaging¹² but other techniques such as ultrasound imaging have also been employed¹³.

In recent literature some effects of temporal and spatial dose variations were reported, such as the out of field radiation quality changes^{14,15} and the influence of the prolonged treatment regime of intensity modulated radiotherapy¹⁶. The significance of these findings for human radiotherapy is at present not clearly understood. The development of novel radiotherapeutic technologies has allowed the field to move into new, unexplored areas of radio-oncological practice, however, these advances have occurred without the corresponding research from the radiobiological perspective.

The growing appreciation for the microenvironment factors and the development of transgenic animal models has led to a major effort to develop small animal imaging technologies (PET, SPECT, MRI, ultrasound, CT, optical), which are mainly being investigated with respect to systemic cancer treatments and biomarkers to e.g. diagnose tumors. A recent review can be found in¹⁷. These imaging techniques have also greatly aided our ability to longitudinally track the response of new pharmaceuticals in small

animal disease models. In contrast, very little effort was invested in animal radiation research, despite the fact that vast numbers of cancer patients are treated with radiotherapy, and despite the fact that this modality is a highly imaging-driven technique (both at the treatment target definition/planning stage and the beam delivery stage various imaging techniques are used). In fact, the majority of animal data acquired from radiation research that is used in clinical radiotherapy (TCP, NTCP etc) was derived from large field, single-beam irradiations where the dose was crudely estimated, although there were, of course, exceptions. This bears almost no resemblance to modern fractionated clinical radiotherapy with multiple, conformal beams with a complex spatio-temporal dose pattern, where the dose is planned with sophisticated dose calculation algorithms, and verified with an arsenal of modern techniques. Tumor response studies in animals were hampered by high dose to the healthy tissue. Extreme hypofractionation schemes (radiotherapy with dose delivery in a single or a few fractions) cannot reliably be studied in animal models because of the poor conformality of the radiation beams to the tumor. It is, therefore, questionable to which extent the existing animal studies still have relevance for modern radiotherapy practice. NTCP knowledge is needed to support dose escalation protocols. However, our current understanding of TCP and NTCP based on animal studies is probably not entirely relevant for modern treatments.

In current radiotherapy practice we usually aim to deliver a uniform dose to the tumor. However, there are proponents of highly heterogeneous dose delivery studies to selectively target radioresistant sections of tumors based on information derived from functional imaging^{18,19}. Before one embarks on such studies with human patients it would be desirable to collect evidence from animal experiments.

From this discussion it is clear that there is an acute need for animal radiotherapy research platforms where human radiotherapy conditions can be faithfully mimicked. This means that radiation beams need to be downscaled in geometry, but also in energy (see further). Such a platform could be used in conjunction with the many small animal tumor models that were developed in recent years. Due to the small size of e.g. mice the demands on technical precision of such a platform exceed the ones for human radiotherapy. The platform should also have imaging capabilities, similar to human radiotherapy, which means that spatial resolution should be sufficiently high to resolve small organs and structures within. This would then allow image-guided radiotherapy (IGRT) for small animals. Ideally, there should also be integrated dose verification technology²⁰. Currently, there are several efforts to develop versatile small animal radiation research platforms which allow unprecedented animal radiotherapy studies. This is the topic of this review. Such platforms will enable a wide range of studies in fields such as radiotherapy, radiobiology, stem cell studies, immunology, to name just a few.

In this review we will first briefly discuss some animal radiation experimental work before the advent of the sophisticated precision irradiator/imager systems for animals which are now becoming available. This mostly involves fairly crude work but also

some interesting endeavors in precision irradiation. This is followed by a section on the requirements for the irradiation and imaging capabilities of an animal radiotherapy platform. Next, an overview is given of the pioneering efforts of which some recently led to commercially available systems. A section on treatment planning considerations follows. Finally, some thoughts on current and future research opportunities are discussed. The review will not discuss microbeam irradiators for cell radiobiology²¹ as e.g. employed in the study of the bystander effect²².

Early and specialized experimental animal radiation work

For many decades animal radiation studies were mostly performed using fairly crude experimental setups with radiation fields that did not conform to only the target. The radiation sources employed were often kilovoltage (kV) x-ray units, ¹³⁷Cs or ⁶⁰Co gamma ray emitting isotopes or linear accelerators producing megavoltage (MV) x-rays. Commonly, these were radiation devices intended for human patient use. These devices typically are only precise at the level of a few mm. They are capable of delivering a sufficiently precise and accurate dose in larger human structures at a certain depth under the skin, in conditions of sufficient dose buildup, but will entail much larger dose uncertainties in centimeter- or millimeter-sized structures in small animals. Many of these experiments were accompanied by little or no dose measurement. Little or no consideration was given to the dose buildup region at beam entrance regions, which can be extended up to several centimeter in MV photon beams. Similarly, little attention is commonly paid to the beam exit region where missing backscatter can significantly influence the dose. A treatment planning system, adequate for dose calculation in small animals, was usually not available and similar systems for human patients are unsuitable due to the use of MV beams and also the much smaller beams in animal systems. Photon scatter in the animals was ignored. Treatment beam energy was usually not a concern. Dose verification during beam delivery was absent. At best, only limited imaging information just before or during radiation delivery was available. Needless to say, the animal radiation work in the past does not bear much resemblance to the sophisticated patient treatments that are now being used in radiotherapy or will be explored in the near future.

Much of the work in the past was necessitated by the lack of dedicated precision radiation equipment for small animals. Examples include studies of lung irradiation by exposing the partial/whole thorax cavity to kV x-rays²³⁻²⁵. Some of these studies involved a camera to image the specimen's position with respect to the beams. In other studies combined chemo-radiation was studied in head&neck cancers in mice by irradiating five mice simultaneously (this is not an uncommon procedure) in a ⁶⁰Co beam with some shielding provided²⁶. Many more similar studies can be found in the literature but they all suffer from one or more of the shortcomings mentioned above. In the past often tumors were implanted sub-cutaneously. These can be relatively easily targeted with a crudely shaped photon beam. Nowadays the interest in targeting orthotopic tumors (i.e. 'in the right place', e.g. a lung tumor in lung) is increasing, which demands higher targeting precision.

A step towards more accurate radiation administration can be found in the recent literature. By making modifications to existing clinical radiation devices often reasonably adequate dose distributions can be achieved. DesRosiers et al.²⁷ built a dedicated rat positioning system for precision irradiation of a rat eye lens using a GammaKnife unit with 201 ⁶⁰Co gamma ray sources. They combined the small precision collimators with Monte Carlo dose calculations and succeeded in selectively irradiating the small eye lens. They also showed differences between dose calculations from the Monte Carlo simulations and the GammaPlan treatment planning system, which is normally used with the GammaKnife unit. While an accurate irradiation system for this application, each new application would require a dedicated setup. Another drawback is that no imaging modality is available to visualize the targets.

By fitting small custom applicators to clinical orthovoltage x-ray units, partial irradiation of rodent legs implanted with xenografts was possible^{28,29}. In one of these studies²⁸ radiochromic film was integrated to measure the tumor entrance and exit dose. Again, no on-board imaging was available. Similarly, a series of studies was undertaken to partially irradiate the rat spine with blocked MV x-ray beams or photons from ¹⁹²Ir^{30,31}.

A final example to be mentioned in this section is another dedicated small animal irradiator^{6,32}. The device (Fig 1) consists of an optical camera, a computerized stage, a radiation monitor, and very fine pinhole collimators (0.5-1.5 mm diameter) to create precise beams of 50 kV x-rays generated by an electronic brachytherapy source (AXXENT from Xoft Inc). The device was conceived especially to irradiate small structures in anesthetized motionless zebra fish, themselves only a few mm long. The camera and stage system ensured accurate positioning, and the radiation monitor enabled precise dose administration and beam alignment. Monte Carlo simulations were used to calculate the output. The system was capable of producing small photon beams with sub-millimeter penumbras. The system was dedicated to studying cell cultures, zebrafish and other small organisms and cannot be used to study radiation response in small rodents, and is therefore of limited use in radiotherapy research.

Figure 1. Dedicated irradiator with optical image guidance for partial irradiation of zebrafish. From ⁶.

Interesting though some of the devices discussed in this section may be, none are fully flexible radiotherapy research systems with high precision and equipped for image guidance. There clearly is a need for such devices in modern animal radiotherapy research.

Current requirements for small animal radiation research platforms [table 1]

In this section ideal requirements for a small animal radiation research system will be discussed. They are summarized in table 1.

Beam delivery requirements

Targeting

Table 1. Ideal requirements for an image-guided small animal irradiator device.

Ideal requirements for a small animal image-guided irradiator device				Notes
Beam energy	100 – 300 kVp			X-ray tube potential
Min dose rate	0.2 Gy/min			based on 10 minutes for 2 Gy skin dose
	Rabbit	Rat	Mouse	
Image resolution	0.33 – 0.66 mm	0.15 – 0.31 mm	0.065 – 0.13 mm	desired isotropic voxel size relative to voxel size of 1– 2 mm for patients
Image temporal resolution	120 ms or 8 fps	75 ms or 13 fps	40 ms or 25 fps	based on one tenth of respiratory period of free breathing animals
Beam diameter	1.6 – 3.3 mm	0.76 – 1.53 mm	0.33 – 0.66 mm	desired beam size diameter relative to smallest pertinent clinical beam diameter of 5 – 10 mm for patients
Targeting accuracy	± 0.3 mm	± 0.2 mm	± 0.1 mm	The ideal targeting accuracy will have zero mechanical beam positional error and be limited only by image resolution

Tumors in humans usually are of the order of a few cm by the time they are treated with radiotherapy. Radiation fields employed in radiotherapy for cancer therapy are rarely of smaller dimensions (they can be much smaller, though, to treat neurological conditions with stereotactic radiation). Most beam delivery systems have a precision of a few mm. To perform similar treatments in small lab animals one must realize that e.g. a mouse lung measures only one or two cm at most in its largest dimension and that tumors within the lung are even smaller. Therefore, a precision (combined positioning, stability and reproducibility) of a few mm does not suffice for mouse radiotherapy studies, where sub-millimeter precision is required, ideally of the order of 0.1 mm. For targeting sub areas in tumors for boost studies better precision may be needed. The smallest possible size of the radiation beam should be 1mm or smaller, if substructure targeting is desired. This puts high demands on the mechanical precision and accuracy of the system.

It should be possible to aim radiation beams from different directions at target structures, similar to clinical radiotherapy in humans. There, motion of the radiation source is usually limited to an arc. This should also be a minimum requirement in an animal system, which in combination with 3D couch motion offers many ballistic degrees of freedom. Arc motion of very small beams which have to be aimed precisely may pose strict mechanical demands on the system. Ideally, dynamic field shape modulation should be possible.

Beam energy and dose distributions

MV photon beams as used in external beam radiotherapy have several characteristics which are unsuitable for irradiating small targets in small animals. At the air-tissue interface in the entrance region of the beam a MV photon beams exhibits dose build up due to electronic disequilibrium³³. This also happens behind e.g. lung-tissue interfaces, a phenomenon which is often termed re-buildup³⁴. At the tissue side of the beam exit at a tissue-air interface missing backscatter would cause a dose depression. The extent of the buildup regions corresponds roughly to the range of secondary electrons in tissue, which amounts to 1-2 cm for MV photon beams (Fig 2, left). Therefore, exposing small animals to such beams would cause dose build-up/down gradients of the order of the animal size itself. This would make it very challenging to e.g. deliver a uniform dose to a tumor. At the external air-tissue interfaces boluses might be used to counteract these non-equilibrium effects but at internal interfaces this would be impossible. Fig 2 also shows that the dose gradient past the build-up is steeper in kV than MV beams. This will put a restriction on the tube voltage range of about 100-300 kV.

Figure 2. Left panel: Monte Carlo calculations of depth dose profiles for a water/air/water/air slab phantom, modeling a low density cavity embedded in tissue. The beam is a 1 mm diameter pencil beam and dose scoring was performed over the whole width of the beam. For the 6 MV beam and the Cobalt photons the buildup can be clearly seen behind both air/water interfaces. The kV beam exhibits only a minimal buildup effect. Missing backscatter is a more subtle effect but is discernable at the water/air interfaces for the Cobalt and 6 MV beam, but is minimal for the kV beam. Right panel: Calculated dose profiles in water at the depth of maximum dose clearly showing the more extensive beam penumbra for the higher energy photon beams. This calculation separates the effect of the beam spreading in water from other beam widening effects (collimator scatter, focal spot broadening).

Another issue is the lateral beam penumbra. These are caused by a combination of the finite source size, collimator photon transmission and scatter, secondary electrons from collimators and the range of secondary electrons in tissue ³⁵. For MV photon beams these may extend several mm beyond the geometric field, which may lead to unacceptable dose distributions in small structures (Fig 2, right).

Figure 3. Mass energy absorption coefficients versus photon energy for human cortical bone and skeletal muscle with their ICRU44 ⁴ compositions, which are assumed to apply to small animals. Data from the NIST website ^{8,9}.

Therefore, to avoid extensive disequilibrium dose regions and wide penumbras, the use of kV photons generated by an x-ray device is required. For accelerating potentials below 250 kV the buildup regions in tissues are less than a few hundred μm . Penumbras are very sharp for kV x-rays, well below one mm. Missing backscatter at the exit side does occur but is limited to a fraction of a mm. A problem that arises when downscaling from the MV to the kV photon energy range is the increasing importance of the photo-electric effect. Figure 3 shows that the mass energy absorption coefficients for human cortical bone and skeletal muscle differ by more than a factor six around 30 keV. Assuming that these human data also apply to small animals, it is clear that assigning all media to water may lead to significant dose calculation errors. Even muscle may differ from water by more than 3% around 40 keV. In contrast, MV dose calculations are far less sensitive to the tissue composition. The largest differences for photons from ^{60}Co up to 24 MV are around 11% for cortical bone compared to water ³⁶. For all other media differences with water are smaller. Therefore, it is important that dose calculation for kV systems irradiating small animals is treated with care. It should be emphasized that large dose enhancements in e.g. bone in kV beams do not correspond to treatments in MV beams, which may invalidate pre-clinical studies

Dose enhancement effects may also be noted when animals are irradiated in the presence of a high atomic number x-ray contrast medium. This is an uncommon situation for humans during radiotherapy (which is not administered as kV x-rays, anyway), but could be possible in combined imaging/irradiation studies in animals. On the other hand, this creates possibilities for studying contrast-enhanced radiotherapy³⁷. This will be discussed in another section.

Photon spectrum and Relative Biological Effectiveness

In addition to these differences in dose distributions, it should be kept in mind that when one uses very low energy x-rays (say, below 50 kV), for irradiation of small animals that the Relative Biological Effectiveness (RBE) of the radiation in tissue exceeds unity. In other words, the dose required to cause a certain level of radiation damage for low-energy kV x-rays is substantially below the dose required to lead to the same effect in e.g. MV x-rays³⁸. This diverging behaviour of low-energy kV and high-energy MV photons may cause extrapolation difficulties from animal experiments to human radiotherapy. On the other hand, it enables RBE studies. Should this capability be desired, the beam delivery system needs to cover a range of photon spectral energies and shapes.

Imaging system requirements

Spatial resolution and dose

CT imaging is the preferred imaging modality for the localization and treatment planning of solid tumors using external beam radiation therapy. Though CT imaging is inherently poor for soft tissue contrast it is still an excellent tool for visualizing anatomical spatial orientation and is well suited to provide electron density data for treatment planning dose optimization. Recently, kV cone-beam CT has been adapted in radiotherapy and attached to the gantry of linear accelerators¹². This adaptation provides a tool for high-precision radiation therapy and reduces patient set-up errors. Similarly, it would be desirable for a small animal irradiator to contain both the imaging x-ray beam and treatment beam in the same space. As the previous section has shown the ideal treatment beam energy for small animals can be delivered by orthovoltage x-ray tubes (100-300 kVp), which is advantageous because both the imaging and treatment component can be delivered by a single x-ray tube.

Typical CT scans used for clinical treatment planning in radiotherapy contain a reconstructed image voxel (3D pixel) spacing of 1 mm in the x/y transverse plane and 2-5 mm in the z trans-axial plane. In order to maintain the same CT image fidelity for small animal imaging, voxel spacings need to be scaled accordingly. Fortunately, nature provides us with convenient allometric ratios based on mass (M) alone that allow one to easily calculate equivalent physiological parameters for animals of differing weights. For instance, body length scales as $M^{1/3}$ ³⁹. Therefore, for equivalent voxels volumes

from 1-2 mm³ of a 70 kg patient, voxel volumes of 330-660 μm³ for a 2.5 kg rabbit, 150-310 μm³ for a 250 gram rat, and 65-130 μm³ for a 20 gram mouse are required.

Modern day micro-CT scanners can routinely acquire image volumes with isotropic voxel spacings well below 100 μm³ ^{40,41}. However, there is a non-linear dose penalty when reducing voxel spacings for the same level of image noise. In general, if the linear dimension along each side of an isotropic voxel in CT is reduced from 200 μm³ to 100 μm³ a 16-fold increase in photon fluence and therefore dose is required to maintain the same level of image noise ^{42,43}. Consequently, micro-CT imaging of the smallest laboratory animals will have a proportionally higher imaging dose than the equivalent CT imaging of patients and it is not uncommon for a single *in vivo* micro-CT scan of a mouse to deliver half a Gray ^{44,45}. In the simplified model presented by ⁴² a standard deviation level of 1 % in the mean linear attenuation coefficient can be achieved at 25 cGy for an isotropic voxel size of 155 μm³ in a realistic micro-CT scanner. Though, strategies like x-ray scatter rejection or correction schemes can be implemented to reduce the image dose without a reduction in image quality ^{3,46}.

Ultimately, for small animal oncological studies the imaging dose must be sufficiently low that it does not interfere with the biological model. According to the International Commission of Radiological Protection there is no evidence that demonstrates either a deterministic effect of tissue damage or a stochastic effect of disease due to whole-body radiation doses of 10 cGy and below ⁴⁷. This level of exposure to ionizing radiation should be safe for repeated micro-CT imaging in small-animal longitudinal studies as previous authors have shown that the repair processes in rodents will allow them to neutralize 25 cGy of radiation per day ⁴⁸. Therefore, a maximum daily whole-body dose of 10 cGy for the combined image dose in an imaging session represents a low toxic level of dose that is unlikely to result in any observable changes to the biological system of interest due to radiation.

Figure 4. Reconstructed micro-CT image slices of a mouse thorax showing the improvement in image quality with increasing entrance dose; the reduced noise and enhanced detail are apparent.

For repeated or serial micro-CT imaging with daily whole-body doses higher than 10 cGy, one study investigating the radiation effects on bone architecture in mice and rats found that “radiation from *in vivo* micro-CT can have a small effect on trabecular bone in mice that are skeletally immature, and no detectable effect on aged rats” for whole-

body entrance doses of 85 cGy for mice and 60 cGy for rats⁴⁹. In a recently released guideline for the use of animals in cancer, the report cautions users that whole-body imaging doses exceeding 1 Gy can affect tumour growth⁵⁰. Therefore, consideration should be made to determine the influence, if any, of the imaging dose on the biological model of micro-CT imaging with entrance doses exceeding 10 cGy. However, a distinction should also be made for research studies involving animals who are to be immediately sacrificed or that have a biological endpoint which can likely tolerate imaging dose greater than 10 cGy. Nonetheless, a trade-off between voxel size and image noise may have to be made for acceptable imaging doses. Fig 4 shows examples of the influence of the imaging dose on the image quality.

Magnification and focal spot size

To achieve micro-CT image volumes with isotropic spatial resolutions between 100-200 μm^3 an important limiting consideration, aside from the imaging dose, is the x-ray tube focal spot size and magnification ratio. Most commercial micro-CT scanners include micro-focus x-ray tubes with focal spot sizes ranging from 5 to 50 μm ⁵¹. However, micro-focus x-ray tubes do not provide sufficient x-ray fluence to be used for both small animal imaging and irradiations within reasonable treatment times. Fortunately, a number of micro-CT devices now include the use of clinical or industrial x-ray tubes that provide greater power loading capabilities and dose rates⁵². For these systems using large array flat-panel detectors, there is an optimum magnification M that is the ratio of the source-to-detector-distance (SDD) and the source-to-axis-distance (SAD), which minimizes the penumbra blurring of the focal spot size d_{fs} within detector sampling limitations.

As in conventional radiology, the limiting spatial resolution cut-off frequency from penumbra blurring due to the finite size of the focal spot in line pairs per mm (lp mm^{-1}) can be determined using the relation,

$$v_{\text{cutoff}}(d_{fs}) = \frac{M}{(d_{fs} \times (M-1))} \quad (\text{Eq. 1})$$

indicating that there is a decreasing in spatial resolution for increasing magnifications⁵³. Yet for a finite detector aperture d_{ap} (i.e. pixel pitch) an increase in magnification gives an increase in limiting sample (Nyquist) cutoff frequency resolution - projected in the object plane - as follows,

$$v_{\text{cutoff}}(d_{ap}) = \frac{M}{(d_{ap} \times 2)} \quad (\text{Eq. 2})$$

indicating there are fundamental trade-offs between objection magnification, focal spot size and detector aperture. Figure 5 illustrates the optimal magnification ratio for the finest spatial resolution which can be achieved for focal spots sizes of 0.5 and 1 mm and for a detector aperture of 0.2 and 0.4 mm. For example, the optimal magnification for the 0.5 mm focal spot size, and 0.2 mm detector aperture is at the point

when the two limiting spatial frequencies are the same (i.e when $M = 2d_{ap}/d_{fs} + 1$) corresponding to a magnification of 1.8 and a limiting spatial frequency of 4.5 mm^{-1} or $111 \mu\text{m}$.

Figure 5. The limiting spatial resolution of an x-ray imaging system in line pairs per mm for a fixed focal spot size d_{fs} , detector aperture (pixel pitch) d_{ap} and magnification, where SDD and SAD are the source-to-detector distance and source-to-axis distance, respectively.

Dose response, timing and artifacts

In addition to voxel size, resolution and image dose constraints, a micro-CT imaging system should be able to accommodate a wide range of tube potentials in order to achieve the optimal contrast-to-noise ratio for an object of interest⁵⁴. It would also be favorable if the detector had a relatively flat detector quantum efficiency or dose response at treatment energies for dose reconstruction techniques, which have been implemented for several electronic portal imaging devices at megavoltage energies⁵⁵. The x-ray detector should also have a high temporal resolution with frames rates around 50 ms to capture the peak inspiration for respiratory gating in murine models⁵⁶. An imaging system flexible to image at a wide-range of energies would also be useful for future developments of dual-energy imaging⁵⁷ or contrast-enhanced dose delivery³⁷.

Notwithstanding scanner requirements, accurate dose delivery and planning depends on reliable CT image data free from artifacts. CT image artifacts or discrepancies between the reconstruction CT data and the true attenuation coefficients of the imaged object can occur in any number of ways but can be broadly categorized as originating from either the physics, patient, scanner, or reconstruction algorithm⁵⁸. A thorough description of the causes of CT artifacts is beyond the scope of this review but given that CT data set are composed from many x-ray transmission measurements the accuracy of the x-ray detector is an important component to achieve quantitative

CT data. Depending on the x-ray detector, it may suffer from geometric distortion³⁹, image lag and ghosting⁵⁹, or need frequent recalibration for pixel offset and gain. Therefore, routine quality assurance should be implemented to ensure the accuracy, stability and reliability of CT numbers⁶⁰.

Overview of recent developments in small animal radiation research platforms

Several groups, all of them based in North America, have recently started the development of research systems which allow precise irradiation of structures in small animals. Some of these commenced from existing technologies, while others built devices from scratch. Some of these devices also have on-board high resolution imaging capabilities, making them potentially powerful research platforms. In what follows we will highlight the efforts of five groups of researchers. Table 2 compares the characteristics of the systems, which also indicates to what extent the systems fulfill the requirements outlined in section 3 (table 1).

Johns Hopkins University system

The first technology we describe, shown in Figure 6, is the Small Animal Radiation Research Platform (SARRP), which was developed collaboratively at The Johns Hopkins University by a group of radiation physicists and mechanical and robotics engineers. The SARRP has since been commercialized by Xstrahl Ltd. (Surrey, UK) and is being continually refined in partnership with the original Johns Hopkins research team. In basic form, the SARRP consists of an x-ray source mounted on a motorized rotating arm, a dual imaging system for CT and planar x-ray imaging for target localization and a set of robotically-controlled stages for positioning the animal⁶¹.

system	Photon energy range (keV)	Field range at treatment site	Fixed fields/ Arcs	Max dose rate (Gy/min)	Image resolution at treatment site (μm)	targeting accuracy (μm)	Refs
SARRP (Johns Hopkins University)	5-225	0.5mmØ-10x10cm ²	F/A	4	130	200	Wong08, Tryggestad09, Matinfar09
X-RAD (Princess Margaret Hospital)	5-225	1mmØ-10x10cm ²	F/A	4	200	200	Clarkson10
Washington University (¹⁹² Ir)	380*	5-15mmØ	F	2.9	N/A	100-180**	Stojadino-vic06,07, Kiehl08
Stanford University	70-120	0.1-6cmØ***	F/A	2	49	100	Graves07, Rodriguez09, Zhou10a
University of Texas Southwestern	5-320	1-20mmØ	F/A	>10	113	65	Song10, Pidikiti11

Table 2. Characteristics of small animal radiation research systems for rodents. All devices are based on x-ray tubes except the Washington University system which is based on an ¹⁹²Ir brachytherapy source.

*mean photon energy of gamma rays

** depending on system axis

*** using an iris collimator

The radiation source for the SARRP is an industrial 225 kVp x-ray tube (dual focal spot sizes of approximately 0.4 and 3 mm according to the IEC 336 standard; maximum current of approximately 13 mA at 225 kV) and is used for both imaging (60-80 kVp, small focus, typically with 1 mm of Al filtration) and therapy (220 kVp, large focus, typically 0.15 mm of Cu filtration). A 20×20 cm² (1024×1024 pixel) amorphous Si flat panel detector is affixed vertically at 52.5 cm from the x-ray source when pointed horizontally (90°). Cone-beam CT is therefore performed in a novel geometry, fixing the source and detector while rotating the animal about the vertical axis at the nominal isocentric location at 35 cm from the source (corresponding to a magnification factor of 1.5). A second digital fluoroscopic imager consisting of a scintillating phosphor screen located directly underneath the animal, a mirror and CCD camera provides planar cine images when the source is pointed towards the floor (0°). The robotic animal positioning system has four degrees of freedom: X and Y (cross-table), Z (vertical-stage) and θ (rotating table), where the latter is used for CBCT acquisition. The radiation source arm is also motorized and controlled robotically. It can be rotated from the vertical position (0°) to 120°.

Figure 6. SARRP system developed at Johns Hopkins University. a) CBCT imaging mode: the source is fixed in the horizontal (90°) orientation and nozzle collimator is removed; the support platform (θ -table) rotates for tomography. b) Treatment mode: nozzle collimator is attached, the flat-panel detector is shielded and the source arm and robotic stages are positioned to irradiate desired target. (Images courtesy of Xstrahl, Ltd., Surrey, U.K.).

Two collimation systems are presently available for the SARRP which attach to the primary imaging collimator (approximately $17 \times 17 \text{ cm}^2$ at isocenter): a nozzle-shaped collimator with interchangeable inserts providing circular radiation field sizes of approximately 0.5 and 1 mm in diameter and rectangular fields of 3×3 , 5×5 and $3 \times 9 \text{ mm}^2$ is used for high-precision, small-field, conformal irradiations, whereas a set of interchangeable tray collimators provide larger square fields of 3×3 , 5×5 and $10 \times 10 \text{ cm}^2$ which are useful for higher-throughput, less-sophisticated experiments (e.g., cell culture or whole/hemi-body irradiations). The source-to-limiting-aperture-distance for the nozzle and tray collimator are 30 cm and approximately 10 cm, respectively. This results in narrow radiation penumbra for the nozzle collimator; typical widths (20%-80%) of approximately 250 microns are achieved for all apertures at shallow depths around the isocenter. This can be reduced to <100 microns if the small focal spot size is used with the 0.5 mm aperture, which may have greater utility for micro-beam applications. Therapeutic dose rates for applicable shallow depths at 35 cm from the source (large focal spot) vary from approximately 2 to $\leq 3 \text{ Gy/min}$ for the set of nozzle apertures, whereas approximately 4 Gy/min is achieved with the tray collimators.

High targeting accuracy and a tight radiation “sphere-of-delivery” under source-arm or θ -stage rotation has been realized by way of a robotic calibration procedure involving a digital beam-finding camera placed near the presumed isocentric position (in X and Y). With the gantry arm positioned at 0° the camera is rotated over 360° by the θ -stage at two different vertical positions; this associates a specific camera point with the axis of θ -stage rotation. Then, for each gantry angle, the robot stages are moved under visual

servo control until the centroid of the x-ray beam intersects this camera point at two distinct positions, thereby locating the beam axis. A least-squares estimation is used to compute an isocentric point for each gantry angle. Thus, a “virtual” isocentric point can be targeted by applying automated corrections to the animal’s position as a function of source-arm position⁶². For continuous arc deliveries (source-arm or θ -stage rotation) this methodology has been extended such that the virtual corrections are also continuous. It has been shown⁶² that the targeting accuracy of the SARRP is 200 microns and that a stereotactic-arc delivery with the 0.5 mm aperture resulted in 1.07-mm-wide high dose region (FWHM).

Princess Margaret Hospital system

Another research effort which led to a commercially available system is the X-Rad 225Cx developed at the Princess Margaret Hospital (Toronto, Canada). This platform was engineered in collaboration with the company which now distributes it; Precision X-Ray Inc. (PXI, North Branford, CT, USA). It combines a heavy duty x-ray tube (5-225 kV) with a cone beam CT imaging panel mounted on a C-arm for IGRT⁶³. The system is equipped with a 3D computer-controlled stage, which can easily be integrated with a gas anesthesia system. Radiation or imaging experiments would usually have the long axis of the animal coincide with the rotation axis of the C-arm, but other configurations are imaginable. The developers refer to their system as a ‘micro-IGRT’ environment. Figure 7 shows the essential elements of the system. It has its own radiation shielding so it doesn’t have to be housed in a shielded room. The focal spot size is either a nominal 0.4 mm (for imaging) or 3 mm (for imaging and radiotherapy), as measured by the IEC 336 standard. The latter allows delivery of dose rates up to 4 Gy/min. Exchangeable x-ray spectral filters are available.

A crucial part of the platform, which contributes greatly to its stability and reproducibility, is the automated stage corrections which are derived from flex map measurements taken prior to irradiation. By automatically compensating for slight sagging motions of the x-ray tube with respect to the isocenter and the imaging panel an accurate radiation delivery is possible. The stage corrections are small and are performed continuously during radiotherapy beam delivery and image reconstruction. It has been shown that the stage corrections provide a better target dose uniformity and sharper 3D penumbras⁶³.

A flat imaging panel consisting of amorphous silicon elements is mounted on the opposite side of the C-arm holding the x-ray tube (Fig 7). The device can be operated as a static imager, for fluoroscopic imaging or for cone beam CT imaging. In its latest version it has a matrix of 1024×1024 pixels with a spatial resolution of about 200 μm . Backprojection techniques allow reconstruction on the fly of cone beam CT imaging during the arcing motion. It is not known presently if the Hounsfield Units derived from the cone beam images are reliable for dose calculations, which is a common problem for cone beam scanners^{64,65}. Possibly photon scatter in these devices needs correction

Figure 7. Schematics of the micro-IGRT animal research platform developed at the Princess Margaret Hospital (Toronto). The left panel shows the C-arm (blue) on which the x-ray tube (top, orange) is mounted, which is stabilized by a support ring (left, grey). On the bottom of the C-arm the cone beam CT imaging panel is visible (dark grey). In between (center) a 3D computer-controlled stage positions anesthetized animals accurately. The right panel is a photograph of the housing and radiation shielding of the system installed at the MAASTRO Clinic (Maastricht, the Netherlands). (Images courtesy of PXI, Ltd.).

A typical irradiation procedure would consist of pre-scanning the animal at low resolution with a wide viewing field, imaging the region of interest in the animal at high resolution, defining the radiation target isocenter with the aid of a software interface, centering the animal so that its target isocenter coincides with the treatment isocenter, selection of a beam collimator from a set provided, followed by radiation delivery during which the couch undergoes continuous small adjustment for flex compensation. The targeting accuracy has been reported to be on the order of 0.2mm⁶³ with excellent geometric stability over 2 years of routine use⁶³.

In addition to the engineering efforts ongoing at Toronto, further developments are currently being made to this system at the Dutch Cancer Institute and the Maastricht Clinic, both in the Netherlands.

Washington University system

Researchers at the Washington University School of Medicine designed and built a small animal irradiator without built-in imaging capabilities^{1,2,67}. Figure 8 gives an overview of the research platform. The system consists of an ¹⁹²Ir source (different sizes were reported) that can be positioned using a standard brachytherapy remote afterloader at various short distances (1-8 cm) from the target structure. Several tungsten collimators were designed to provide field sizes of 5-15 mm. Fixed beams can

be aimed from four different directions at a mouse, with intervals of 90° . Positioning of the animal is done with a computer-controlled stage. Anesthesia of the animals was achieved through injection ⁶⁷. Since the device has no onboard imaging capabilities a procedure involving fiducial markers on the stage is used to register the CT treatment planning scans (acquired on a human CT scanner ⁶⁷). An irradiation accuracy of 0.3 mm was reported ⁶⁷. The research platform has a software interface for steering the stage, and a treatment planning system (microRTP) that is derived from CERR, a Matlab based in-house research treatment planning system ⁶⁸. Dose rates of 90 cGy/min are possible.

Based on Monte Carlo simulations the authors developed a fast analytical dose calculation scheme that assumes a uniform water geometry, i.e. tissue heterogeneities are not taken into account. They estimate that this assumption causes only small dose errors, mostly in lung. The photon energy of the ¹⁹²Ir isotope ranges from a few keV to 885 keV, with an average energy of 380 keV. Therefore, the differences in absorbed dose in the different mouse tissues are not as large as for kV x-rays, but still their dose calculation method could improve. A more accurate approach taking into account heterogeneities was considered ¹ but it is not obvious how the parametrized dose calculation model may handle this. The fast algorithm was reported to result in dose errors limited to 10% in water, near beam edges and close to the entrance region for small fields (<10mm). These researchers ² reported a buildup region of less than 1.5mm. From their papers it is clear that the beam penumbra extends up to 3 mm. The Washington University researchers are presently working on an in-line tandem combination of a micro cone beam CT scanner and a high power 320 kV x-ray tube capable of providing a dose rate of 20 Gy/min ⁶⁹⁻⁷¹. Both systems rotate around a common axis.

Provided an ¹⁹²Ir afterloader is available, the original solution provided by the Washington University group allows small animal radiotherapy studies in a cost-effective fashion. No commercial system was – as far as we are aware of - developed out of these efforts.

Figure 8. Small animal irradiation under development at the Washington University, microRT. The left panel shows the collimator assembly which can accommodate the ¹⁹²Ir source. The middle panel shows a sketch of the tungsten collimator and the source. The right panel depicts the computer controlled mouse stage in relation to the collimator (from ^{1,2}).

Stanford University system

A team at the Departments of Radiation Oncology and Medicine at the Stanford University started the development of a small animal research system from an existing commercial micro-CT scanner (eXplore RS120 microCT scanner, GE Medical Systems, London, ON, Canada)^{3,72,73}. They designed a collimator which can produce variable pseudo-circular field sizes (0.1-6 cm) through the use of a sophisticated camera-like iris geometry. Figure 9 shows the design of the brass iris and gives a general overview of the device. The iris collimator is one of the unique features of the Stanford system. It consists of twelve pentagonal lead-brass blocks arranged in two planes above each other, forming two stages of collimation. Each stage consists of sliding brass blocks which form a hexagonal aperture. The two hexagonal apertures are arranged coaxially, offset by 30°, each driven by a linear stepper motor. Position sensors attached to each stage measure the aperture dimensions. The total shielding of the collimator exceeds 95%.

Since the basis of this platform is a CT scanner dedicated to small animals, the spatial imaging resolution is very good (0.1 mm). Small radiation beams can administer therapy level doses in a co-planar fashion with an accuracy within 0.1mm³. The system operates at 120 kV with a maximum current of 50 mA and can irradiate animals in a CT-like arc fashion or from discrete directions. To deliver therapy level dose rates (approximately 2 Gy/min) more work appears needed to alleviate heating problems of the generator and x-ray tube. These problems required a delivery time of about 40 minutes for an 8 Gy dose⁷³. The radiobiology of protracted pulsed irradiation should be investigated.

Accurate positioning of the radiation beams was possible through careful calibration and alignment procedures involving a 3D stage. Animals can be anesthetized with a gas system. Sub-millimeter sized beam penumbras were reported for small fields (0.5-2 cm) at 1 mm depth⁷³. At greater depth penumbras were somewhat wider. Treatment planning was based on an in-house developed program, *RT_Image*, which can make use of Monte Carlo dose calculations. To circumvent heating problems the system may operate with a second, larger, focal spot but this will result in larger beam penumbras.

Figure 9. Stanford University modified micro-CT scanner for irradiation of small animals. On the left a part of the iris collimator is shown (there is another similar collimator above it, rotated 30°). The dodecagonal beam cross section from the two stacked irises is a close approximation to a circular field. On the right the main components of the system are shown. From bottom to top: x-ray tube, collimator in its holder, plexi glass CT bore that holds a translation stage and the animal, and CT detector. From ³.

A micro-CT scanner is an expensive piece of equipment but provided one is available, as is the case in many institutes, the modifications needed to allow small animal radiotherapy studies are fairly limited in cost. No commercial product has been developed yet from this work, as far as we know.

University of Texas Southwestern system

Recently, another small animal research platform was introduced which was specifically designed for stereotactic irradiation of e.g. brain and lung, and for normal tissue studies ^{74,75}. It consists of a rigidly fixed 320 kV industrial x-ray tube, capable of high dose rate delivery. Small cylindrical collimators (1-10 mm) shape the stereotactic fields. Image guidance is done with a fixed imaging panel with high resolution. The animal is mounted on a 3D precision stage, which may also hold a cylinder in which the animal can be immobilized and made to rotate around its long axis, perpendicular to the vertical beam axis. This way, stereotactic arc therapy can be administered in discrete steps. They developed software for accurate animal positioning, which finds the target at every discrete treatment angle. The operating mode of this device raises questions about organ motion during rotation of the animal due to gravity which may occur even when the animal has been tightly immobilized.

Treatment planning for small animal irradiation system

For accurate planning of irradiation of structures in small animals there are several similarities with human radiotherapy. Segmentation of structures may be done based on images acquired with the on-board imager (if available) or with a separate, preferably micro-, CT, PET, MRI device. Similar image registration techniques as employed in human radiotherapy may be applied (e.g. fiducial markers). To simplify image manipu-

lations the DICOM imaging standard should be implemented as much as possible. Tissue assignment may be done based on animal atlases^{76,77}. There are also clear differences with human radiotherapy planning. All modern small animal research platforms discussed above use either kV x-rays or iridium γ -rays. Issues such as (re)buildup, missing backscatter and penumbra sharpness were already discussed (section 3.1). In particular for kV x-rays animal tissues are not water-equivalent due to the increasing dominance of the photo-electric effect at lower photon energies. The probability of this effect depends strongly on the atomic number of the tissue to a power between three and four (Fig 3). Thus, there is a need to accurately assign the right tissues to the voxels. This is especially the case for Monte Carlo dose calculations (see further), which may require special imaging modes such as dual-energy CT to avoid dose errors^{57,60}. The atomic number dependence of photon absorption may, on the other hand, be exploited in contrast-enhanced radiotherapy^{37,78,79}. Other differences with human radiotherapy are that animal fields usually have simpler, static shapes, and that multileaf collimators are, as yet, unavailable.

No commercial treatment planning system can currently handle accurate dose calculations for small animals, let alone they would be capable of performing inverse planning for treatments like intensity modulated radiotherapy. The discipline of small animal radiotherapy would benefit from standardization of treatment planning procedures, but thus far the literature has reported diverse approaches which will be discussed briefly in the next sections. Ideally, a treatment planning dose calculation for a small animal should take into account the different tissues, and model small fields accurately.

Measurement based or analytical dose calculations

Depending on the complexity of the radiation model required, simple 1D dose calculations assuming water as the absorbing medium may provide substantial utility. A purely measurement-based point-dose calculator was developed for the Johns Hopkins University system (SARRP – described in Section 4.1). This calculator uses parameterized results from a measured set of water commissioning data. The apparatus for these measurements, which is based on Gafchromic EBT film dosimetry, has been described⁸⁰. A set of nine EBT films are sandwiched in a stack of water-equivalent plastic and exposed simultaneously, the experiment is repeated a number of times, changing the source-to-surface (SSD) and collimators. Percent-depth-dose (PDD) curves, relative to surface dose, are parameterized over the sampled depths, taking either 2D-region-averaged or peak doses for each of the measured depths. Typically a phantom stack is irradiated at three SSDs per collimator; the measured surface dose points allow for a parameterization of the output as a function of SSD. Combining this information, the dose rate for any depth in water for any given SSD is then approximated using the Mayneord F-Factor formalism⁸¹. Provided the effective depth, size and location of the target is known, the 1D dose can be prescribed for single, multi or arc-beam treatment plans.

Investigators at the Brigham & Women's Hospital have developed a general-purpose analytical 3D dose computation tool for kV applications that assumes a homogenous medium for efficiently estimating dose delivered for arc therapies using rectangular cones. 3D dose for an arbitrary arc can be reproduced with good accuracy more efficiently by subdividing the arc into a set of sub-arcs. Inputs to such a system can be either measured or Monte Carlo-generated 2D beam profiles as a function of depth⁸².

Similarly, the Washington University group^{1,2,67} developed for their micro-irradiator built around an iridium brachytherapy source an analytical dose calculation scheme based on a parametrized model in water. It uses functions which were precalculated from Monte Carlo simulations. They used the BEAMnrc Monte Carlo package^{83,84} to simulate the brachytherapy source (without encapsulation) and the collimation system for various field sizes. Their customized dose calculation platform is based on the computational radiotherapy environment CERR⁶⁸. As with the previous two methods, no tissue heterogeneities were considered but this is probably not a serious problem for the relatively high photon energies of ^{192}Ir ⁸⁵. Nevertheless, the authors estimated that errors of 15% could occur in mouse lung due to homogeneous tissue modelling². Much larger errors will occur in bone (see further). An advantage of the approaches mentioned in this section is obviously calculation speed.

Convolution/superposition for kV energies

Convolution/superposition (C/S) is the current standard for MV dose computation, given its faster computational speed compared with Monte Carlo techniques. C/S consists of two parts: First, energy fluence is transported through the patient to compute the Total Energy Released per unit Mass (TERMA) in the volume. Then superposition spreads the TERMA by a dose deposition kernel to determine the final dose at each location. To allow the dose deposition kernel to scale realistically with tissue heterogeneities, the radiological distance is traditionally used, differentiating superposition from convolution. Accuracy limitations of C/S, which are traditionally most prevalent at lung/tissue boundaries, are accepted in favor of treatment planning practicality. The predominance of Intensity Modulated Radiation Therapy (IMRT) and, more recently, modulated arc therapy (e.g., IMAT, RapidArc and VMAT), which require increasingly intensive iterative dose computation in inverse optimizations, has amplified the need for efficiency. Meanwhile, as part of an “adaptive” radiotherapy paradigm, there is a desire in the community for repeated dose computation for each patient, either for plan re-optimization or for dose accumulation during the course of radiotherapy. CPU speeds are no longer increasing at a pace which matches these demands.

Nowadays graphical processing units (GPUs) have gained in popularity and are becoming increasingly flexible for running general purpose algorithms. Recently, researchers at The Johns Hopkins University have implemented C/S on a GPU. Taking full advantage of the parallel architecture of GPUs, they have significantly improved the dose

computation accuracy for MV applications. The main innovations are: 1) using multi-spectral primary fluence attenuation in the TERMA calculation; 2) a back-projected TERMA; 3) dose deposition from a tilted poly-energetic kernel (compared with the more typical approach employing a non-tilted kernel) using volumetric mip-maps¹ to approximate solid-angle ray casting. They have been able to achieve speedups of nearly a hundredfold using a commercially available system as the benchmark (Pinnacle v9.0, Philips Medical Systems, USA)⁸⁶. These authors have since extended their GPU method for kV applications, specifically for the SARRP at Johns Hopkins⁸⁷. This involved changes to the source model. Using a traditional primary or extra-focal source model (as in the MV application) proved to be insufficient for modeling the SARRP nozzle collimators. Instead, an analytical source model was used to determine the fluence exposed to each voxel. The large-focal spot was modeled as a uniform, rectangular area source, whose bounding size was approximated by pin-hole images of the SARRP source. The fluence was found from the area of intersection between the source and the collimator's top and bottom projected back to the source plane. For the circular nozzle collimators, they found the complexity of analytically integrating the intersection of a square with two circles to exceed efficient GPU computation. To handle this problem, they developed a hybrid numerical-analytical integrator which integrated the intersection of a line with two circles. This proved to be fast, simple and efficient on the GPU. The collimator transmission was negligible and was not modeled, which allowed for a drastic reduction in the number of TERMA attenuation values computed.

Figure 10. Results for the kV GPU C/S algorithm in water compared with EBT film measurements and BEAMnrc/DOSXYZ⁵ calculations for the 3×3 mm² SARRP nozzle collimator: a) profiles at two depths (5.1 and 71.1 mm); b) depth-dose.

Using the SARRP x-ray-tube geometry, an x-ray phase space file was calculated using the BEAMnrc Monte Carlo simulation package⁸⁴ at the exit plane of the primary imaging collimator (10.5 cm from the source)⁸⁰. The region bounded by the opening of this collimator was subdivided into 100 bins (10 each in x and y) and an average x-ray energy spectrum was computed for each location. The TERMA calculation treated

1 Maximum Intensity Projection

these (x,y) bins as point samples and the spectrum for any traversing ray was approximated using bi-linear interpolation. The TERMA calculation is multi-energetic; the material properties (μ_{tr}/ρ lookup table for 11 different materials) along the ray-trace were used to attenuate each energy bin separately. In this case, the derived 225 kVp spectrum for each ray was binned into 21 energy bins. Dose deposition from each TERMA voxel was then computed using a poly-energetic x-ray energy deposition kernel, where the assumed spectrum was that of the central-axis ray at the same scoring plane in the previous description. For this, energy deposition kernels were calculated for water in steps of 2.5 keV from 2.5 keV to 225 keV using the EDKnrc module in EGSnrc^{88,89}. For each kernel, 4×10^7 photon interactions were forced at the center of a 10-cm-radius sphere and energy deposition was recorded in a voxelized radial and azimuthal geometry. Sample results from the SARRP kV GPU C/S dose engine in water compared to previously-reported measured and Monte Carlo data⁸⁰ for the $3 \times 3 \text{ mm}^2$ nozzle collimator are shown in Figure 10. Although not shown for brevity, a single normalization factor allows that the GPU engine match measured profile and depth-dose data for the set of rectangular collimators over two measured SSDs.

Efforts are now underway to fully benchmark this kV dose computation for arbitrary in-water phantom arrangements. The next step will then involve experiments with phantoms containing known heterogeneities. It is not clear at this time how well this algorithm will perform in this latter context. Similar to kV Monte Carlo simulations, accuracy of this algorithm is likely to be limited by CT-related uncertainties, as mentioned before.

Monte Carlo simulation techniques

Monte Carlo simulations offer arguably the most accurate dose calculations, provided accurate models for radiation source and animal are used^{90,91}. This is especially the case for kV x-rays where conventional dose calculations in water fail to take the various tissues into account^{92,93}. Several groups have investigated Monte Carlo techniques for small animal radiotherapy. Besides being the most accurate dose calculation technique, it is usually also the slowest.

The Toronto group developed a graphical user interface, DOSCTP^{7,94}, which allows basic treatment planning of kV beams of 0.5-5 cm diameter. DOSCTP performs Monte Carlo dose calculations with the code DOSXYZnrc⁵. CT images of animals with voxels down to one tenth the size of human voxels can be loaded and material assignment is via a CT ramp calibration procedure⁵. The system is capable of creating 3D conformal plans; an example of an arc beam delivery is shown in Fig 11. The results may be exported to commercial treatment planning systems for more detailed analysis. DOSCTP was validated against a commercial treatment planning system for MV beams⁷, and later also for kV beams⁹⁵. The same authors studied the dose increase in mouse bone due to kV x-rays with Monte Carlo simulations in a slab phantom⁹⁶. They found

dose enhancements over fivefold for 100 kV x-rays, at the entrance side of the beam. The effect was observed to depend on kV and depth of the bone in tissue. Beam hardening was invoked as a partial explanation but this needs confirmation. Proximity of lung was also found to have an effect on the bone dose. Presumably dose to the medium bone was not converted to water in this paper. The authors later extended their study to real mouse geometries where hot spots in bone were reported, but very little effect due to the presence of lungs was noted⁹⁵.

In these types of studies the dose in the bone could also be reported as dose to water^{36,97}, which may have advantages to compare to conventional treatment planning systems or to measurements. Therefore, it is important to mention clearly how dose is reported in kV beams to avoid confusion.

Figure 11. Arc irradiation of a small target in a mouse chest with 225 kVp x-rays, planned on a cone beam CT image⁷.

The group at Johns Hopkins University has explored 3D kV Monte Carlo treatment planning for their SARRP platform. In their unpublished work, the solution was to couple dose computation using DOSXYZnrc⁵ with beam definition and dose display performed in Pinnacle. In-house C-code, interactively interfacing with the user via the Pinnacle user-scripting environment, is used to automatically generate the input files required for DOSXYZnrc: (beam angle geometry and beam weighting) and the CT phantom creation. Once the dose is computed by DOSXYZnrc scripts, C-codes are used

to automatically convert the output file into a Pinnacle-format dose image for display. The system uses a set of pre-computed phase space files for each of the available SARRP nozzle collimators generated with BEAMnrc⁸⁰. An example 3D RTP result generated by this platform using 8 co-planar, equally-weighted beams for a mouse bearing a very large pancreatic tumor is shown in Figure 12.

The Stanford group also developed a Monte Carlo model for their micro-irradiator, designed around a modified 120 kV micro-CT scanner. They provided a good source of information on how to optimize the choice of variance reduction techniques (essentially, speed-up techniques)⁵⁶. Simulation times of up to about 100 hrs were reported to achieve a 1% statistical uncertainty. It is conceivable that larger uncertainties are acceptable for small animal radiotherapy. The same group used Monte Carlo simulations to investigate the dose conformity to small spherical and ellipsoidal targets in a phantom delivered by discrete beams spread out in an arc⁹⁸. They studied centered and off-centered targets in a phantom and they also performed planning for a tumor in a mouse lung. They showed dose volume histograms that were close to human lung tumor cases.

Figure 12. a) Sample Monte-Carlo-based 3D treatment plan for a mouse bearing a large pancreatic tumor. Eight co-planar, equally-weighted $5 \times 10 \text{ mm}^2$ beams were used. 5×10^8 photons were simulated in total on a CT-based dose-grid resolution of $400 \mu\text{m}^3$ voxels. b) Co-registered PET image showing the PET-avid tumor rim.

It is clear, however, from these kinds of studies that only fairly simple treatment planning is feasible currently and that both beam delivery techniques and treatment planning for small animal need further development to achieve the full potential of these research platforms. In most of the studies highlighted in these sections human tissues were assigned to small animals, an assumption which needs further scrutiny. For example, most studies assign only one (human) bone type. An in-depth study of the influence on the dose of different bone types was reported⁹⁹, indicating that 47 different bone types are needed to limit dosimetry errors to 2%. Optimal tissue seg-

mentation techniques in general need more attention. Dose reporting in water or other media also requires further study.

Current and potential research with small animal radiotherapy platforms

Small animal radiotherapy research is a young emerging field. Besides the pioneering efforts discussed in this review, to our knowledge eleven radiotherapy centers have recently acquired a small animal research platform and many more are considering such technology. This shows that the radiotherapy community has fully understood the urgent need for this type of research. To highlight just one of the new developments, a team at the University of North Carolina recently reported on the use of carbon nanotubes in field emission cathodes. This technology may be harnessed for ultrafast imaging (e.g. cardiac-gated in small animals) and high-precision irradiation in multi-pixel source arrangements¹⁰⁰⁻¹⁰² but needs further developments to attain higher photon energies.

Not many studies with these advanced irradiation/imaging systems have been published yet and papers are scattered widely over journals and research fields. Our review elucidated that treatment planning to target small structures within animals is far from routine, and much more work is needed before complex dose distributions can be administered with confidence. This is one of the reasons why it may still take a while before the field reaches its full potential.

We will briefly mention here a few published studies that used small animal radiotherapy platforms, albeit not all of them in the field of radiotherapy. Several planning studies showed the feasibility of irradiating small animal structures such as lesions in the mouse lung with thirty beams⁹⁸, or half/whole mouse brains with single or parallel-opposed beams⁶⁷. Biological endpoints were not discussed in these papers. The precision irradiation capabilities of a small animal platform⁶¹ have been used to induce radiation necrosis in rat brain hemispheres¹⁰³. An innovative MRI imaging modality based on an endogenous contrast technique using amide proton transfer imaging was then employed to distinguish radiation necrosis from recurrence of glioma in the brain. This study, reported in the journal *Nature Medicine*, holds promise of offering new ways to distinguish with non-invasive imaging between very different disease outlooks. In the controlled environment of a small animal irradiator combined with advanced imaging techniques, these kinds of studies may be performed with a reasonable throughput to assess therapeutic response. Another group¹⁰⁴ succeeded in developing an orthotopic mouse lung tumor model introduced as a solitary pulmonary nodule, which is a highly desirable model for human lung cancer. Combined with bioluminescence imaging to monitor tumor progression and precision irradiation with high doses⁷⁵, this constitutes an opportunity to probe the biological mechanisms of lung radiotherapy in a pre-clinical setting. The knowledge gained may be beneficial for stereotactic body radiotherapy treatments of lung cancer. Since damage to the remaining healthy lung volume is often the limiting factor in lung radiotherapy, the same group¹⁰⁵ also performed a study of radiation induced damage to healthy mouse lung to study

its normal tissue toxicity. These studies may help to develop an understanding of the radiobiology of hypofractionated radiotherapy, which may ultimately aid in optimizing therapy.

Another area of active study relevant for this new technology is the radiation bystander or “abscopal” effect^{106,107} and the related clinical translational approach of spatially fractionated or “grid” radiotherapy¹⁰⁸. The technology provides the capability to irradiate small sub-regions of tissue, potentially with sharp beam edges, and has therefore been proposed as ideal for studying these phenomena *in vivo*.

In a study in a totally different area, the treatment of Huntington’s disease with selective serotonin reuptake inhibitors¹⁰⁹, precision irradiation of the mouse brain was performed with one of the platforms mentioned in this review⁶¹. The radiation served to block neurogenesis, which was found in this experiment to be a requirement for the drug to be successful. This indicated that neurogenesis-enhancing drugs might be beneficial for the disease investigated in this work. In another study¹¹⁰ radiation was used in combination with other agents to prevent inhibition of apoptosis in pancreatic cancer. The study of synergistic therapies is ideally suited for small animal radiation research platforms.

This non-exhaustive discussion of a handful of papers clearly shows the immense research potential of small animal research platforms². Much work lies ahead of us to develop versatile research platforms and to devise research protocols from which we may draw important conclusions concerning human disease. On the technological side it may be envisaged that coordinated stage/beam motion may enable true intensity modulated radiotherapy in small animals, potentially with highly heterogeneous dose distributions for dose painting studies. Motion compensation with gating signals (respiratory, cardiac and others) is another technology that is already well established in small animal MRI imaging and may be adapted to precision irradiation as well. Treatment planning is in its infancy for these kinds of studies. Much work is needed to be able to do inverse planning, for example. Precise tissue characterization is a prerequisite for the dosimetry of the kV beams used. Being able to reconstruct the truly delivered dose and compare it to the planned dose is also a desirable asset. On the biological side, it is clear that a vast range of research topics can be investigated. One example is the use of gold nanoparticles to enhance both CT image contrast and therapeutic dose deposition¹¹¹⁻¹¹⁴. Other examples include the effect of radiation on novel transgenic tumor models, the synergy of radiation with either radiosensitizers (for tumors) or radioprotectors (for normal tissue), the exploration of novel fractionation schedules, or RBE studies. Precision small animal radiation technology may also open many other, perhaps not yet imagined, new avenues of radiobiological research.

2 The papers on applications of small animal radiation research platforms are so widely scattered throughout the literature that it may be a good idea to organize a web-based repository for these papers.

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CHAPTER

3

Multi-institutional dosimetric and geometric commissioning of image-guided small animal irradiators

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ABSTRACT

To compare the dosimetric and geometric properties of a commercial x-ray based image-guided small animal irradiation system, installed at three institutions and to establish a complete and broadly accessible commissioning procedure. The system consists of a 225 kVp x-ray tube with fixed field size collimators ranging from 1 mm – 44 mm equivalent diameter. The x-ray tube is mounted opposite a flat-panel imaging detector, on a C-arm gantry with 360 degree co-planar rotation. Each institution performed a full commissioning of their system, including half-value layer, absolute dosimetry, relative dosimetry (profiles, percent depth dose and relative output factors) and characterization of the system geometry and mechanical flex of the x-ray tube and detector. Dosimetric measurements were made using Farmer-type ionization chambers, small volume air and liquid ionization chambers, and radiochromic film. The results between the three institutions were compared. At 225 kVp, with 0.3 mm Cu added filtration, the first half value layer ranged from 0.9–1.0 mm Cu. The dose-rate in-air for a 40 x 40 mm² field size, at a source-to-axis distance of 30 cm, ranged from 3.5–3.9 Gy/min between the three institutions. For field sizes between 2.5 mm diameter and 40x40 mm², the differences between percent depth dose curves up to depths of 3.5 cm were between 1-4% on average, with the maximum difference being 7%. The profiles agreed very well for fields > 5mm diameter. The relative output factors differed by up to 5% for fields larger than 10 mm diameter, but differed by up to 49% for fields ≤ 5mm diameter. The mechanical characteristics of the system (source-to-axis and source-to-detector distances) were consistent between all three institutions. There were substantial differences in the flex of each system. With the exception of the half-value layer, and mechanical properties, there were significant differences between the dosimetric and geometric properties of the three systems. This underscores the need for careful commissioning of each individual system for use in radiobiological experiments.

INTRODUCTION

Preclinical models are an important component of translational research in radiation therapy. In studies investigating orthotopic tumor models, tumor micro-environment, and dose-volume effects in normal tissues, a very high level of precision and accuracy is necessary to appropriately target anatomical or tumor-specific regions of interest. In recent years, a number of groups have started to investigate high precision, image guided irradiation systems, designed specifically for small animal applications. Such systems offer conformal, multi-angle beam arrangements with collimated fields down to 1 mm, as well as x-ray image-guidance capabilities and automated specimen positioning to support accurate placement of radiation fields.

Many different designs have been implemented by these groups¹. These include a brachytherapy-based system using an Ir-192 source², the SARRP system (XStrahl Ltd., UK) which consists of a 225 kVp tube, a rotational stage, and a flat-panel detector for imaging³⁻⁵, and a system which is based on the GE microCT scanner⁶⁻⁸. The system described in this manuscript (X-RAD 225Cx, Precision X-Ray Inc., USA) has a 225 kVp x-ray tube mounted on a rotational gantry opposite a flat-panel imaging detector. The tube is used for both imaging and treatment, with manually placed fixed collimators⁹. In addition to these systems, efforts are underway by many groups to develop systems with similar operational capabilities^{10,11}.

There are a number of publications that describe the characterization of different small animal irradiation systems¹²⁻¹⁴. In this work, we describe the characterization of the X-RAD 225Cx and the comparison of its properties for systems at three separate institutions. The design goals and basic characteristics of the system, along with extensive dosimetric and mechanical measurements, are presented. These systems were installed in April 2010, April 2010, and Nov 2010 at Maastric Clinic, the Netherlands Cancer Institute and Princess Margaret Cancer Centre, respectively. The scope of this paper is to evaluate the consistency of the dosimetric and mechanical characteristics between the three systems at these three institutions, and to investigate the possibility of establishing a set of reference data for commissioning of individual systems.

MATERIALS AND METHODS

The goal of the commissioning process at each institution has been to adequately characterize the dosimetric and mechanical properties using measurements alone of a small animal micro-irradiation so as to be able to compute and deliver a point dose in an animal within an accuracy of 5%. The value of 5% is chosen to align with the commonly quoted value for clinical radiation therapy¹⁵. The absolute dose (in-air and water) for a 40x40 mm² field has been measured. Relative dose distributions, including profiles and percent depth dose, have been characterized for a range of field sizes

from 2.5 mm diameter to 40x40 mm² square Dosimetry presented here has all been performed at 225 kVp, as this is a commonly used energy for small animal irradiations.

System Description

Hardware and Software

The system consists of a 225 kVp x-ray tube (Comet MXR-225/22 tube, GE Isovolt 225 Titan generator) and an amorphous silicon flat panel detector (PerkinElmer XRD 0820 AN3 ES (1024 x 1024) digital x-ray imager)⁹. These are mounted on a C-arm gantry with additional support ring for stabilization and mechanical rigidity, the tube and flat panel detector rotate 360 degrees while the object remains stationary. The x-ray tube can operate from 5 to 225 kVp, with a maximum power of 3 kW, although useful beams are not produced below about 20 kVp. Dual focal spots make this tube appropriate for both imaging and treatment. The position and intensity distribution of the focal spot in an x-ray tube become important for radiation fields having sizes comparable to the focal spot itself¹⁶. The actual focal spot sizes along the longest axis provided for each system by x-ray tube manufacturer were 0.8-0.9 mm (small focal spot), and 3.4-3.8 mm (large focal spot). These are smaller than the nominal values quoted by the manufacturer of 1 mm and 5.5 mm (small and large focal spots according to EN 12543).

Typically, the maximum energy and current (225 kVp, 13 mA, with 0.3 mm added Cu filtration) is used for irradiation, while a lower energy and filtration (40-120 kVp, 2mm Al added filtration) is used for image acquisition. The flat-panel detector consists of a CsI scintillator screen with 1024x1024 active elements (200 μ m pixel pitch), and a maximum readout of 30 frames-per-second when the elements are binned 2x2. The source-axis distance (SAD) of approximately 30 cm and source-detector distance (SDD) of 62 cm give an effective pixel pitch of 0.1 mm at isocenter. The system has a 3-dimensional translational stage with encoded motors.. Cone-beam CT images are reconstructed using a standard Feldkamp algorithm¹⁷. Projections from each angle are corrected on a pixel-by-pixel basis for dark field, panel non-uniform response and bad-pixel data, as well as angularly dependent mechanical flex of the system. Secondary collimation of the radiation field is achieved through interchangeable cones, with field sizes varying from 1 mm to 40 mm, and without any secondary collimator, the field is approximately 125x125 mm² at isocenter. The fixed collimators are a two-stage, non-tapered cone collimator. They consist of an upper lead aperture that truncates the primary radiation field to a size just slightly larger than the lower lead aperture and brass protective aperture, which together define the field. An image of the collimators can be found in¹⁶. The distance between the final collimating aperture and the isocenter is 70 mm. The dosimetric data for a subset of the available collimators was analyzed and compared in this study. The 6 collimators chosen for comparison were: 2.5 mm, 5 mm and 10 mm diameter circular collimators, and 10x10 mm², 20x20 mm² and 40x40 mm² square collimators. System calibration, image acquisition and reconstruction, image-guidance, and irradiations are managed through accompanying software,

which includes database management of experimental and imaging data. Details of the imaging and image-guidance capabilities of the system have been previously described⁹.

Radiation Safety

The system is housed in a self-shielded cabinet. The x-ray tube is always appositional to the flat-panel detector, with a maximum field size of 125x125 mm² at isocenter. Lead placed behind the flat-panel detector (6.3 mm) serves as a primary shielding barrier. The leaded door (9.5 mm) and lead inside the steel enclosure (7.9 mm) serve as secondary shielding barriers. Shielding surveys were conducted around the exterior of the system, including at the door hinges, leaded glass window, and the tube maze for anesthesia and cabling. The dose rate outside is < 1 µSv/h at 10cm. The door of the system is interlocked against radiation and mechanical motion. The system has multiple beam-off and emergency power-off buttons, all which must be tested as a part of acceptance of the system.

Absolute Dosimetry

Absolute dosimetry was performed following the recommendations outlined in report of the American Association of Physicists in Medicine Task Group #61 (TG61), using the “in-air” and “in-phantom” methods for orthovoltage x-ray tubes to determine the dose-to-water at the surface of a water phantom and the dose-to-water at 2 cm depth in water, respectively¹⁸. Measurements were done at the maximum energy, which is most commonly used for irradiation at each institution, 225 kVp with 0.3 mm Cu filtration. All measurements were made at the isocenter. Absolute dosimetry was performed using calibrated ionization chambers that could be traced to measurements at Accredited Dosimetry Calibration Laboratories (ADCL). All centers used a 0.6 cc Farmer-type ionization chamber (NE 2571 and PTW30012) for calibration, with a comparison between Farmer ionization chamber measurements and a plane-parallel ionization chamber (PTW soft x-ray chamber 0.02 cm³, Freiburg, Germany) at one institution. Chamber calibration factors were available for energies from 50-270 kVp. Correction factors in TG-61 for temperature and pressure were applied.

Due to the nature of small animal irradiators, the relevant field sizes and fixed geometries, it is not possible to follow the recommendations in TG61 without the following accommodations: (1) The reference field size is not the recommended field size of 100x100 mm² but rather that collimated by using a 40x40 mm² square field; and (2), the half-value layer (HVL) measurements are performed with the detector at a distance less than 50 cm. The first accommodation to the TG61 guidelines requires extrapolated values for the chamber perturbation($P_{Q, \text{cham}}$) and the mass energy-

absorption coefficient ratio $\left(\frac{\bar{\mu}_{\text{en}}}{\rho} \right)_{\text{air}}^{\text{water}}$ using the in-phantom method, but both can be estimated from graphs provided within TG61.

First and second HVL measurements were made with the x-ray tube oriented vertically upwards and adding increasing thicknesses of pure copper on top of the tube collimated to just encompass the ionization chamber. Due to the geometrical constraints of this system, the maximum distance between the ionization chamber and surrounding structures achievable was 20 cm. The influence of scatter from the attenuating material outside the field was quantified by varying the source, ionization chamber and detector distances as much as possible, and deemed negligible. Both first and second HVLs were measured and compared between all systems.

Relative Dosimetry

Measurement Conditions

Relative dosimetry measurements have been performed using various measurement devices, including small volume air and liquid filled ionization chambers and film. Relative dosimetry was performed in water or solid water phantoms, available at the different institutions. Measurements were made in large ($30 \times 30 \times 30 \text{ cm}^3$) and medium ($25 \times 25 \times 15 \text{ cm}^3$) water tanks as well as large (area perpendicular to the beam of $20 \times 20 \text{ cm}^2$) and small ($4 \times 8 \text{ cm}^2$) solid water phantoms, as those will be addressed in this manuscript. Large phantoms are used to reproduce the clinical commissioning conditions. The use of a small phantom to represent a typical size of scatter for small animal experiments was also employed.

Film measurements were performed using EBT-2 Gafchromic film (Ashland Inc, Covington, KY, USA) following standard protocols¹⁹. Because EBT-2 film may have some energy dependence²⁰, calibration was performed on the XRad systems or on an orthovoltage system with similar beam quality. EPSON Pro V750, 1680 and 10000XL scanners (Seiko Epson Corp, Suwa, Japan) were used, and 48-bit images were acquired at 225-300 DPI (pixel sizes of 0.085-0.11 mm). All film measurements were performed in slab phantoms, with the films oriented perpendicular to the beam axis.

X-Ray Tube Linearity with Current and Time

Linearity with current and linearity with time of the x-ray tube output were tested using in-air measurements. At 225 kVp, currents from 0.1 – 13 mA were tested, and times of 1-60 seconds

Percent Depth Dose

Percent depth dose (PDD) measurements were performed using EBT-2 film and ionization chambers, in both water and solid water. The results are shown only up to a depth of 3.5 cm, as this is typical of the thickness of a small animal (i.e. a mouse). All film measurements were performed with films perpendicular to the beam axis. Some of

the PDD data at one institution was acquired using the PTW μ Lion chamber. The μ Lion chamber is a liquid filled ionization chamber, with a sensitive volume of 0.0017 cc (radius of 1.25 mm, active length of 0.3 mm). The chamber was oriented with the length parallel to the beam axis, such that the averaging was over the active length for depth dose measurements.

Relative Output Factors

Relative Output Factors (ROF) were measured in the small solid water phantom, at a depth of 0.5 cm. The ROF for the smallest field sizes (<0.5 cm diameter) were measured with film alone; for larger field sizes, results with film and ionization chamber were compared at one institution. A Capintec PR-05P (Capintec Inc, Ramsey NJ, USA) ionization chamber (0.07 cc, 2 mm inner radius) in a custom-made solid water holder was used for the measurements. The reference field for all relative output factor measurements was the 40x40 mm² square field.

Dose profiles

All field size, penumbra, and profile data was acquired based on film measurements in solid water. From the 2D film measurements, profiles were taken through the center of the field. The profiles were evaluated in terms of field size, 80-20% penumbra, flatness and symmetry. Flatness and symmetry were calculated over the central 80% of the field (where the full field is defined by the 50% dose) at 0.5 cm depth in-phantom, as follows²¹:

$$\text{Flatness} = (D_{\text{max}} - D_{\text{min}}) / (D_{\text{max}} + D_{\text{min}}),$$

$$\text{Symmetry} = \max(|D(x) - D(-x)|) \text{ for any pair of symmetric points } (x \text{ and } -x).$$

Inverse Square Law

The agreement with inverse square falloff was quantified, and the location of the virtual source was calculated. This was done for a collimated field size of 40x40 mm². for which the maximum practical deviation is ± 5 cm from isocenter. Over this distance, the field size changes by 25%.

Out-of-Field Dose

The out-of-field dose, due primarily to phantom scatter, was measured by EBT-2 Gafchromic film in the small (4x8 cm²) solid water phantom, for field sizes ranging from 2.5 mm to 40x40 mm².

Mechanical operation and performance

Mechanical operation and performance of the system are a key component in the ability to deliver precision irradiation on an appropriate scale for small animal studies. Particularly for small fields, collimator alignment and gantry flex can cause substantial dosimetric errors for multiple beam or arc delivery. In addition to ensuring that routine irradiations are performed accurately, an adequate characterization of the system is crucial for accurate Monte Carlo simulations, including a good understanding of the source geometry^{16,22}. This includes measurements of the SAD and SDD, characterization of the focal spot distribution, and quantification of the system flex.

Magnification

The magnification of the system, and the SAD and SDD were quantified using a phantom consisting of 16 ball-bearings (bbs) arranged in a 2-circle geometry²³. The phantom size was modified (diameter of the bb-ring was 65mm, distance between the two rings was 65mm) to be appropriate for the field-of-view of this system. This phantom can be used to quantify an arbitrary cone-beam CT (CBCT) geometry, and analysis of the results gives SAD and SDD as a function of gantry angle. The results presented here are averaged over all gantry angles.

Gantry Flex during Imaging and Radiation Delivery

The x-ray tube and flat panel detector are mounted on a C-arm gantry with a stabilizing ring at one end, which reduces the flex of the system but does not eliminate it completely. Therefore, for accurate image reconstruction and radiation delivery, the mechanical flex of the system must be quantified through a projection map²⁴ and Winston-Lutz test²⁵. The projection map quantifies the flex of the x-ray source relative to the flat panel detector, and is then used to correct individual projection images during CBCT reconstruction. From the Winston-Lutz test, residual motion of the radiation beam relative to a fixed point in space is quantified and then uploaded to the hardware controller which controls the stage position. During radiation delivery, the stage then moves by predefined amounts, as a function of gantry angle, to compensate for the mechanical flex of the system. Both the magnitude and reproducibility of the flex of the system are important to ensure good image quality, and accurate, precise radiation delivery. To quantify how well the stage motion is compensating for the mechanical flex of the system, the software has the capability to acquire the Winston-Lutz test with “tracking-on” during which the stage motion is enabled. Perfect compensation for the flex of the system would result in a residual Winston-Lutz with zero offsets between the center of the radiation field and the fixed point in space across all gantry angles.

A set of three repeated projection maps and Winston-Lutz tests were acquired with and without stage corrections for a single sized collimator at each institution. Tests were repeated a number of months apart, to demonstrate the reproducibility of the

system. The results for these are displayed in the coordinate system of the flat-panel detector, with the u-axis orthogonal to the axis of rotation of the system and v-axis aligned with axis of rotation.

RESULTS

The results for all three institutions are shown labeled Institution A, B and C.

Absolute dosimetry

Table 1 shows the 1st and 2nd HVL measured at the three institutions. The error is estimated by error propagation of the linear fit, and the value represents the first confidence limit.

	Institution A	Institution B	Institution C
1st HVL	0.98	0.91	1.02
2nd HVL	1.95	1.89	2.14

Table 1. 1st and 2nd half value layer (mm Cu) for 225 kVp, 0.3 mm Cu added filtration. The uncertainty in these measurements is < 0.02 mm Cu.

Table 2 shows the results of absolute calibration in-air and in-phantom for all three institutions. To compare the measured output in-air and in phantom, the Tissue-Phantom-Ratio (TPR) was calculated from the PDD at 2 cm depth. If the in-air and in-phantom methods were in perfect agreement, the TPR at 2cm depth would be equal to the phantom/air output.

40x40 mm ² square			
	A	B	C
output in-air (Gy/min)	3.9	3.6	3.5
output in-phantom (Gy/min)	3.4	3.1	3.0
PDD at 2cm depth (%)	77	75	79
TPR at 2cm depth (calculated)	0.88	0.85	0.90
phantom/air output	0.88	0.87	0.86

Table 2. Absolute output in-air and in-phantom for the 40x40 mm² collimator at the system isocenter. The percent depth dose, measured in phantom, are used to calculate the TPR at 2 cm depth. The ratio of output in-phantom to output in-air is shown to demonstrate the agreement between in air and in phantom measurements.

The uncertainty in absolute dosimetry estimated in the AAPM TG61 protocol is 3.6%.
Relative Dosimetry

The uncertainty in the film measurements included in the following sections is estimated to be 2-3% for individual measurements, which corresponds to 3-4% for composite measurements (i.e., PDDs and ROFs). The uncertainty analysis of the film dosimetry was done following the method of Devic et al¹⁹. An uncertainty of <1% may be achievable using further uncertainty analysis²⁶. Error bars are not shown on individual data-points for clarity of data presentation.

Measurement conditions

One of the concerns about the dosimetric characterization of these systems is that in the small animal context, the scatter conditions for experimental irradiations may be much different than in the calibration situation, which is typically a large field. Monte Carlo calculations in BEAMnrc²⁷ were used to determine the influence of scatter as a function of beam diameter, phantom diameter, and depth within the phantom for a 225 kVp beam. These simulations demonstrated that the proportion of the dose along the beam axis due to scatter is primarily dependent on the beam size and independent of the object size except for the case where the beam size is greater than the object size. For all fields (up to 40 mm diameter), Monte Carlo simulations showed only small difference (<3%) between the dose in small and large phantoms, as long as the phantom was at least as large (area and depth) as the beam diameter, which is particularly relevant when irradiating small specimens such as mice using large fields (>10 cm) as recently quantified²⁸. The contribution to dose due to scattered radiation at the surface and at 2 cm in the water phantom for increasing beam size with respect to the primary dose are shown in Table 3. The influence of scattered radiation was found to be non-negligible (>5%) for all beam diameters and has consequences for dose calculations based solely on the primary dose calculation or dose-to-medium to dose-to-water conversion strategies that rely on knowledge of the spectrum. As a result of these simulations, all relative dosimetry measurements using film were done using a small (40x80 mm² area) solid water slab phantom (5mm thick slabs).

	2 mm Ø	10 mm Ø	25 mm Ø	40 mm Ø
Surface	0.024	0.069	0.141	0.192
2 cm depth	0.054	0.184	0.393	0.506

Table 3. Monte Carlo results for scatter-to-primary dose ratio at the surface and 2cm depth shown for a cylindrical phantom having a diameter and height of 7 cm. The backscatter factor can be obtained for the surface S/P ratios by adding 1 to this ratio.

Linearity

For currents between 0.1 and 13 mA, for 30 seconds irradiations, a linear curve was fitted to the ionization chamber reading (dose) vs mA and gave an R^2 value of 1.0. The y-intercept was 0.25% of the maximum reading. The timer error, for irradiation times of 1-60 seconds, at a current of 13 mA, did not exceed 0.2 seconds for any of the three systems, and so for practical use this is ignored in the dose calculation. It should be noted, however, that the generator can be configured by the end user to have different ramp-up times, and so that end-effect must be tested for each system to confirm that it is negligible.

Figure 1. Percent depth dose curves for the (A) 2.5 mm $\varnothing$ collimator, (B) 5 mm $\varnothing$ collimator, (C) 10 mm $\varnothing$ collimator, (D) 10x10 mm² collimator, (E) 20x20 mm² collimator and (F) 40x40 mm² collimator. The curves labeled A, B, and C are for the three institutions for each field size. In figures A, B, C, and F, the institution C curve was measured using the μ Lion ion chamber.

PDD

Figure 1 shows the PDD curves for all three institutions for a range of field sizes. Data for institutions A and B were measured with EBT-2 GafChromic film in solid water and for Institution C were measured using both film and the μ Lion chamber in water. Good agreement was found between the two measurement techniques at Institution C Table 4 shows a quantitative comparison of the PDD curves, with the average and maximum difference between datasets from each pair of institutions.

Collimator Size	Institution A vs B	Institution A vs C	Institution B vs C
2.5 mm $\varnothing$	2.5 (5.7)	1.6 (3.6)	1.5 (3.7)
5 mm $\varnothing$	3.1 (5.8)	0.7 (2.4)	3.7 (5.9)
10 mm $\varnothing$	0.9 (2.3)	3.5 (5.0)	3.2 (4.4)
10x10 mm ²	1.3 (3.0)	0.8 (2.7)	1.5 (4.2)
20x20 mm ²	1.0 (1.9)	1.4 (3.2)	1.3 (3.7)
40x40 mm ²	1.3 (3.7)	1.9 (4.4)	2.9 (7.0)

Table 4. Comparison of the difference between PDD curves from the three institutions. The average (maximum) differences (%) over the depth range 0-3.5 cm are shown. The differences are quantified as a percent of the PDD curve, not as a local percentage difference.

ROF

Table 5 shows the relative output factors for selected field sizes, relative to the 40x40 mm² collimator. The differences in relative output factor are <2.5 % for the square field sizes, 7.5% for the 10 mm circular field size, and 49% for the 2.5 mm field size. To calculate the ROF, a region of interest on the films of 2.5x2.5mm² was used for all fields > 5mm, and a region of interest of 0.5x0.5 mm² for fields ≤ 5mm. The standard deviation of pixel values within these regions was <3.0%, except for the 2.5 mm field size, where the standard deviation < 4.5%.

	Institution A		Institution B	Institution C
	Film	Film	Ionization Chamber	Film
2.5 mm $\varnothing$	0.27	0.76	N/A	0.44
5 mm $\varnothing$	0.79	0.82	N/A	0.65
10 mm $\varnothing$	0.83	0.83	0.87	0.82
10x10 mm ²	0.85	0.83	0.89	0.86
20x20 mm ²	0.94	0.89	0.94	0.94

Table 5. Relative output factors (normalized to the 40x40 mm² square field) measured at 5mm below the surface in solid water.

Figure 2. Normalized dose profiles for the (A) 2.5 mm collimator, (B) 5 mm collimator (C) 10 mm collimator , (D) 10x10 mm² collimator, (E) 20x20 mm² collimator and (F) 40x40 mm² collimator. The curves labeled A, B, and C are for the three institutions for each field size.

Dose profiles

Table 6 shows a summary of the dose metrics from the beam profiles. Figure 2 illustrates the profiles for all collimators. All the profile data shown here is aligned along the cathode-anode axis of the x-ray tube. The orthogonal profiles (data not shown) show slightly larger field sizes and beam penumbra (on the order of 0.1-0.3 mm), due to the asymmetric shape of the focal spot.

The flatness and symmetry metrics are shown for completeness, but their value in this context may be limited. They are quite sensitive to the processing of the film results (resolution, smoothing), and also to the choice of “central” region used for analysis. In this data, the central 80% of the curve was used, but for some of the fields shown (10 mm circular field, for example), the shoulders of the curve start to fall off within the central 80% region. For the 2.5 mm circular field, the flatness and symmetry results are not presented, because there is not any central flat region in these fields. For all quantifications using the films, a median filter was applied to the curves to attempt to achieve a similar level of noise. Although some of the numerical differences in flatness and symmetry between systems are very large, qualitatively, the profiles are very similar, as can be seen in Figure 2. The exception to this is the 2.5 mm circular collimator, which has a very different shape between the three different systems. This is due to the interplay between the size of the focal spot and the alignment of the collimator. This issue is further highlighted in the discussion.

		Institution A	Institution B	Institution C
2.5 mm Ø	Flatness (%)	---	---	---
	Symmetry (%)	---	---	---
	Field Size (mm)	2.5	2.3	2.8
	Penumbra (mm)	0.7	0.5	0.4
5 mm Ø	Flatness (%)	2.0	3.0	9.5
	Symmetry (%)	1.0	0.5	0.5
	Field Size (mm)	5.0	4.9	6.7
	Penumbra (mm)	0.5	0.5	0.7
10 mm Ø	Flatness (%)	1.4	2.1	2.8
	Symmetry (%)	0.7	4.1	1.3
	Field Size (mm)	10.1	9.7	10.2
	Penumbra (mm)	0.7	0.5	0.5
10 x 10 mm ²	Flatness (%)	1.5	1.8	1.8
	Symmetry (%)	0.3	2.5	0.4
	Field Size (mm)	10.2	10.2	10.4
	Penumbra (mm)	0.7	0.6	0.6
20 x 20 mm ²	Flatness (%)	3.1	3.6	2.9
	Symmetry (%)	1.3	2.2	1.0
	Field Size (mm)	21.0	20.7	20.9
	Penumbra (mm)	0.8	0.6	0.7
40 x 40 mm ²	Flatness (%)	3.8	5.0	5.4
	Symmetry (%)	1.5	3.6	6.7
	Field Size (mm)	41.6	39.2	43.7
	Penumbra (mm)	1.3	0.7	2.3

Table 6. Beam profile characteristics at 5mm depth in-phantom.

Inverse Square Law

Measurements were made with the 40x40 mm² square collimator. For distances within ± 5 cm of the isocenter, the fitted value for the effective SSD was 29.5, 28.9, 28.8 for institutions A, B, and C, with an R² > 0.98.

Figure 3. Dose beyond the edge of the radiation field. The results are displayed as a function of distance from the field edge, quantified as 50% of the central axis dose.

Out-of-field Dose

Figure 3 shows the out-of-field dose as a percentage of the dose on the central axis, for field sizes ranging from 2.5 mm to 40x40 mm², starting at a distance of 0.5 cm from the field edge. The field edge is defined point where the dose is 50% of the central axis dose. At distances > 0.5 cm from the field edge, the dose is < 2%, and as expected, the out-of-field dose decreases with decreasing field size. The data shown is from only one system (Institution B).

Mechanical Operation

Magnification

The results in Table 7 show the SAD and SDD determined for all three institutions. Note that this is the average over all gantry angles.

Table 7. Geometric characteristics of the systems, as quantified by a 16 bb phantom [reference]. Results are the mean ± 1 standard deviation (in cm), quantified over 360 degrees rotation of the system.

	Institution A	Institution B	Institution C
SAD	30.2 ± 0.1	30.1 ± 0.1	30.2 ± 0.1
SDD	62.0 ± 0.1	62.1 ± 0.2	62.3 ± 0.1

System Flex during Imaging and Radiation Delivery

Figure 4. Projection map showing the flex of the x-ray tube relative to the flat panel detector in the u , v -coordinate system of the detector. Results are back-projected to the system isocenter (mm at isocenter). Data are shown at three time points for each system. (A) shows the results in the u -axis (orthogonal to the axis of rotation of the system) and (B) in the v -axis (parallel to the axis of rotation). Note the scale of (A) and (B) is not the same.

Figure 4 shows the projection maps for all three institutions, with the u - and v - coordinates (detector coordinate system) of the isocenter as a function of gantry angle, at the SAD. The magnitude of the flex in both u - and v - is $< 0.5\text{mm}$, with the flex in u - being slightly smaller ($< 0.2\text{ mm}$) for all three systems. The shape of the curves is very reproducible between time-points for each system, although the shape between different systems is variable. The shift in the mean value of the v - curves depends on the location that the ball-bearing is placed during the flexmap. This does not represent

variability in the actual flex of the system, and is corrected for when the flexmap is applied. Quantitatively, the maximum peak-to-peak variation in any give curve (u , v) is A: 0.15 mm, 0.40 mm; B: 0.11 mm, 0.48 mm; C: 0.10 mm, 0.34 mm. The maximum variation between any two curves (u , v) for any one institution (for any given gantry angle) is A: 0.09 mm, 0.48mm; B: 0.03 mm, 1.19 mm; C: 0.02 mm, 0.25 mm.

Figure 5. Winston-Lutz results at three time points for all three systems. The results are in u , v - coordinate system of the flat panel detector, and quantified at the radiation isocenter. (A) show the u -axis and (B) shows the v -axis. Winston-Lutz with tracking on results for all three systems. The results are in u , v - coordinate system of the flat panel detector, and quantified at the radiation isocenter. (C) show the u -axis and (D) shows the v -axis.

Figure 5(a, b) shows the Winston-Lutz test results for the difference in u - and v - coordinates (detector coordinate system) between the center of the radiation field and the isocenter as a function of gantry angle. The magnitude of the flex in both u - and v - is < 1.5 mm, with the flex in u - being slightly smaller for all three systems. The shape of the curves is very reproducible between time-points for each system, although the shape between different systems is quite variable.

The shifts in the mean position of the curves at different time-points (within each institutions dataset) are due to reproducibility in positioning the collimator within its assembly. Quantitatively, the maximum peak-to-peak variation in any give curve (u , v) is A: 0.63 mm, 1.44 mm; B: 0.66 mm, 0.71 mm; C: 0.79 mm, 1.10 mm. The maximum

variation between any two curves (u , v) for any one institution (for any given gantry angle) is A: 0.47 mm, 0.59 mm; B: 0.18 mm, 0.51 mm; C: 0.23 mm, 0.15 mm.

The results for the Winston-Lutz tests with tracking on are shown in Figure 5(c,d). There is a show a maximum residual motion of ± 0.19 mm in u and ± 0.33 mm in v for any of the 3 institutions. The Winston-Lutz results with tracking on were acquired at each time point immediately following the Winston-Lutz acquisition. Therefore, these represent the best possible targeting achievable, as they do not incorporate the errors caused by removing and replacing the collimator.

DISCUSSION

This study involved collecting and comparing commissioning data from three institutions each using the same small-animal micro-irradiator system, the X-RAD 225Cx. Part of this project was to establish, between all three groups, a set of important data to collect and a process for commissioning that was similar enough to allow comparison. Our aim was to evaluate both the numeric results themselves, as well as the agreement between the different systems. We have presented here the results for basic dosimetric and mechanical characterizations of the three systems. Table 8 summarizes the agreement between the measurements of different quantities, and recommendations about data acquisition for individual systems. We briefly discuss some of the key points and make some recommendations. The measurements are classified as those which have direct impact on the resulting dose (i.e., change in dose is linear with change in measurement quantity) and those which have an indirect impact on the resulting dose. The agreement is classified as good versus poor if the impact on dose is $\leq 5\%$ for direct quantities. For indirect quantities, we quantified the agreement as good versus poor if the quantity changed by $\leq 10\%$, or if the expected impact on dose would be $\leq 5\%$. Overall, the agreement between the three institutions was good for most quantities. The notable exceptions are for the 2.5 and 5 mm diameter collimators, for which alignment and focal spot occlusion can cause substantial variation between the different systems.

Table.8. Recommendations

Quantity	Impact	Agreement	Remarks
Absolute Output	Direct	Poor	Must measure at each institution using ionization chamber traceable to primary standards and calibrated at an appropriate beam quality; using the appropriate backscatter factor
Relative output factors	Direct	Good for fields > 5mm Ø Poor for fields ≤ 5mm Ø	Must measure at each institution
PDD	Direct	Good	Efforts should be taken to reduce the measurement uncertainty as much as possible
HVL	Indirect	Good	Could use consensus values, or those provided in SpekCalc ²⁹
Profiles	Indirect	Variable across field sizes	Sensitive to collimator alignment and individual focal spot shape
Magnification and Effective SSD	Indirect	Good	Could use consensus values
Flex Reproducibility	Indirect	Good	Must be measured at each institution

Absolute Dosimetric Quantities

The HVL measurements were very consistent between all three institutions indicating that the beam quality for each x-ray tube is similar. For commissioning of a new system using this tube, therefore, the HVL values could be taken from the manufacturer, or calculated from spectrum generating code, provided the thickness of the added filtration provided by the manufacture is within specifications. However, as monitoring the HVL annually to safeguard against changes in beam quality may be a part of routine quality assurance, establishing a baseline at each institution may still be beneficial. The sensitivity of the PDD to changes in HVL was investigated by Monte Carlo simulations, which showed that a 15% change in HVL (0.86 mm Cu vs 1.02 mm Cu) lead to a < 1% change in PDD at 2 cm depth.

The agreement between in-air and in-phantom measurements of the absolute output varied somewhat between the three institutions. The differences between in-air and in-phantom absolute dose-rates were less than 5%, although there was some variation between institutions. For the absolute output a maximum variation is noted between the three systems of about 12%. We have found that the tube output decreases slightly over time and as the calibration measurements were done after different frequencies of use, this may account for some of the differences. However, there is clearly still the need for careful and accurate absolute calibration of each system independently.

Relative Dosimetric Quantities

For field sizes ≥ 10 mm equivalent diameter, there was good agreement observed in the percent depth dose, relative output factors, and profiles between all three sys-

tems. The beam penumbra is $< 1\text{ mm}$ for field sizes $< 40 \times 40\text{ mm}^2$, and the actual field sizes agree well with the nominal field sizes. The profiles agree well qualitatively between all three systems. Some differences in flatness and symmetry between the three systems are due to the noise in the film data, rather than actual differences in the dose profiles. Some differences in field size were also noted. For the small fields ($\leq 5\text{ mm}$), this may be due to alignment of the collimator with the focal spot, as well as machining of the collimators themselves. For collimators $\geq 10\text{ mm}$, the maximum differences in field size were 2.1 mm . Some of this difference is due to differences in the machined collimating apertures.

However, the smallest fields tested (2.5 mm and 5 mm diameter) pose certain challenges. Because these fields are smaller than or approaching this size of the x-ray tube focal spot, and because the system is using a two-stage collimation (upper and lower collimating apertures), there is strong sensitivity to the alignment of the collimator relative to the focal spot^{16,30}. After further inspection the aperture in the upper collimator between institutions was inconsistent despite originating from the same supplier for the specified beam size and similar lower collimator aperture size. Institution A had an upper collimator with an aperture diameter of 1.5 mm , while institution B, and C, had aperture diameters of 2.8 , and 1.8 mm , respectively. This explains the large difference in relative output factor for this collimator between Institution A and Institution B. When a measurement at Institution B was taken without the upper collimating aperture, the output increased by $< 5\%$. For Institution A, the change in removing the upper aperture was an increase in output of 43% . A similar result has been found at Institution C for the 5 mm collimator. The shape of the 2.5 mm collimator profile for Institution A also supports the hypothesis that the upper collimator is occluding the focal spot, resulting in an asymmetric profile and reduction in output. Furthermore, this profile shape impacts the quantification of the PDD for the small collimators.

The relative dosimetric data provide a useful reference for the PDD, profiles and relative output factors for this system. Generally the three systems agree to within 5% ; however, some differences for these quantities exceed 5% . As this is already larger than what one would like to achieve for overall dosimetric accuracy, using nominal values rather than measuring them for each individual system will introduce undesirable uncertainties into the system calibration. We note that it appears that the differences in $40 \times 40\text{ mm}^2$ PDD curves are increasing as a function of depth between Institution B and Institution C. We hypothesize that this may be due to scatter conditions. The measurements for Institution C for the $40 \times 40\text{ mm}^2$ field were acquired in a water phantom, with the μLion chamber, whereas those for Institution B were acquired in $40 \times 80\text{ mm}^2$ solid water phantom, with a total thickness of 40 mm . A smaller variation is observed in the 10 mm collimator. For simple point-dose calculations, it is clear how differences in relative dosimetry propagate to the final results. However, the measured data may also be used as input to more complex dose-calculation or modeling (such as superposition/convolution or Monte Carlo treatment planning methods). In this case, the impact of differences in relative dosimetry must be further investigated.

One previous publication has characterized the dosimetry for the X-RAD 225Cx¹². Overall, the results shown here are in agreement with those, although the objectives of that work were to compare point, 2D and 3D methods for commissioning.

Mechanical Quantities

The mechanical data from the 16-bb phantom characterizing the SAD and SDD for each system agree very well. The maximum difference between the three systems is 0.5% (1 mm for SAD and 3 mm for SDD). This indicates a consistency in this aspect of manufacturing of the system, and is important for image reconstruction, as well as corrections for displacements of the object about the isocenter. Based on the consistency of these three systems, standard values for SAD and SDD could likely be used for all systems.

For the projection map and Winston-Lutz test, the magnitudes of flex are similar for all three systems. The data shows high reproducibility within each system between different time points. Furthermore, when the Winston-Lutz tests are uploaded to the hardware controller and used to compensate for the flex of the system, the maximum residual targeting errors were within 0.33 mm in both u- and v. This demonstrates that the system has the potential for highly accurately radiation targeting for radiobiology studies. Comparing the shape of the projection map and Winston-Lutz curves between the three institutions, it is clear that these mechanical calibrations must be done independently on each system, as is current practice. Also, the variations over time are much smaller than the magnitudes of the flex, but there is still a need to routinely monitor the mechanical characteristics of the system.

CONCLUSIONS

A conformal, image-guided small animal irradiation platform has the potential to deliver complex radiation distributions that attempt to mimic the clinical delivery of radiation therapy. Accurate characterization of the absolute dosimetry, relative dosimetry and mechanical properties of these systems are crucial for their effective use in pre-clinical radiobiology experiments. In the present study, dosimetric and mechanical commissioning has been completed on three X-RAD 225Cx small animal irradiators installed at three separate institutions. Part of the motivation for this comparison was to investigate the possibility of establishing a set of reference data that would reduce the workload for commissioning of individual systems. We recommend following the methodology for commissioning described in the present study. Since certain differences in commissioning data exist between individual institutions/systems, it is important to note where these variations can be expected to occur when commissioning a new system and to recognize which parameters are essential for ensuring accurate and precise radiation delivery.

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Conflict of Interest

Some of the authors (PEL, DAJ) of this work are listed as inventors of the system described herein. This system has been licensed to Precision X-Ray Inc. for commercial development. The NKI receives license fees from Precision X-Ray Inc and Xstrahl Ltd.

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CHAPTER

4

A combined dose calculation and verification method
for a small animal precision irradiator based on
onboard imaging

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ABSTRACT

Novel small animal precision micro-irradiators (micro-IR) are becoming available for pre-clinical use and are often equipped with onboard imaging (OBI) devices. We investigated the use of OBI as a means to infer the accuracy of the delivered treatment plan. Monte Carlo (MC) modeling of the micro-IR including an elliptical Gaussian electron beam incident on the x-ray tube was used to score dose and to continue photon transport to the plane of the OBI device. A model of the OBI detector response was used to generate simulated onboard images. Experimental OBI was performed at 225 kVp, gain/offset and scatter-glare were corrected. Simulated and experimentally obtained onboard images of phantoms and a mouse specimen were compared for a range of photon beam sizes from 2.5 cm down to 0.1 cm. Simulated OBI can be used in small animal radiotherapy to determine if a treatment plan was delivered according to the prescription within an uncertainty of 5% for beams as small as 4 mm in diameter. For collimated beams smaller than 4 mm, beam profile differences remain primarily in the penumbra region of the smallest beams, which may be tolerable for specific pre-clinical micro-IR investigations. Comparing simulated to acquired OBI during small animal treatment radiotherapy represents a useful treatment delivery tool.

INTRODUCTION

Pre-clinical, small-animal studies have been identified as an invaluable tool for advancing radiotherapy research as they allow detailed investigations into biological processes that could otherwise not be investigated easily in radiotherapy patients.¹ To bridge the gap between the complex spatio-temporal beam delivery schemes in modern clinical radiotherapy and the common norm of imprecise irradiation with crudely estimated dose calculations in small animal irradiation studies, new image-guided conformal micro-irradiators (micro-IR) are becoming available for pre-clinical research and are often equipped with high resolution x-ray onboard imaging (OBI) devices.²⁻⁴ Accurate small animal radiotherapy studies with these devices require accurate dose calculations. However, present treatment planning software is not easily adapted to small animal micro-IR devices making accurate dose calculations challenging.

Currently, some groups use Monte Carlo (MC) simulations, analytical models, or a combination of both for small animal micro-IR dose predictions.⁵⁻⁷ However, the actual delivered treatment dose is not verified against the planned dose. Furthermore, the accuracy of the delivered treatment plan becomes more critical at low photon energies (<300 keV) and small beam sizes (down to 1 mm) due to e.g. potential errors in the assignment of tissue compositions in the treatment planning process and the influence of mechanical tolerances for accurate beam delivery. Therefore, a method to validate the intended treatment dose against the truly delivered dose would be valuable. In clinical radiotherapy, such methods now exist e.g. based on portal dosimetry.⁸

Portal dosimetry techniques can be used to verify the pretreatment plan (e.g. catch multi-leaf collimator errors),⁹ recalculate the delivered dose to the patient using the most recent cone-beam CT and the recorded photon beam fluence,¹⁰ or verify whether the intended treatment plan was the delivered treatment plan at the position of the OBI.¹¹ The intent of this paper is the latter by means of treatment verification using a recently reported MC photon transport algorithm^{12,13} that continues photon transport through objects to the plane of the OBI device providing the ability to generate simulated onboard images.

However, OBI devices commonly make use of large array a-Si CsI flat-panel detectors that are known to suffer from several drawbacks including ghosting,¹⁴ scatter-glare,¹⁵ and image lag¹⁶ that cause image intensities to be time variable and field size dependent. Consequently, in addition to characterizing the energy-response of the OBI device, strategies are required to minimize image intensity variability between simulated and experimental OBI. Therefore, the goal of this study is to develop a MC model of a novel conformal small animal micro-IR including the OBI device and demonstrate its ability to verify that the intended treatment dose agrees with the delivered treatment dose based on agreement between 2-D simulated and experimentally acquired OBI.

METHODS

Figure 1. Schematic illustrating the MC simulation workflow, required inputs, software used and simulation outputs e.g. in the form of phase space files (PSF). Fig. 1(A1, A2, A3) shows a cross-section of the x-ray tube in the MC program BEAMnrc (A1), a depiction of the voxelized simulated phantom contained within an egspant file (A2), and an illustration of the phase space file at the position of the OBI (A3). Fig. 1(B1, B2, B3) shows a photograph of the x-ray tube and C6 collimator (B1), small spherical test object located at the micro-IR SAD (B2) and the flat-panel onboard imager (B3).

Small animal irradiator

The small animal micro-IR (225Cx, Precision X-ray Inc., North Branford, CT) used in this study system is similar to the one recently described by *Clarkson et al.* (2) with a few minor improvements, including a higher resolution imaging panel and a different x-ray tube.² The source-to-axis distance (SAD), source-to-detector distance (SDD) and maximum field-of-view (FOV) at the isocenter can be seen in figure 1. The system consists of a dual-focus x-ray tube with a maximum tube potential of 225 kVp (225 Cx, Comet, Switzerland). The x-ray tube contains a static, water-cooled, tungsten target angled at 20°. Photons are filtered through a 0.8 mm Be exit window. The micro-IR OBI device is an indirect flat-panel detector that consists of a structured CsI scintillator coupled to an active amorphous-silicon 1024×1024 matrix having a pixel pitch of 200 μm (XRD0820AN, Perkin Elmer, Germany). Several collimators can be interchanged to produce x-ray beams as small as 1 mm in diameter at the isocentre. For the purposes of this study, the six supplied circular collimators were chosen for investigation and are identified here as C1-C6 with entrance and exit aperture diameters listed in table 1.

Table 1. Lists entrance and exit aperture diameters that were measured and modeled in BEAMnrc for labeled collimators (C1 to C6) located at distance to the source of 7.6 cm and 21.8 cm (except C3 at 21.0 cm), respectively.

Collimator label	Collimator entrance aperture diameter (mm)	Collimator exit aperture diameter (mm)
C1	1.50	0.75
C2	1.50	1.90
C3	9.90	3.80
C4	8.50	7.70
C5	8.50	11.50
C6	8.50	19.00

An 80-kVp imaging protocol with an acquisition rate of 10 frames/s with 2×2 pixel binning and a gantry rotation of 1 revolution/min was developed for all imaging with the micro-IR. Projections were acquired and reconstructed using the Feldkamp filtered backprojection (XRAD v 1.1.18, Precision X-ray Inc., North Branford, CT).¹⁷

Absolute dosimetry

A 225 kVp x-ray beam (0.32 mm added Cu filtration) was used for the dosimetric characterization throughout this study and represents a typical animal treatment photon beam. Absolute dosimetry was performed following the recommendations of the report of AAPM task group 61 using the in air method for orthovoltage x-ray tubes to determine the dose-to-water at the surface of a water phantom.¹⁸ A parallel plate ionization chamber (PTW 0.02 cm³ soft x-ray chamber type 23342 Freiburg, Germany) was used. The center of the sensitive air cavity was placed at the isocentre of the micro-IR as determined by OBI. The field was collimated using the largest circular collimator (C6), which covered the parallel plate ionization chamber's collecting volume. Half value layer (HVL) measurements were made with the x-ray tube vertically aligned and collimated by C6. The imaging and irradiation protocols of the micro-IR, used in this study, correspond to 80 kVp (2.14 mm Al), 1.3 mA, small focal spot ($d \approx 0.8$ mm) and 225 kVp (0.32 mm Cu), 2.5 mA, large focal spot ($d \approx 3.4$ mm), respectively.

Radiochromic film EBT2 (ISP, Wayne, NJ) was used in this report to measure dose due to its high spatial resolution. Several film pieces from a single batch of EBT2 film (lot # F12170901A) were irradiated at the treatment energy for 8 known doses and scanned using a high-quality flatbed scanner (V750-M Pro, Epson, Tokyo) and its accompanying software (SilverFast HDR 6.6, Epson, Tokyo). Precautions using a flat-bed scanner and the film readout procedure were implemented.¹⁹ Sheets of EBT2 film were cut into 5.1 x 6.4 cm pieces for all calibration irradiations. No lateral scanner correction was applied due to the small size of the film pieces. A transparent template with a 4.8 x 6.1 cm cutout was used to lift the film slightly off the scanner glass to eliminate Newton rings and to ensure a consistent scan orientation and position. The scanner bed was cleaned prior to each scan. Films were digitized as TIFF images and acquired at 225 dpi in 48 bit RGB transmission mode, 16 bit per color channel, with all image enhance-

ments turned off. Films were pre-scanned before irradiation and 24 hours post-irradiation with the same orientation and side. A calibration curve was fit to characterize the change in net optical density (netOD) versus dose using a recently proposed rational function and single intensities read from the red color channel.²⁰

Figure 2. Absolute depth dose curves measured using EBT2 film (points) in 1 cm intervals between slabs of PMMA and the simulated depth dose curves (solid lines) for collimators C1-C6 as indicated. The error bars represent the standard deviation of the mean value and reflect the deviation of the measured dose values from EBT2 film or the statistical uncertainty from the MC simulation. The source to surface distance in the experimental configuration was 5.3 cm.

OBI

Unprocessed image frames ($IMAGE_{raw}(i,j)$) from the OBI device were acquired through Matlab EXecutable (MEX) code and C++ commands to the frame grabber (XRD-FG, Perkin Elmer, Germany). Parameters for OBI acquisitions were set to low gain, 33.1 ms/frame, and pixels binned 2×2 for a final image frame size of 512×512 pixels with a pixel pitch of 400 μ m. An average image ($DARK(i,j)$) consisting of 100 frames prior to irradiation was used for a dark pixel (offset) correction on all data acquired using the OBI. To eliminate spatial variations in pixel sensitivity, a gain image $G(i,j)$ was acquired of the unobstructed x-ray flood field at 2.5 mA and the acquisition parameters described above. Therefore, raw image frames were gain/offset corrected by the following equation:

$$IMAGE_{cor}(i,j) = G(i,j) \times (IMAGE_{raw}(i,j) - DARK(i,j)) \quad (1)$$

In our approach, the useful area of the OBI was restricted to the central half of the detector where the field profile from the flood field would be flat to within 1 % along central axis of the OBI device and pixel sensitivities would dominate in the gain correction rather than any field non-uniformity.

Figure 3. Beam profiles measured using EBT2 film (solid line) and simulated (dashed line) along the cathode-anode direction for collimators C1-C6 at the isocentre of the small animal micro-IR. The profiles are scaled according to their respective dose output factor.

Minimizing OBI perturbations image; lag and scatter-glare

Image lag is an observed phenomenon whereby image intensities decay exponentially in subsequent image frames following the image frame in which it was initially acquired. Image lag is often difficult to correct without the equipment necessary to measure the response of the OBI device to an x-ray impulse.²¹ Therefore, to reduce the effects of image lag during irradiations of the OBI device without a complicated correction model, an average image consisting of 100 frames is acquired following the first 300 frames of irradiation so that image intensities would approach a steady-state condition. To access the influence of image lag for this acquisition protocol a falling-edge response step response function was used by rapidly cutting power to the x-ray tube and measuring the residual image signal (i.e. lag).

Scatter-glare is a low frequency imaging phenomenon that contributes image signal over the whole detector area and is largely attributed to scatter from within the detector. We implemented a technique to correct for the scatter-glare image intensity contribution based on a recently proposed method.²² It consists of fitting a function to

the tails of a point spread function called a glare spread function (GSF). The GSF proposed in *Poludniowski et al.*²⁰ is reproduced here as:

$$GSF(r) = \frac{(1-a)\delta(r)}{2\pi r} + \frac{a}{2\pi b^2} \frac{1}{(1+r^2/b^2)^{3/2}} \quad (2)$$

where a and b are fit parameters and r is the radial distance. In this paper, the point spread function was determined using an edge-spread technique²³ with a 2.5 cm thick iron block that covered half of the OBI. The block was placed at a distance to the source of 40 cm. The GSF was obtained for both the imaging and irradiation energy of the micro-IR.

A first-order correction to the removal of the scatter-glare contribution in collimated beam images was performed as,

$$IMAGE_{sgcor} \approx IMAGE_{cor} - IMAGE_{cor} \otimes GSF(r), \quad (3)$$

where the subscripts *cor* and *sgcor* denote images that have been gain/offset corrected and those that have been both gain/offset and scatter-glare corrected.

Figure 4. (A) Experimentally acquired gain/offset corrected image profiles of beams collimated using C3-C6. (B) The same profiles as in (A) but corrected for scatter-glare.

MC model

The micro-IR was modeled using the MC package BEAMnrc/EGSnrc^{24,25} based on measurements, manufacturer supplied documents and in a manner similar to *Tryggestad et al.*²⁶ by first generating device phase-space files (PSFs) for specific collimator geometries as inputs for subsequent dose calculations. The focal spots were modeled using the apparent focal spot size (as seen from the central axis) according to manufacturer supplied data with the small imaging focal spot having a width and length of 0.76,

and 0.89 mm, respectively, and the large radiotherapy focal spot having a width and length of 3.41, and 3.09 mm, respectively, where the length is defined along the source-to-anode direction. These dimensions are then projected towards the primary electron source according to the target angle. Therefore, the small focal spot was modeled in BEAMnrc as a rectangular uniform electron beam with the half dimension of y-beam and z-beam being 0.38 and 1.22 mm, respectively. A more realistic elliptical Gaussian model for the intensity distribution of the large focal spot was developed to reproduce the dosimetric behaviour of small beams (e.g. focal spot occluded beams).

One way to achieve an electron beam targeted on the XTUBE component module with a shape other than a uniform rectangle or ellipse within BEAMnrc is to first create a PSF containing the desired source intensity distribution. Using an infinitesimally thin slab of air, a PSF with an elliptic Gaussian and 2.5 million particles was created using source 19 (“elliptical beam with Gaussian distributions in X and Y, Parallel or with Angular Spread”), that is – as always in BEAMnrc – scored perpendicular to the z-axis (the beam axis). Alternatively, a user could create a PSF outside of BEAMnrc with any electron source distribution. The width and length of the elliptical Gaussian was derived by considering the apparent width and length of the large focal spot (the largest dimension usually reported as d) equal to $\pm 2\sigma$. Therefore, the full width half max (FWHM) dimensions of the Gaussians were calculated from the relationship between a normal distribution’s standard deviation and FWHM multiplied by a tuning factor δ i.e.

$$FWMH = 2\sqrt{2\ln 2} \cdot \delta \cdot d / 4 \quad (4)$$

The tuning factor was incremented in steps of 0.05 until reaching an agreement of the relative output factor of the smallest beam within 5 %.

In order to incident the elliptical Gaussian electron beam PSF on the XTUBE component module, a “dummy” component module (e.g. slabs) is require above the XTUBE component module and source 24 (“phase-space source, incident from user-specified angle”) is used. Source 24 also requires that a negative distance to point of rotation equivalent to the half z dimension of the XTUBE target is used and the PSF is rotated by 90° with respect to the y-dir to model a source impinging from the side (along the x-axis) to the target.

Figure 5. The normalized (ramped) detector response generated for the a-Si Csl flat-panel OBI. The small spike around 30 keV is due to the k-edge of iodine.

A cross-section of the MC modeled micro-IR components can be seen in figure 1. The six circular collimators were modeled and one model without any collimation, representing the imaging beam. All models were run with 5×10^9 electrons hitting the target and the particle transport parameters provided in the appendix table A1 including variance reduction techniques as recommended by *Ali and Rogers*²⁷ for bremsstrahlung cross-section enhancement (BCSE) with directional bremsstrahlung splitting (DBS).

Figure 6. Beam profiles measured using the OBI (solid line) and simulated (dashed line) along the cathode-to-anode direction for collimators C1-C6 at the position of the OBI in the micro-IR. The profiles are scaled according to their respective image intensity output factor.

A modified version of DOSXYZnrc^{12,13} that continues photon transport past the irradiated object was used to simulate photons through user-generated phantoms to score dose and to generate a PSF at the plane of the OBI device. Phantoms in DOSXYZnrc are constructed based on voxelized geometries that contain the composition and density in an “egsphant” file for each voxel. For all DOSXYZnrc simulations egsphant files were created with isotropic resolutions of $0.2 \times 0.2 \times 0.2$ mm. Experimental micro-CT data was used to obtain the phantom geometries. Voxels were assigned compositions and densities for all micro-CT images of phantoms with known materials or assigned based on a CT calibration curve.²⁸ Photon transport was performed in DOSXYZnrc with 5×10^8 histories with the transport parameters in provided in the appendix table A1.

A normalized energy-weighted (ramped) detector response is required to simulate OBI based on the PSF produced at the plane of the OBI device. The normalized detector response was calculated based on a simulation of the quantum detection efficiency for our detector and published data of the light output response of CsI (i.e. Swank factor).²⁹ The simulation was performed by tabulating the percentage of energy deposited for monoenergetic photons in steps of 1 keV from 0 to 225 keV for our detector model. The detector model consisted of two layers, a top layer of 50 μm thick Al and a bottom layer of 550 μm thick CsI with densities of 2.7 and 3.73 g/cm^3 , respectively. An experimental validation of the detector response is provided in appendix A.

A separate C++ code¹³ was used to generate simulated OBI. The code extracts the energy and location for each photon in the phase-space file and assigns the photon an image intensity value equal to the normalized MC-derived detector response and a pixel location. For each pixel location, image intensity values are summed and divided by the incident number of particles simulated from the original source target. Simulated full field (512×512) projection images were calibrated using the following equation,

$$PROJECTION(i, j)_{sim} = \frac{MC\ IMAGE_{transit}(i, j)}{MC\ IMAGE_{flood}(i, j)} \quad (5)$$

where the $MC\ IMAGE_{transit}$ was simulated through an object and the $MC\ IMAGE_{flood}$ through air.

Simulated OBI of collimated beams were calibrated to experimentally obtained OBI of collimated beams. For each of the collimators, three images were acquired, averaged, gain/offset corrected and the scatter-glare contribution removed according to section II.C.1. A MC conversion factor $F_{MC \rightarrow OBI}$ was calculated as the ratio of the mean image intensity (from 80 % to max) between the experimentally obtained and scatter-glare corrected average image using collimator C6 and the corresponding MC simulated image. OBI correction factors CF_{OBI} were used to improve agreement for the smallest collimated beams due to the sensitivity of collimator alignment. The OBI correction

factors (CF_{OBI}) were determined as the ratio of the mean image intensity between the experimentally obtained and corrected average image acquired for all collimators and their corresponding MC simulated image adjusted by the conversion factor $F_{MC \rightarrow OBI}$. In this manner, the correction factor (CF_{OBI}) for C6 is unity. Therefore, simulated images were calculated as,

$$IMAGE(i, j)_{sim} = F_{MC \rightarrow OBI} \times CF_{OBI} \times MC\ IMAGE_{transit}(i, j) \quad (6)$$

MC validation

The experimental validation of our MC model as well as the MC dose calibration procedure has been excluded from the materials and methods section in this manuscript for brevity but has been provided in appendix B for interested readers. In it, one finds the calibration procedure from dose per particle to dose per mAs, transport parameters, descriptions of comparisons between simulations and experiments for HVL measurements, depth dose curves and beam profiles that are reported in the results section.

Figure 7. (A) A picture of four CIRS inserts (adipose, lung, muscle, and bone core containing average bone) placed on the stage of the micro-IR. (B) A cropped section of an experimentally acquired and corrected onboard image (scatter-glare removed) taken of the inserts. (C) A cropped section of a simulated calibration onboard image taken of the inserts. (D) Line profiles of the highlighted region in (B) and (C) showing a good agreement between the simulated and experimentally acquired onboard attenuation profiles.

OBI image verification was performed using an experimentally obtained projection image of tissue mimicking cylinders and compared to a simulated projection image generated under the same experimental conditions. The cylinders consisted of tissue substitutes mimicking adipose, lung, muscle, and average bone with known composi-

tions and densities (CIRS, Norfolk, VA). The tissue substitutes were micro-CT imaged. A MC simulation was performed and a calibrated image was generated.

A measured 2-D dose profile for a three-beam irradiation plan resembling a “bull’s-eye” was compared to MC simulations. The experiment consisted of film sandwiched between 1-cm slabs of PMMA and irradiated at a fixed gantry position using three concentrically collimated beams (C1, C4, C6). Irradiations were performed for 90 seconds each at 2.3 mA (approximately 2 Gy total dose). OBI images were acquired. The PMMA sandwich was micro-CT imaged to generate an egsphant file. A MC simulation was performed with 5×10^8 particles for each of the three beam collimators.

Animal experiment

To demonstrate the proof of concept of the combined dose calculation and verification model using the OBI for a realistic animal model, a single beam irradiation was planned for an intact dead mouse (strain fvc/j, 10 weeks old). The intended target – the head – was placed at the isocentre of the micro-IR. The largest circular collimator C6 delivered x-rays for 90 seconds at 2.3 mA (approximately 2 Gy total dose). OBI images were acquired for the duration of the irradiation with the mouse present. A single calibrated image was generated from the average of a sequence of 100 image frames.

Micro-CT imaging of the dead mouse was performed and the 3-D imaged volume converted into an egsphant file using a 4-tissue conversion scheme. Mouse compositions and densities are not well known; therefore, the same CIRS tissue mimicking substitutes mentioned in section above were deemed to be an adequate surrogate for mouse tissue assignments for this proof of concept. Tissue assignment included air, adipose, muscle, and average bone and was based on the HU thresholds, -1000, -650, -70, 300, 2500. Densities were assigned based on linear interpolations of the densities of 0.00125, 0.98, 1.06, and 1.6 g/cm³ for the 4 media between the CT HU thresholds. A MC simulation was performed to generate an onboard image of the collimated beam through the mouse head. The experimentally acquired onboard image was compared to the simulated onboard image using a 2-D gamma calculation with local criteria of (5%; 0.8 mm). The gamma distance to agreement criterion was chosen based on scaling down a clinically relevant distance to agreement between 3-5 mm and clinical CT scanner voxel spacing of 1 mm³.

RESULTS

MC validation

The measured and MC-derived HVLs of the micro-IR for the irradiation protocol agreed within 1 and 3 % for the HVL₁ and HVL₂; they were 11.75, 11.6 mm Al for HVL₁ and 13.91 and 13.6 mm Al for HVL₂ between the measured and MC-derived HVL, respectively.

A value of 1.30 for the MC tuning factor δ was found to replicate the relative output of the smallest simulated field (C1) within 1% by modeling the electron beam intensity distribution as elliptical Gaussian. Therefore, the width and length of the projected FWHM sizes used in simulations were 7.15 and 2.37 mm.

Measured and simulated depth dose (DD) curves agreed within 4% for all but collimator C2, which had a combined deviation of 5.6 % from the film measurements (see ure 2). The relative dose at the surface of the depth dose PMMA stack (i.e. dose output factor) were 0.39, 0.36, 0.90, 0.87, 0.90 and 1 for collimators C1 to C6, respectively. A least-squares fit procedure was used to obtain correction factors (CF_{Dose}) to minimize differences between the simulated DD curves and the experimental data. The correction factors are relative to the largest circular collimator C6 for which the absolute dosimetry procedure was performed and are listed in table 2. The use of the correction factors resulted in residual deviations between of the film measurements and the simulated data of less than 1% for all collimators.

Table 2. A comparison of dose measurements at 2 cm below the surface of a PMMA phantom between experimentally acquired EBT2 film measurements and MC-derived depth dose values for the six collimators. A least-squares fit procedure was used to obtain correction factors (CF_{Dose}) to minimize differences between the simulated DD curves and the experimental data. A comparison of the mean image intensity values between experimentally measured collimated beams from the OBI against MC-derived simulated OBI beam profiles for the six collimators investigated. To improve agreement between the MC-derived simulated OBI beam profiles and the experimentally acquired collimated profiles an OBI correction factor (CF_{OBI}) was used.

Collimator label	Beam radius (80 % to max, mm)		Penumbra – along tube axis (20-80 %, mm)		Penumbra – across tube axis (20-80 %, mm)		Percent difference using isodose boundaries of	
	EBT2	MC	EBT2	MC	EBT2	MC	20 %	80 %
C1	0.42	0.3	0.26	0.40	0.38	0.42	23	12
C2	0.97	1.2	0.36	0.35	0.55	0.40	12	8
C3	2.2	2.2	0.51	0.62	0.74	0.68	4	2
C4	4.7	4.7	0.45	0.51	0.66	0.58	4	2
C5	7.3	7.3	0.34	0.55	0.67	0.66	1	1
C6	12.3	12.1	0.43	0.68	0.68	0.87	1	1

Beam shape differences (e.g. radius, penumbra size) were quantified by the 20 % isodose contour for the penumbra and the 80% isodose contour for the radius.³⁰ Beam

radii, penumbras, and the percent difference between the experimental and simulated beam profiles within the 20 and 80 % isodose boundaries are listed in table 3 and were found to be less than 5 % for collimators C3 to C6 but larger differences of 8 % and 12 % for C2, and C1, respectively, were observed for the 80 % isodose boundary. For all beams the radii agreed within 0.3 mm. Beam profiles orthogonal to the cathode-to-anode at the SAD can be seen in figure 3.

OBI

Image lag was found to have a maximum influence of 2.9 % in the first frame following irradiation which reduced to 0.4 % in the 300th frame. Between the 300th to 400th frame the change in image lag was negligible and only a small 0.1 % drift in the signal intensities was observed.

The fit parameters a and b in the glare-spread function (equation 2) were determined to be 0.065 and 3.1, respectively for the imaging mode of the micro-IR and 0.061 and 3.0, respectively for the treatment mode of the micro-IR. For those beams that are not focal spot occluded (C4-6) as determined by the geometry, beam intensities become approximately field size independent, as expected, using our first-order correction for scatter-glare (figure 4).

MC simulations (object and detector model)

The MC conversion factor $F_{MC \rightarrow OBI}$ was found to be $(7.87 \pm 0.02) \times 10^{11}$ particles per mAs. The OBI correction factors (CF_{OBI}) for each individual collimator are listed in table 2. The MC derived normalized detector response for the OBI can be seen in figure 5 and its verification against a flat detector response is shown in figure A1.

Measured and simulated OBI beam profiles parallel to the cathode-to-anode direction agreed well for all collimators (see figure 6). A small asymmetry was observed in the beam profiles observed near the edge of the beam of the largest collimator that was equally visible in the beam profiles acquired using film at the SAD.

A comparison between the measured and simulated projection images of the tissue-equivalent inserts resulted in a difference of 0.6% along a central image profile that can be seen in figure 7.

Differences between simulated and measured OBI of the three-beam “bull’s-eye” treatment plan resulted with 85, 100, and 98 % of the pixels (within the beam) passing a local gamma criterion of (5%; 0.8mm) for collimators C1, C4, and C6, respectively (Fig 8). The total dose for the combined three-beam treatment plan resulted with 91 % of the pixels (within the beam) passing using a local gamma criterion of 5%, 0.8 mm between simulations and experiments (figure 8).

Mouse experiment

The 2-D gamma comparison of the simulated and measured calibrated mouse image for the single beam using collimator C-6 resulted in 92 % of the pixels (within the beam) having a value of less than 1 using a local gamma criterion of 5%, 0.8 mm. The simulated and experimental onboard images as well as the gamma image can be seen in figure 9.

Figure 9. (A) A simulated onboard image of a treatment beam collimated using C6. (B) The measured calibrated onboard image. (C) A 2-D gamma difference image with a local gamma criterion of 0.8 mm 5%.

Table 3. Beam radius, penumbra, and percent differences of the integrated area contained within the 20 and 80 % isodose lines between MC simulated and experimentally obtained beams at the isocenter of the micro-IR for the six collimators used.

Collimator label	Beam radius (80 % to max, mm)		Penumbra – along tube axis (20-80 %, mm)		Penumbra – across tube axis (20-80 %, mm)		Percent difference using isodose boundaries of	
	EBT2	MC	EBT2	MC	EBT2	MC	20 %	80 %
C1	0.42	0.3	0.26	0.40	0.38	0.42	23	12
C2	0.97	1.2	0.36	0.35	0.55	0.40	12	8
C3	2.2	2.2	0.51	0.62	0.74	0.68	4	2
C4	4.7	4.7	0.45	0.51	0.66	0.58	4	2
C5	7.3	7.3	0.34	0.55	0.67	0.66	1	1
C6	12.3	12.1	0.43	0.68	0.68	0.87	1	1

DISCUSSION

In this manuscript, we have demonstrated good agreement between measured and simulated OBI of collimated beams of a novel small animal conformal micro-IR. We have shown that OBI can be used in small animal radiotherapy to determine if a treatment plan was delivered according to the prescription within an uncertainty of 5% for beams as small as 4 mm in diameter.

To achieve good agreement between simulated and measured *DD* curves for beams collimated down to 1 mm in diameter it was necessary to include and tune a non-

uniform intensity distribution for the incident electron beam. It was found that a tuning factor of $\delta=1.30$ resulted in good agreement for all collimated beams with the largest difference between the simulated and measured *DD* curves of 5.6 % for collimator C2. Therefore, if a manufacturer reports a focal spot size of d then a good start for modeling beams as small as 1 mm would be to create a Gaussian electron intensity distribution having a FWHM size of $0.77 \times d$ projected by the target angle.

The small difference between the simulated and measured *DD* curves for collimator C2 is due to the skewness of the beam (figure 3) and emphasizes the utility of introducing small correction factors (CF_{DOSE} and CF_{OBI}) to account for differences between the micro-IR and the model that are difficult to incorporate in the MC model (e.g. collimator alignment). Two separate correction factors were required (one at the isocenter and one at the level of the OBI) because each correction factor is applied at a separate distance and therefore refer to different fields.

The dose correction factor only improves the output agreement of the collimated beams (i.e. *DD*) and does not correct for beam profile disagreements. The beam profiles were found to deviate by less than 5 % for all but for the smallest fields (C1, C2) using the integrated area bounded by the 20 % isodose boundary (table 3). The smallest fields (C1, C2) were found to deviate by 23 and 12 % using the integrated bounded area by the 20 % isodose boundary and by 12 and 8 % using the integrated area bounded area by the 80 % isodose boundary, respectively. The improved agreement of the beam profiles for the smallest fields between the 20 and 80 % isodose boundaries means that most of the disagreement is in the penumbra region.

Beam profile differences were also observed through the use of the gamma comparison as seen in Fig.8 and Fig.9 near the edges of the beams. We had chosen a gamma criterion of 5% 0.8 mm that appears to be a good criterion to elucidate differences between the simulated and experimental plan for our particular system, others may find other criteria more appropriate. Moreover, the choice of the passing criterion is institution and study specific, what we have provided in this manuscript is a framework for investigating appropriate dose comparison metrics.

To generate accurate simulated OBI of collimated beams through objects it was necessary to include a scatter-glare correction and to minimize the effects of image lag. We found that the relative contribution of scatter-glare to the total image intensity values was approximately 6 % when the OBI is completely exposed (e.g. intended for projection imaging). We also included a delay of 300 frames in analysing image frames acquired from the OBI due to image lag and the change in signal intensities over time. Therefore, our approach has been limited to static beams and requires that the time interval for which an OBI is acquired should be fixed. Both these consequences could be overcome with an adequate image lag correction as has been proposed.²¹

In our method, 100 frames were averaged to decrease noise and capture a “snapshot” of the treatment delivery, including that of a dead mouse. In future implementa-

tions, this number may be reduced in an effort to capture intra-fraction motion of small animals during treatment. The potential to assess dose-delivery errors due to intra-fraction motion would be advantageous over more rudimentary dose calculations approaches, like point dose calculations using tables.³¹ An additional advantage of our method may be the ability to assess the accuracy of the CT number to tissue conversion scheme by being able to compare simulated OBI with experiment OBI. This topic needs further research.

This investigation highlights challenges in the MC modeling of a small animal micro-IR, particularly when x-ray beams are collimated to field sizes smaller than the focal spot size. This may guide both the MC modeller and designers of small animal micro-IRs, particularly to pay attention to incident electron intensity distribution. Small asymmetries for both small and larger field sizes were observed that we believe are primarily due to small misalignments between the collimator mounting device and the focal spot location, or between the collimator and collimator mounting device. Further study may be helpful to provide recommendations on the influence of collimator design, alignment, and the influence of the electron beam intensity distribution.

CONCLUSION

This manuscript demonstrates the proof of concept of a combined dose calculation/verification method for a small animal precision irradiator based on onboard imaging. This manuscript also presents a Monte Carlo model of a small animal micro-IR with attention to details such as a non-uniform electron source intensity distribution, precision collimator geometry, and imaging detector response. The model proved accurate for field sizes of 4 mm and larger. Refinements to the MC model may consider small mechanical tolerances in the collimator design and source alignment, but these remain difficult to incorporate and alternative techniques for representing the true beam profiles should be considered.

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Figure A1. A comparison between two theoretically determined and measured image intensities for increasing x-ray tube potential normalized at the midpoint of mean spectral energy of 64 keV for the x-ray tube potential of 120 kVp (0.32 mm Cu). The two theoretical image intensities were calculated based on the MC-derived detector response and a flat detector response model representing the worst-case scenario. Even though the MC-derived detector response performed much better than the flat detector response model, the differences are relatively small for a completely inaccurate model and demonstrate that the image intensities from the OBI are largely output and not energy determined.

APPENDIX A: EXPERIMENTAL VERIFICATION OF THE OBI DETECTOR RESPONSE

We investigated the OBI response by recording image intensities over a range of x-ray tube potentials and comparing them to theoretical image intensities using the spectrum and output (including characteristic and Bremsstrahlung photons) from a separate verified x-ray generating program SpekCalc³² and the MC-derived detector response. The OBI was exposed to increasing tube potentials from 0 to 225 kVp and images acquired. A mean value was calculated for a central block of pixels (100 x 100) averaging 100 frames. Measured and predicted image intensities from the OBI were compared. Predicted OBI intensities were calculated by multiplying each SpekCalc spectrum by the MC-derived detector response and integrating the result at each tube potential. The theoretical OBI intensities were normalized to the image intensity measured from OBI by a constant determined by minimizing the sum of square errors. The detector response was also compared against an energy independent response model, representing the worst-case scenario.

The MC detector response for the OBI can be seen in figure A1. A comparison of the measured and theoretical image intensities resulted in deviations of less than 5 % from 50-225 kVp. This was compared to the theoretical flat detector response model that was found to deviate by up to 15 %, as can be seen in figure A1. This is still considered a reasonable agreement for this worst-case detector response model. This demonstrates that image intensities from the OBI are largely determined by output and not by energy. This observed OBI response means that changes in the x-ray spectrum due to e.g. the heel effect, will not drastically alter the image intensities from the OBI.

APPENDIX B: MC MODEL VALIDATION

In order to be able to extract meaningful dose values from MC simulations, a conversion factor between Gray per incident particle to Gray per mAs is required for each kV/filtration combination. This MC dose conversion factor can be determined by relating absolute output measurements to a MC simulation that replicates the idealized geometry (e.g. the dose to water at the surface of a water phantom, including the backscatter factor). In this case, BEAMnrc was used to score the dose to water the surface of a 10 cm water slab positioned at the SAD of micro-IR using the collimator geometry of C6 as was performed in the absolute dosimetry (section II.B). The dose to water was scored at the surface of the water phantom in a 2.2 cm diameter circle, 0.1 cm deep region and to a statistical uncertainty less than 1%. The calibration factor was calculated as the absolute output measurement in Gray per mAs divided by the dose at the surface of a water phantom in Gray per incident-particle.

HVLs, depth dose (*DD*) curves, and beam profiles were used to validate the MC models. Measured and simulated first and second HVL_1 and HVL_2 were compared for collimator C6. The MC-derived HVLs were determined from the x-ray spectrum obtained from the PSF file.³³ Measured and simulated *DD* curves were compared for all collimators. *DD* curves were measured using a stack of 10 square slabs of PMMA plastic (10 x 10 x 1 cm) with film pieces placed at the surface and between each layer. The surface to collimator distance was 5.3 cm and irradiations took 60 seconds. The dose-to-PMMA was converted to dose-to-water (in PMMA), using the mass energy absorption coefficient ratios of PMMA and water, to compare the film measurements to the simulations.

Transport parameters (EGSnrc inputs)	BEAMnrc v. 1.22 04/2011 (source)	DOSXYZnrc v. 1.11 05/2002 (phantom)
Boundary crossing algorithm		EXACT
Electron step algorithm		Presta-II
Spin effects		On
Electron impact ionization		On
Brems angular sampling		KM
Brems cross section		NIST
Bound Compton scattering		On
Photoelectric angular sampling		On
Photo cross-sections		XCOM
Rayleigh scattering		On
Atomic relaxations		On
Global electron cutoff energy – ECUT (MeV)		0.521
Global photon cutoff energy – PCUT (MeV)		0.01
Variance reduction		
Electron range rejection		Off
Directional bremsstrahlung splitting (DBS)	2000	-
DBS splitting radius and z location from source	2cm, 10 cm	-
Bremsstrahlung cross-enhancement (BCSE)	100	-

Table A2. General source and transport parameters defined in the BEAMnrc and DOSXYZnrc MC simulations including variance reduction techniques.

Measured beam profiles were compared to simulated beam profiles at the SAD and SSD for all collimators. At the SAD, measured beam profiles were acquired using film pieces suspended in air and irradiating for 360 seconds at 2.6 mA. Simulated beam profiles were extracted from the dose to a thin sheet (0.3 mm) of water placed in air at the SAD, replicating the experimental conditions. At the SSD, measured beam profiles were acquired using the OBI device (section II.C). Simulated OBI of collimated beam profiles were generated by continuing photon transport to the plane of the OBI according to section II.D.

To reduce pixel-to-pixel variation, a 5×5 pixel adaptive wiener filter was applied to all beam profiles both simulated and measured. Beam profiles were normalized to the average value of the beam contained within 80% to the maximum value in the beam. The field size was measured based on the 80 % isodose lines. An absolute percent difference was calculated for the integrated area contained within the 20% isodose boundaries between the normalized experimental and simulated 2-D beams acquired at the SAD.

Differences between MC calculated dose predictions and simulated OBI to experimentally acquired dose values and OBI of collimated beams were evaluated through the use of a gamma comparison.³⁴ The conversion factor from dose-per-particle to dose-per-mAs at the irradiation energy was determined to be $(7.87 \pm 0.2) \times 10^{15}$ particles/mAs. Each of the six PSF generated at the collimator exit took 30 hours to run on a 2.26 GHz quad-core Intel Xeon processor running Ubuntu 9.04 with 16 GB of memory. No efforts were made to optimize MC calculation times in this work.

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CHAPTER

5

On the use of an analytic source model for dose
calculations in precision image-guided small animal
radiotherapy

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ABSTRACT

Precision image-guided small animal radiotherapy is rapidly advancing through the use of dedicated micro-irradiation devices. However, precise modeling of these devices in model-based dose-calculation algorithms such as Monte Carlo (MC) simulations continue to present challenges due to a combination of very small beams, low mechanical tolerances on beam collimation, positioning and long calculation times. The specific intent of this investigation is to introduce and demonstrate the viability of a fast analytical source model (AM) for use in either investigating improvements in collimator design or for use in faster dose calculations. MC models using BEAMnrc were developed for circular and square fields sizes from 1 to 25-mm in diameter (or side) that incorporated the intensity distribution of the focal spot modeled after an experimental pinhole image. These MC models were used to generate phase space files (PSF_{MC}) at the exit of the collimators. An AM was developed that included the intensity distribution of the focal spot, a pre-calculated x-ray spectrum, and the collimator-specific entrance and exit apertures. The AM was used to generate photon fluence intensity distributions (Φ_{AM}) and PSF_{AM} containing photons radiating at angles according to the focal spot intensity distribution. MC Dose calculations using DOSXYZnrc in a water and mouse phantom differing only by source used (PSF_{MC} vs. PSF_{AM}) were found to agree within 7% and 4% for the smallest 1 and 2-mm collimator, respectively, and within 1% for all other field sizes based on depth dose profiles. PSF generation times were approximately 1200 times faster for the smallest beam and 19 times faster for the largest beam. The influence of the focal spot intensity distribution on output and on beam shape was quantified and found to play a significant role in calculated dose distributions. Beam profile differences due to collimator alignment were found in both small and large collimators sensitive to shifts of 1-mm with respect to the central axis.

INTRODUCTION

Preclinical precision small animal radiotherapy is a new field of research that continues to advance¹. At present, we estimate there are 30 research sites all in USA, Canada, and Europe, with precision small animal image-guided micro-irradiators (micro-IR) installed. This number is rapidly growing. The introduction of these type of devices provides researchers the ability to monitor disease progression (e.g. tumor growth) combined with localized radiation treatment in high-throughput large cohort studies. However, modeling of these devices for use in model-based dose-calculation algorithms such as Monte Carlo (MC) simulations continues to present challenges (e.g. long calculation times) but also in accurately reproducing collimator geometry and alignment.

Recently, our group presented a method to capture differences between the MC calculated and the measured dose distribution through the use of portal imaging². Using our best effort to model our micro-IR at the time, including tuning of the approximate elliptical size and intensity distribution of the focal spot, we still found 8 to 12% differences in beam profiles between measurements and simulations for the smallest collimated fields below 4 mm in diameter. Because there are too many degrees of freedom to adapt a detailed MC model (e.g. collimator position, focal spot size and location) through successive simulation iterations that lead to prohibitive calculation times, it is difficult to improve the MC model.

Therefore, we believe that the source generation (i.e. a description of the radiation field before the specimen) may benefit from alternative analytical techniques since the majority of the calculation time in MC algorithms is spent on electron transport and bremsstrahlung generation in the x-ray target. This is potentially unnecessary given that fast photon spectrum generating codes now exist³. Di Sopra *et al.*⁴ used a similar strategy for larger x-ray photon fields for a radiotherapy x-ray unit, but they did not investigate smaller, potentially focal-spot occluded, fields. In addition, generation of a smooth intensity distribution may improve calculation time by reducing any latent variance present in the MC generated phase space file. Furthermore, if future generations of image-guided micro-IRs would incorporate the use of non-static fields like those used in intensity modulated radiation therapy (IMRT), a fast source model would be desirable.

The purpose of this investigation is to introduce and demonstrate the viability of a fast analytic photon source model either for use in faster source generation and subsequent dose calculations or for use in investigating improvements in the design of static collimators or other beam-modulating devices. We will present the analytical model algorithm and compare it to the full MC model calculation.

MATERIAL AND METHODS

Micro-IR

A more thorough description of the small animal micro-IR (225Cx, Precision X-ray Inc., North Branford, CT) modeled and used throughout this study can be found in Clarkson *et al.*⁵. Only details that are germane to the study are described. The micro-IR consists of a dual-focus x-ray tube with a maximum tube potential of 225 kVp (225 Cx, Comet, Switzerland). The x-ray tube contains a static, water-cooled, tungsten target angled at 20°. Photons are filtered through a 0.8 mm Be exit window and 0.32 mm Cu removable filter cassette. The x-ray tube acts as both the source for imaging and irradiation. A set of circular and square collimators ranging in size and shape from 1 mm to 25 mm were investigated (see table 1 for dimensions). For all collimators, the source to surface distance of the entrance and exit apertures, D1 and D2 (see figure 1), are fixed at 90.0 and 224.9 mm, respectively. The apertures consist of 6.4 mm thick lead with the exit aperture incorporating an additional 3.2 mm brass insert to protect the lead from damage. Figure 1 illustrates the position and location of the collimator apertures in the MC model and analytical model further described below. For consistency, all figure orientations used in this manuscript use the coordinate system indicated in figure 1 with the z-axis defined as the central axis.

Figure 1. Illustrations of the MC and Analytic model of the small animal micro-IR.

Modeling of the micro-IR

The MC model of the micro-IR provides an accurate description of the radiation field - by way of phase-space files (PSF) - that contain individual particle information for specific collimator geometries. These PSF are used for subsequent dose calculations in voxelized phantoms of a simple water slab and a more complicated one of a mouse specimen. The micro-IR was modeled in BEAMnrc^{6,7} based on measurements and manufacturer-supplied data as previously reported² with a single exception - we incorporated the machine specific electron source intensity distribution targeted on the X-ray tube through the use of a separate PSF_e . Figure 1 illustrates the MC and analytical models of the micro-IR including the location of the electron source intensity distribution for reference.

Modeling the primary electron source

The electron intensity distribution was modeled by generating an electron beam PSF using a pinhole camera image of the focal spot supplied by the manufacturer. This was acquired according to the European standards document EN-12345⁸ and is a mirrored and scaled image of the focal spot as seen from the central axis. The PSF_e was generated by centering the focal spot image based on its center-of-mass, normalizing the maximum intensity to unity, mirroring, and accounting for the projection of the electron source by the target angle along the cathode-to-anode direction. An illustration of the position of the focal spot pinhole image and its relation to the generated PSF_e can be seen in figure 2.

The PSF_e was generated according to the BEAMnrc manual as a binary file (mode0 format, i.e. without a ZLAST variable) for 2.5 million electrons using the rejection method for their position. Positions on the normalized and modified focal spot pinhole image were randomly sampled to determine whether the source intensity at that location was greater or lesser than a randomly generated number with equal probability between 0 and 1. For those locations that passed the acceptance criterion, electrons were added to the PSF_e , and given an energy of 225 keV with a unit directional vector towards the x-ray target and a particle weight of 1 until the total number of particles was reached. This way, the position probability distribution of PSF_e will exactly reflect the focal spot image.

In addition, to study the influence of more simplistic focal spot shapes, two other focal spot intensity distributions PSF_e were generated: a 2-dimensional (double) Gaussian with different in x- and z-dimensions and a rectangular electron intensity distribution. The double Gaussian was modeled as seen along the central axis to the source plane (i.e. from the position of the focal spot image in fig 2) with the width and length of the FWHM sizes used in simulations of 7.15 and 2.37 mm, respectively, where the length is defined along the cathode-to-anode direction (i.e. y-axis). The rectangular intensity distribution was modeled as seen along the central axis with a width and length of 3.41, and 3.09 mm. These different intensity distributions PSF_e shall further be denoted

$PSF_{e,fs}$, $PSF_{e,gauss}$, and $PSF_{e,rect}$ for the focal spot pinhole image-based, Gaussian and rectangular intensity distributions and can be seen as viewed from the central axis in figure 3. Unless otherwise indicated, the $PSF_{e,fs}$ was the primary source used and reported throughout this study.

Figure 2. An illustration of the focal spot pinhole image orientation with respect to the input electron intensity phase space file (PSF_e).

Simulation of the photon phase space file

Simulations in BEAMnrc were performed using variance reduction techniques as described by Krawakow *et al.*⁹ and further explored by Bazalova *et al.*¹⁰ for small animal radiotherapy. Directional bremsstrahlung splitting (DBS) was set to 80,000 with a splitting radius of 2.5 cm at a SSD of 30 cm and bremsstrahlung cross-section en-

hancement (BCSE) was set to a factor of $100^{*11,12}$. The electron transport cutoff ECUT and the photon transport cutoff PCUT energies were set to 10 keV. All relevant particle processes such as electron impact ionization, atomic relaxations, Rayleigh scattering, and bound Compton scattering were included in the simulations.

Figure 3. The top leftmost panel shows the pinhole image of our focal spot acquired by the manufacturer and the subsequent generation of the $PSF_{e,fs}$ displayed with 10000 particles and oriented according to the pinhole focal spot image for illustration purposes (B). The bottom panels display the two additional electron beam intensity distributions investigated $PSF_{e,gauss}$ and $PSF_{e,rect}$ (C) and (D), respectively.

A variable number of primary histories were launched for each collimator depending on the approximate beam diameter at the isocentre and the number of required particles in the MC generated PSF (PSF_{MC}) to reach a statistical uncertainty of 1% in high dose regions in submilliter voxel dose calculations, as outlined in Bazalova *et al.*¹⁰. The number of required photons (n_{PSF}) in the PSF_{MC} can be found in Table 1. The

* BCSE was not implemented correctly in previous versions to V4 2.3.2 as discovered by the authors and has been since addressed by the NRC

scoring plane for the PSF_{MC} was placed beyond the exit aperture of the collimator (fig 1) at a source to scoring plane distance (SSPD) of 26.5 cm.

To better understand the influence of the collimator on the beam and its penumbra at kV energies, the largest circular collimator (C25) was modeled with regions identified by particle tagging (a *latch* variable). Simulations were run twice, once for tagging identification by interaction and secondly for tagging identification by region¹³.

Figure 4. Flow chart of the analytical model (AM) illustrating the discrete stages.

The analytical source model

An analytical model (AM) of the micro-IR was developed in Matlab (Mathworks, MA, USA) that consists of two distinct stages. The first stage is the generation of a photon fluence intensity distribution (Φ_{AM}) beyond the final collimation at the SSPD, while the second stage consists of generating - at the same position - the AM PSF (PSF_{AM}) from the photon fluence intensity distribution. The inputs to the AM include the electron beam PSF_e , a pre-calculated x-ray spectrum, and the collimator-specific entrance and exit aperture geometries. An illustration of the AM contrasted beside the MC model is shown in figure 1.

Figure 4 shows a flowchart of the steps of the AM algorithm used to derive Φ_{AM} and PSF_{AM} . In the first stage of the AM, the user defines the Φ_{AM} scoring plane resolution

and SSPD. Isotropic scoring plane resolutions between 8 and 50 pixels per mm^2 were used depending on the size of the beam under investigation. The scoring plane resolution also defines the AM source resolution Φ_{prim} . The Φ_{prim} is generated using PSF_e as an input, undoing the projection of the target, and renormalizing so that the integrated intensity of Φ_{prim} equals 1 in the source plane.

The next step is to define the geometry of the collimators since they primarily shape the beam. The only required geometrical inputs are the width and diameter of the collimator entrance and exit apertures and the respective distances from the source of their front (D1, D2) and back faces (D1+ 6.4 mm, D2 + 9.6 mm) with respect to the photon beam direction. Then a search grid is determined by projecting a beam from the source origin (O in figs 1 and 2) to the scoring plane defined by the distal side (back) of the exit aperture. To account for the penumbra size in the search grid, the projected beam is enlarged by an empirical factor between 1.15 and 2.0 depending on the size of the penumbra for that collimator and SSPD or until the expansion factor is large enough to encapsulate the full beam after an initial simulation and visual inspection of the beam (Φ_{AM}).

In the search grid of the scoring plane, the four interfaces perpendicular to the beam central axis of the entrance and exit apertures are projected upwards from each search location position as binary masks into the AM source plane Φ_{prim} . The intensity values and the locations of the visible source within the intersection of the aperture surfaces are stored per search location in an array. This is denoted by the photon fluence $\Phi_{\text{prim}}^{\text{vis}}$. The sum of the visible source intensity values ($\sum \Phi_{\text{prim}}^{\text{vis}}$) at each search position $\Phi_{\text{AM}}(i,j)$ in the scoring plane is assigned. This stage of the Φ_{AM} beam image generation has been parallelized in Matlab using a Parfor loop for eight CPUs because each search location (i,j) is independent of one another and to decrease the Φ_{AM} beam image generation time. The $\Phi_{\text{AM}}(i,j)$ beam image will contain values ranging from 0 to 1, 1 for a location from where the focal spot is completely visible and 0 for a completely occluded focal spot location in the source plane. Values in between 0 and 1 are for locations (i,j) for which Φ_{prim} is partially occluded. The Φ_{AM} beam image can also be saved for later inspection.

Once the Φ_{AM} beam image has been obtained the second stage is to generate the PSF_{AM} for use in subsequent dose calculations. The PSF_{AM} is generated by first considering a rectangular region encompassing the beam and randomly generate photons from it using the rejection method. Positions on Φ_{AM} are randomly sampled to determine whether the intensity at that location is greater or lesser than a randomly generated number with equal probability between 0 and 1. For those locations that pass the acceptance criterion, photons are added to the PSF_{AM} , and assigned an energy, a directional vector and a particle weight of 1 until the total number of desired particles is reached.

The photon energies are assigned from a pre-calculated x-ray spectrum. The program SpekCalc^{3,14} was employed to generate an x-ray spectrum at a 1 keV resolution accord-

ing to our x-ray tube parameters (Section A) and has been recently demonstrated to reproduce within tenths of a per cent the beam quality parameters HVL_1 and HVL_2 ^{15,16}. Energies are assigned using a random number generator and a table-lookup method from the cumulative distribution function of the pre-calculated x-ray spectrum.

The photon direction vectors are calculated by first determining the position of the corresponding search location of the photon during the Φ_{AM} beam image generation. These locations now need to be connected with a location at the level of Φ_{prim} , visible from the level of the search location. At each search location both the positions and intensities of the visible Φ_{prim} source are recalled from an earlier stored array (Φ_{prim}^{vis}). The sum of the Φ_{prim}^{vis} source intensities are normalized to unity and a cumulative distribution generated where the x-axis corresponds to the location index of the Φ_{prim}^{vis} source and the y-axis corresponds to the probability of a photon originating from that source location. A random number is generated and the corresponding index location provides the x and y location. Direction vectors u and v with respect to the x- and y-axis are calculated as:

$$u = \frac{(x - x_{fs})}{\sqrt{(x - x_{fs})^2 + (y - y_{fs})^2 + SSPD^2}} \quad (1a)$$

$$v = \frac{(y - y_{fs})}{\sqrt{(x - x_{fs})^2 + (y - y_{fs})^2 + SSPD^2}} \quad (1b)$$

where x, y is the position of the photon to be generated and x_{fs}, y_{fs} is the calculated origin location.

Dose normalization

MC dose calculations report dose-per-incident particle (Gy/particle) originating from the initial electron source (i.e. the PSF_e), which is later converted to dose-per-mAs (Gy/mAs) based on ion chamber measurements using a conversion factor for a specific collimator geometry. Because in our AM there are no incident particles on the target, a dose normalization is required for subsequent dose calculations using the PSF_{AM} . We have introduced a dose normalization factor (DNF) in a similar way to that of our MC simulations by calculating the relative amount of incident particles required to generate a PSF_{AM} for a specific collimator PSF denoted $PSF_{AM,C}$ or $PSF_{AM,S}$ for the circular or square collimators relative to the largest collimator $PSF_{AM,C25}$. The largest circular collimator denoted C25 is also the collimator that is typically used in experiments to determine the conversion factor between dose-per-particle and dose-per-mAs. The DNF for specific collimators PSF_{AM} is calculated the following way:

$$DNF = \left(\frac{\sum \Phi_{AM, Cor S}}{\sum \Phi_{AM, C25}} \right). \quad (2a)$$

The DNF is then used to calculate the number of incident particles ($N_{incident}$) required to generate the PSF_{AM} for each collimator by multiplying the DNF by the number of desired photons to be generated in the PSF_{AM} . A second conversion factor ($CF_{AM>MC}$) is also required to be able to compare the two calculation methods, which was calculated as:

$$CF_{AM>MC} = \left(\frac{D20_{C25}(PSF_{AM, C25})}{D20_{C25}(PSF_{MC, C25})} \right), \quad (2b)$$

where $D20_{C25}$ is the average dose of the top highest doses in simulations using the $PSF_{AM, C25}$ and $PSF_{MC, C25}$. Therefore, $N_{incident}$ is calculated for each collimator as

$$N_{incident} = DNF \cdot CF_{AM>MC} \cdot n_{PSF} \quad (2c)$$

where the DNF is a specific normalization factor per collimator and n_{PSF} is the number of required photons to be generated in the PSF_{AM} .

Limitations of the analytical model

Inherent in the AM are limitations and assumptions in the idealized MC generated beam. In this section we will briefly outline what those limitations and assumptions are and indicate a means to evaluate their potential impact in the idealized beam.

Scatter assumption: There is no or minimal pre-object scatter

There is no, or little contribution from scattered – what we are calling pre-scattered photons - generated from within the x-ray tube head assembly or collimators in the PSF_{MC} . This assumption can be tested using the latch variable tag as earlier mentioned. Even if scattered photons exist in the PSF_{MC} , as long as these are uniformly distributed in the PSF_{MC} their impact on the AM approach will be reduced when using the dose conversion factor to obtain dose-per-mAs. The contribution of pre-scattered photons will be presented in the Results section.

Perfect absorber assumption: The collimators act as perfect x-ray absorbers

There is no attenuation correction performed through materials (i.e. ray tracing) in the AM and therefore the collimator apertures – particularly the brass insert – are assumed to be a perfect x-ray absorber. While this may be a good approximation for the lead collimators, it may not hold for the brass exit aperture plug. This assumption can

be tested using the latch variable for photons that have passed through either the lead or the brass aperture. The contribution of these photons that end up in the PSF_{MC} and contribute to the penumbra of the beam will be presented in the Results section.

Energy assumption: The energy of the photons does not vary widely across the field

The heel effect¹⁷ causes the hardening of x-rays along the cathode-to-anode axis and is assumed to be negligible for the small field sizes considered in this paper. Only a single 225 kV x-ray spectrum is used for the generation of the PSF_{AM} , which results in a single mean energy across the field. The mean energy of the full MC PSF_{MC} along the cathode-to-anode axis can be plotted against the mean energy from the PSF_{AM} to test this assumption.

Fluence assumption: The photon fluence of the fields does not vary widely across the field

Also due to the heel effect, x-ray fluences are greater towards the cathode portion of the field than the anode portion of the field. In the AM, off-axis variable fluences along the cathode-to-anode axis are not incorporated. We assume that for the field sizes considered this approximation is valid. Photon fluence profiles will be plotted against the AM generated Φ_{AM} image.

Dose calculations in phantoms

Dose calculations were performed in a simple cubic water phantom and in a more complicated CT-derived phantom of a mouse specimen centered at the SSD. The water phantom was 3 cm³ cubic phantom with an isotropic voxel size of 200 μm surrounded by 0.5 cm of air. Dose calculations were performed using DOSXYZnrc (rev 1.47, 2011)¹⁸ using the same transport parameters as in the MC model simulation with the exception that electrons below 110 keV ($\text{ECUT} = 621 \text{ keV}$) were not transported, corresponding to a cut-off range of 0.1 mm in water. The number of histories launched was equal to the number of photons in the PSF and each photon was split (N_{split}) 250 times and ensured that the average statistical error of the twenty highest doses (D20) would be less than 1%. No photons were recycled. MC dose calculations using the AM vs. the full MC model differed by only the PSF used (PSF_{AM} vs. PSF_{MC}).

A micro-CT image (80kVp, 2.5 mA) of an anesthetized adult male mouse thorax (C57Bl/J6) was used to generate a realistic phantom (205 μm isotropic voxel spacing) for subsequent small animal MC dose calculations. Animal facilities and experiments were in accordance with local institutional guidelines for animal welfare and were approved by the Animal Ethical Committee of the University (number 2012-006). A 4-tissue CT-to-material and-density conversion scheme was used for air, lung, soft tissue, and bone with thresholds of -665, -102, 383 HU. A single treatment plan consisting of 8 beams, each separated by 45 degrees, were targeted in the lung of the mouse phan-

tom. Simulations were performed using DOSXYZnrc for collimator C5 using the same physical transport parameters per beam as defined above, meaning that 192 million (24×8) photons in total were launched.

Importance of focal spot alignment on beam shape and output

To investigate the importance of collimator alignment and the focal spot location we shifted the focal spot location in our AM by 0.5 and 1 mm in the both the x- and y-axis and generated beam profiles Φ_{AM} for all of the collimators. Beam profiles parallel to and perpendicular to the cathode-to-anode source axis were plotted for all collimators in the AM. Phase space files $PSF_{AM,C1+0.5}$ and $PSF_{AM,C1+1}$ were generated for the smallest circular collimator C1, where effects are expected to be the largest, to further investigate the impact on dose calculations.

Figure 5. Effect of the assumptions made in the AM compared to the detailed MC model. (A) shows the effect of pre-object scatter from various components depicted in the embedded illustration. (B) shows the magnitude of “leaky” photons from the collimator. (C) shows the change in mean energy of the beam along the cathode to anode axis between the MC and AM for a 1-mm wide central section of the beam. (D) shows the change in the beam photon fluence along the cathode-to-anode axis between the MC and AM for the same 1-mm wide central section of the beam.

RESULTS

AM vs. MC model beam profiles for different focal spot intensity distributions

The effect of the focal spot intensity distribution on the beam photon fluence was investigated for the three intensity distributions studied ($\text{PSF}_{e,fs}$, $\text{PSF}_{e,gauss}$, $\text{PSF}_{e,rect}$; fig 3) with the most pronounced beam shape changes observed for the smallest collimator C1 shown in fig 5A, B, and C, respectively. Beam profiles parallel to and perpendicular to the cathode-to-anode axis of the AM generated beam images (i.e. $\Phi_{AM,C1}$) were compared to the MC generated $\text{PSF}_{MC,C1}$ (fig 8D, E). The MC profiles were normalized to the peak intensity distribution of the $\Phi_{AM,C1}$ beam images for comparison. Only small differences at the edges of the penumbra for the rectangular source intensity distribution ($\text{PSF}_{e,rect}$) were observed between the AM and MC generated beam profiles. Continuation of $\text{PSF}_{MC,C1}$ to the water phantom resulted in differences in the average of the twenty highest dose values (D20) of 32% and 35% dose decrease for the double Gaussian and rectangular focal spot intensity distributions (i.e. $\text{PSF}_{e,gauss}$ and $\text{PSF}_{e,rect}$) compared to the pinhole image generated focal spot intensity distribution ($\text{PSF}_{e,fs}$) were found, respectively.

AM assumptions

Scatter assumption

Using the latch variable to store defined geometric regions where photons have undergone an interaction for the largest circular collimator C25 we found, predictably, that the majority of photons reaching the scoring plane are primary photons originating from the source that have not scattered. However, a small percentage, accounting for a maximum 0.4% of the total number of photons has been scattered. The largest contribution of these pre-object scattered photons originates from the primary Cu filter used to harden the spectrum. The makeup of the pre-object scattered photons can be seen in figure 6A.

Perfect absorber assumption

Again using the latch variable, but this time for particles that have transited geometric regions for the largest circular collimator C25, we found that a portion of the penumbra did consist of photons that have traversed the edges of the distal collimator, particularly through the brass insert component. Relative to the central axis of the beam, these “leaky” photons contributed to a maximum of 12.5% of the number of photons in an annular region approximately 1 mm wide situated at the edge of the C25 collimator beam (fig 6B).

Figure 6. Dose ratios in two perpendicular planes (colored panels), depth dose curves (second column) and dose profiles (last column), at the surface of the water phantom normalized to 10Gy for the largest field (C25) at the surface for the collimators investigated.

Energy assumption

The hardening of x-rays due to the heel effect was barely perceivable in the largest circular collimator along the anode to cathode source axis at 30 cm from the source (fig 6C). The influence of scattered photons though, could be observed at the edges of the beam when compared to the AM beam profile, which does not include scattered irradiation. Overall there was good agreement between the AM and the full MC model, indicating that a single x-ray spectrum is sufficient in applications of collimated small fields. In addition, comparison of calculated half value layers (HVL) between MC-derived vs SpekCalc spectral HVLs were found to agree within 0.7 and 0.1% for HVL1 and HVL2¹⁹.

Fluence assumption

Similarly due to the photon energy heel effect, a fluence gradient could only barely be perceived in the largest circular collimator along the cathode/anode axis (fig 6D).

AM vs. MC model

Dose calculations in phantoms.

The $CF_{AM \rightarrow MC}$ was found to be $6.87 \cdot 10^{-6}$. Using this conversion factor in addition to the calculated collimator specific *DNFs*, which were calculated to be 670.3, 101.3, 25.0, 6.2, 4.9 and 1.2 for collimators C1, C2, C5, C10, S10, and S20, respectively, AM generated dose calculations could be compared to MC generated dose calculations using the procedure outlined in section II.C.1. The *DNF* for C25 is unity per definition. Because dose-per-particle is somewhat difficult to interpret, dose calculations were scaled to 10 Gy (i.e. the maximum dose in the MC generated dose calculation for the largest collimator was scaled to 10 Gy and all other dose calculations normalized by the identical factor). Dose calculations in the simple water phantom using the AM generated PSF_{AM} compared to the MC model generated PSF_{MC} were found to largely agree with dose differences less than 1% for all but the smallest two collimators (C1, C2) that reached maximum dose differences up to 7 and 3%, respectively (fig 7A). However, the integrated dose distribution difference for the smallest collimator C1, was found to be slightly smaller at 6% as seen in a dose profile through the top of the water stack (i.e. dose maximum at $z = 1.5$ cm with respect to the SAD) as seen in the last column of (fig 7A).

Comparisons of dose distributions in the mouse phantom from simulations differing by only their PSF used for each collimator were found to agree very well even in highly heterogeneous regions such as in the bone (fig 8). Visualization of dose ratios between the AM and MC dose calculation reveal mostly noise between the two methods for the union of pixels that contain 2% or greater than the maximum dose (fig 8C).

Figure 7. 2D axial dose slices through the central targeted region of the mouse phantom calculated using the $PSF_{AM,CS}$ and $PSF_{MC,CS}$ in (A) and (B), respectively. Panel (C) displays a dose ratio slice of (A) and (B) for doses 2% and greater of the target dose.

Applications

Result of collimator and focal spot alignment on beam shape

The effect of a focal spot misalignment with respect to the central axis on beam shape was investigated for all the collimators by shifting the position of the focal spot image by 0.5 and 1 mm in both x- and y-axis. Significant beam profile shape changes were observed for collimators C1, C2, C25, and S20 but not for collimators C5, C10, and S10. Line profiles parallel to the cathode-to-anode direction of the shifted focal spot locations were plotted against the stationary (centered) focal spot shown in Figure 9.

Result of collimator and focal spot alignment on output factor

The effect of a focal spot misalignment on dose calculations was further investigated for the smallest collimator where we expected the largest dose differences. The smallest collimator saw only a small dose difference of a 1% drop on both the peak dose and the integrated beam dose at the surface of the water phantom for the 0.5 mm x/y shifted focal spot compared to the stationary focal spot dose calculation. Much larger dose differences of a 13% drop on the peak dose and a 21% drop on the integrated beam dose at the surface of the water phantom for the 1 mm x/y shifted focal spot compared to the stationary focal spot dose calculation were observed.

Calculations times

AM vs. MC generation times

Calculation times for the AM are broken down into the two distinct stages, Φ_{AM} and PSF_{AM} generation, as well as the total calculation times for each collimator in table 2. The total CPU time for the Φ_{AM} generation is reported to give a fair comparison over

the MC model, which is also reported in total CPU time since MC simulations are also parallelized. Though these calculations times are evidently long, in practice Φ_{AM} is calculated on 8 cores, similarly are the MC calculations. The speed up advantage in generating the PSF_{AM} vs. PSF_{MC} is approximately 1200 times faster for the smallest beam and 19 times faster for the largest beam. One should also consider that Φ_{AM} only has to be calculated once for each collimator, if the main application is dose calculation in specimens.

AM vs. MC dose calculation efficiency gains due to latent variance

By generating the PSF_{AM} from a smooth fluence intensity distribution Φ_{AM} , the PSF sources have inherently less latent variances and therefore should produce smoother dose distributions. This effect was quantified by calculating the efficiency gain in dose calculations using the figure of merit:

$$\varepsilon = \frac{1}{\sigma^2 T}, \quad (3)$$

where σ^2 is the average statistical variance in the highest 20 doses achieved in a CPU time T . The average efficiency gain in simulations of the water phantom between the AM and the MC model (i.e. $\varepsilon_{AM}/\varepsilon_{MC}$) for all the beams investigated was found to be 1.20 ± 0.02 . This means we see a 20% efficiency gain or conversely a 16% reduction in dose calculation time using the AM vs. full MC model using an isotropic dose calculation grid size of 200 μm .

Therefore, the combined advantages in using the PSF_{AM} over the PSF_{MC} to achieve similar dose accuracy is then 1392 (1200×1.16) and 22 (19×1.16) times faster for the smallest to largest collimator (C1, C25) when considering both the phase space file generation and the improvement in the latent variance of the phase space file.

DISCUSSION

In this study, we have introduced an analytical source model for use in small animal dose calculations, which has been demonstrated to be highly accurate for beams that are not focal spot occluded, and less accurate for focal spot occluded beams (C1, C2) resulting in integrated dose differences of 6 and 3%, respectively. Dose differences for the focal spot occluded beams (C1, C2) between the AM and the MC model can largely be attributed to photon transmission through the walls of the brass insert at the distal end (i.e. exit side) of the collimators not accounted for in the AM. Therefore, the implementation of an AM source model for use in small animal dose calculations

having total dose calculation times reduced by 1392 to 22 times for the smallest to largest collimator has been found to be both an effective and efficient tool.

Figure 8. 2D contour plots of $\Phi_{AM,C1}$ for the smallest collimator at 30 cm SSPD using the three different electron intensity distribution studied $PSF_{e,fs}$, $PSF_{e,gauss}$ and, $PSF_{e,rect}$ in A, B and C, respectively. Panels D and E show line profiles of A, B, and C, parallel to (y-axis) and perpendicular (x-axis) to the cathode/anode source axis compared against the MC generated beam profiles $PSF_{MC,C1}$. MC profiles were normalized to the peak intensity distribution from $\Phi_{AM,C1}$ for comparison purposes.

The AM has elucidated several important aspects with regard to performing small animal dose calculations of collimated beams with dimensions on the order of the focal spot size. These include: the influence on beam shape, output and alignment with respect to the focal spot. As the aperture size is reduced to a size smaller than the focal spot, and for increasing distances away from the point of collimation, the beam will begin to reflect the intensity distribution of the source (i.e. the collimator will start to act like a pinhole device). Therefore, for focal spot occluded beams, the beam shape will be a product of the focal spot intensity distribution used. However, for most of the geometries used in small animal micro-IR devices, these beam profile differences are less important for total dose calculations than the change in dose output. Decreases in D20 of 32% and 35% between the double Gaussian and the rectangular focal spot

intensity distributions (i.e. $PSF_{e,gauss}$ and $PSF_{e,rect}$) compared to the pinhole image generated focal spot intensity distribution ($PSF_{e,fs}$) were found. These drops in output may help to explain our previous low experimental measurements of output factors for the two smallest beams (C1, C2) of 0.39 and 0.36 where a correctly focal spot aligned model would have been closer to 0.6 and 0.7, respectively, based on the dose calculation in water.

AM simulations of shifted focal spots reveal strict tolerances on the alignment of the focal spot with respect to the central axis of collimation. Shifts or misalignments of the smallest collimator (C1) as small as ± 1 mm can lead to a peak dose output drop of 13% and are difficult to predict without knowledge of the focal spot intensity distribution as changes in the output will reflect the irregular shape of the source. These changes would be difficult to catch in day-to-day routine small animal treatment without an external validation method e.g. EPID dosimetry². Interestingly, the larger collimators (C25, S20) also reveal sensitivities on beam profile changes to small shifts or misalignments as small as ± 1 mm. These profile changes result from narrow-fitting collimation in the upper entrance aperture and are likely the result of being engineered based on a point source model or convenience given that all collimators share similar upper aperture collimator diameters (e.g. C5, C10 and C25) as seen in Table 1.

Though we achieved good agreement between the AM and the MC model, improvements are possible. For example, the coding could have been done in C++ instead of in Matlab which would likely increase the beam calculation and phase space generation time. A ray-tracing method could be investigated to account for the “leaky photons” that pass through the brass insert. Alternatively, the brass insert could be machined to be smaller, which would greatly improve the current model for the smallest beams. Better still, a thinner brass insert would also reduce the mechanical penumbra of the beams.

Based on the work in this paper, improvements to the design or function of these micro-IR could be to establish an alignment procedure that could include the use of a pinhole device together with the onboard imager to both acquire the intensity distribution of the source for use in the AM or MC model and to determine the alignment of the source with the collimation on a more routine basis. Additional consideration should also be given to the size of the entrance aperture, which should be enlarged to increase the tolerance on collimator alignment. Another possibility could be to use the small focal spot, which is primarily used for imaging purposes but could be used to reduce the tolerance on alignment as well as to decrease the sensitivity of model based dose calculation engines reliance on the focal spot intensity distribution. Though, this would require that the interchangeable collimators can be centered on either the small or large focal spot position. Additionally, heating issues could prevent prolonged use of the small spot.

In this work we have demonstrated that the use of a *DNF* enables correct dose calculations without resorting to beam calibration specific factors that would effectively

render the AM useless if they were required. To produce real dose calculations the conversion factor ($CF_{AM \rightarrow MC}$) would simply be replaced by a measured dose calculation factor (Gy/mAs) as is the traditional approach. The flexibility of the proposed AM means that a variety of field shapes could be generated easily and could be useful in a dedicated small animal treatment planning system. The AM also will allow investigators to more easily adapt their source model until they achieve better agreement between MC dose calculations and measurements. Though in practice and based partially on this work, provided a realistically shaped focal spot intensity distribution is used we believe that an output factor measurement using film can to first order, provide a correction to the MC source models that will result in sufficiently accurate dose calculations for most institutions.

Figure 9. Illustration of the effect of moving the source 0.5 and 1 mm in the positive x/y direction on line profiles Φ_{AM} parallel to the cathode-to-anode direction for all collimators compared to the stationary beam profiles.

CONCLUSION

We have demonstrated that a simplified analytic source of a small animal micro-IR model proved accurate for all but the smallest 1-mm collimated beam. Refinements to the analytical model may consider incorporating small transmissions through the exit aperture. This study also demonstrates the sensitivity of the focal spot intensity distribution on output and alignment, which can have an effect on beam profiles for even the largest collimated beams in this small animal micro-IR. Phase space generation

times were approximately 1200 times faster for the smallest beam and 19 times faster for the largest beam. AM generated phase space beams had an efficiency gain of 20% due to improvement in the latent variance of the phase space beams.

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CHAPTER

6

Development and Validation of a Treatment Planning System for Small Animal Radiotherapy: *SmART-Plan*

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ABSTRACT

Background and Purpose: Image-guided equipment for precision irradiation of small animals for pre-clinical radiotherapy research became recently available. To enable downscaled radiotherapy studies that can be translated into human radiotherapy knowledge, a treatment planning system for pre-clinical studies is required. A dedicated treatment planning system (SmART-Plan) for small animal radiotherapy studies was developed. It is based on Monte Carlo simulation of particle transport in an animal. The voxel geometry is derived from the onboard cone beam CT imaging panel. SmART-Plan was validated using radiochromic film (RCF) dosimetry in various phantoms: uniform, multislabs and realistic plasticized mouse geometry. **Results:** Good agreement was obtained between SmART-Plan dose calculations and RCF dose measurements in all phantoms. For various delivered plans agreement was obtained within 10% for the majority of the targeted dose region, with larger differences between 10-20% near the penumbra regions and for the smallest 1mm collimator. Absolute depth and lateral dose distributions showed better agreement for 5 and 15-mm collimators than for a 1-mm collimator, indicating that accurate dose prediction for the smallest field sizes is difficult. SmART-Plan offers a useful dose calculation tool for pre-clinical small animal irradiation studies.

INTRODUCTION

There is a limited amount of information that can be gleaned from clinical trials with radiotherapy patients and it has been hypothesized that greater insight can be obtained from downscaling radiotherapy to the scale of small animals such as mice and rats ¹. This is because of the rapid development of realistic preclinical orthotopic tumor models in animals and because many radiobiological experiments in the past were conducted using radiation conditions that are not representative for the current radiotherapy practice. Most past animal experiments used fairly large radiation beams, didn't have image-guidance and had imprecise or no treatment planning. Hence the recent development of dedicated image-guided precision irradiation research platforms (see ² and references therein) and other bespoke devices based on modified micro-CT devices ³ for use in preclinical studies.

The latest commercial small animal radiation research platforms consist of a heavy duty x-ray machine (typically 225 kV), precision photon beam collimators (down to 1mm field size), a 3D moving animal stage and an x-ray imaging panel for image-guidance. These devices are computer-controlled and are capable of delivering precise static or dynamic arc radiation beams to small targets, with significant sparing of healthy tissue compared to older experiments on which our radiobiology knowledge is built.

While these devices constitute a major technological advance, another element is needed to ensure that complex radiation dose distributions can be delivered to small animals. A dedicated treatment planning system (TPS) that can plan beam delivery to arbitrarily shaped targets with complex beam arrangements is needed to downscale human radiotherapy to the small animal level. Current clinical TPS can usually not handle dose calculations for kV x-rays, very small beams, and are often not equipped to deal with multimodality images with very small voxels. Hence the need for a TPS which may handle these aspects and can also be used for high-throughput animal experiments for example in drug-radiation synergy studies. This work describes the ongoing development and validation of a dedicated small animal TPS, *SmART-Plan* (where *SmART* stands for Small Animal RadioTherapy).

There are significant differences between treatment planning for small animals and human patients. Chief among them is the use of very small beams of kV x-ray photons. In the commercially available devices a broad x-ray spectrum of about 225 kV is used for radiotherapy studies with collimators of a fixed beam size of between 1mm and several centimeters. The x-ray tube is mounted on a movable support to enable irradiation of the specimen from different sides. The same x-ray tube, but with a lower kV setting is used to create a cone beam CT image (CBCT) of the specimen. The imaging panel typically has a high resolution of about 100µm, resulting in 3D CBCT images with a large number of voxels easily ranging in the tens of millions, imposing demands on computer memory.

For kV photons the interaction coefficients in tissues depend on both composition and density. Therefore, whereas for radiotherapy patient dose calculations it usually suffices to consider the geometry to consist solely of water with a density derived from the CT images, for kV x-ray dose calculations in the 40-225 keV photon energy range require knowledge of the x-ray spectrum and the tissue composition. Appendix A1 discusses this in more detail. Currently, animal dose calculations use human tissue compositions due to a lack of available data on animal tissue compositions.

Another issue related to small animal dose calculation is the use of very small fields (1mm – few cm). The output of an x-ray device drops significantly for small collimator sizes expressed as radiation dose rate per unit x-ray tube current. This is due to a lack of scatter equilibrium in tissue for the smallest fields but also due to obscuring of the electron focal spot on the x-ray target⁴.

MATERIALS & METHODS

Development of treatment planning platform

SmART-Plan was developed in MATLAB (v 7.11.0.584, R2010b, The MathWorks Inc., Ma) and is distributed as a compiled executable program on a Linux-based platform (Ubuntu 12.04 or later). Figure 1 shows the design of SmART-Plan. It performs absolute dose calculations on user-generated voxel phantoms from reconstructed CBCT images by interfacing with the Monte Carlo (MC) program DOSXYZnrc (v 1.47 2011, NRCC, Canada). MC simulations of photon and electron transport are performed. The dose calculations are performed on a parallel computing architecture with 24 processors (12 core 2.67 GHz Intel Xeon with 24 threads total). The x-ray device and its collimator are modeled by a photon source (i.e. phase space files) from a previous MC simulation⁵. Currently only the most common treatment photon spectrum is modeled (225 kVp, 0.32 mm Cu filtration). SmART-Plan is validated in this paper for a specific research platform (X-Rad 225Cx; Precision X-Ray Inc., North Branford, CT), but should be equally usable with other similar systems.

CT image reconstruction

CBCT scanning was performed using the research platform and the accompanying PilotCal software (Precision X-Ray) at 80 kVp, typical for small animal micro-CT platforms to produce a sufficient signal at the detector without excess imaging dose to the animal⁶. 3D images (210 μ m sided voxels) were reconstructed by filtered backprojection. A Hounsfield Unit (HU) to density calibration of the CBCT scanner was performed with a phantom with known tissue substitute inserts (Model 467; Gammex RMI, Middleton, WI).

Figure 1. Flowchart of SmART-Plan. The tasks outlined on the left are to be performed sequentially top/down.

Workflow of treatment planning platform

The flowchart of the SmART-Plan process is shown in figure 1. Dose distributions and the final treatment plan are based on a CBCT image acquired with the animal in the treatment position. The CBCT image is imported in the CT upload module and can be cropped and resized to speed up MC dose calculations. Assignment of materials and densities is an essential part of the process not found in a human TPS and the CT2MD (CT to Material and Density) module is dedicated to this step. Any number of materials can be assigned based on thresholds in the HU-density histogram; densities are assigned based on the HU to density calibration curve. Required material photon interaction data is read from a database of human tissues, since the atomic composition of animal tissue is poorly known. A voxel phantom is created that serves as input for the MC dose calculation. Optionally, structure contours can be created to aid treatment plan evaluation. The planning of isocenters and static/dynamic beams is performed in the beam definition module. An estimated number of histories to be simulated is

calculated based on the desired statistical uncertainty. A default selection of transport parameters is provided for the user based on previously published work of kV dose calculations^{5,7} but can be edited by the user. The dose calculation module takes care of the complete interface with the MC dose engine and dose distributions are imported and displayed automatically. Thereafter, dose distributions and dose volume histograms can be evaluated and optimized by adjusting beam weights. Finally, an *ini file* is generated which serves as input for the PilotCal software to execute the irradiation. See Appendix A2 for examples of screenshots of some SmART-Plan modules.

Validation of treatment planning platform

Absolute dose reporting in SmART-Plan was performed by deriving a calibration factor from a comparison of a MC dose calculation in a water phantom and an absolute dose measurement using an ionization chamber with a reference field of 30×40 cm at source to surface distance 29.9 cm. By modeling the exact same geometry as used in the experiment the conversion factor from dose per particle (MC) to dose per mAs (experiment) can be determined. This conversion factor is then valid for all future simulations with the same photon spectrum⁵. The measurements determined the dose to the surface of the water phantom by following a medium energy x-ray dosimetry protocol⁸.

Field size diameter (mm)	OF (RCF)	OF (SmART-Plan)	OF correction factor
1	0.53 ± 0.01	0.67 ± 0.02	0.79 ± 0.05
5	0.85 ± 0.02	0.86 ± 0.02	-
15	0.90 ± 0.01	0.90 ± 0.03	-
30x40 (ref.)	1 ± 0.01	1 ± 0.02	-

Table 1. Measured (RCF) and calculated (SmART-Plan) OF for three circular collimators and their uncertainty.

Validation of SmART-Plan was performed by examining calculated absolute depth dose curves and dose distributions from multiple-target irradiation plans in uniform and heterogeneous phantoms, compared against radiochromic film (RCF; type Gafchromic EBT2, GafChromic, Ashland Specialty Ingredients, Wayne, NJ) measurements for 1, 5, and 15-mm circular collimators. RCF was used throughout this study for dose verification due to its superior spatial resolution, near water-equivalence for dosimetry, and the avoidance of a pre-scan when using the triple-channel calibration method⁹. The RCF was calibrated with the research platform for a range of doses. Films were read out (225 dpi in 48 bits, 16 bits per color channel) 24 hrs after exposure with a flatbed scanner Epson PRO V750 (Seiko Epson Corporation, Japan)⁹.

To validate absolute depth dose distributions for the three collimators, first a CBCT image was acquired of a solid water slab phantom. A target dose of 5 Gy was prescribed to the surface of the phantom and MC dose calculations were launched to reach a statistical uncertainty of less than 2%. Next, the *ini file* (Fig 1) is generated that includes the beam-on time and the target isocenter location that the positioning stage

moves towards prior to irradiation. Depth dose measurements for the collimators were then performed and repeated three times by exposing five RCF pieces on top and at different depths in the phantom. Measured and calculated depth dose distributions were compared by taking the average dose in a region from the maximum dose value to 80% thereof in both datasets.

Figure 2. Measured (RCF) and computed (MC, SmART-Plan) absolute depth dose (A) and lateral dose profiles, parallel to the cathode/anode direction (B). Panels (C) and (D) display local differences (measured – calculated) dose.

More treatment plans were created for three other phantoms. The first two slab phantoms consisted of three RCF pieces sandwiched on top and between three pieces of 10-mm thick solid water ($5 \times 8 \text{ cm}^2$) slabs, with the second phantom differing only by replacing the middle solid water slab by bone substitute (SB3, Gammex-RMI, Middleton, WI). Treatment plans for these two phantoms made use of the three collimators under different irradiation angles and with two target isocenter locations each targeted with a dose of 5 Gy. Finally, a treatment plan was applied to a plasticized heterogeneous mouse specimen (*PlastiMouse*) developed by our anatomy department. The *PlastiMouse* phantom was sliced coronally to allow insertion of RCF. 3 Gy was prescribed from a static 5-mm collimator beam targeted from above.

RESULTS

Validation of the treatment planning system

Following the TG61 guidelines the dose rate at the surface of a solid water phantom was found to be 0.246 Gy/min/mA. Table 1 compares measured and calculated output factors (OF) at the surface; for the smallest beam an output correction factor is required.

Measured and calculated absolute dose distributions and lateral dose profiles (Fig 2) showed good agreement within 5% for the 5-mm and 15-mm collimator at all depths. For the smallest 1-mm collimator a maximum difference of 13% at 21 mm depth was obtained. Lateral dose profiles showed good agreement except in the penumbra regions. Differences seen in the penumbra region occur due to several reasons. The “choppiness” of the top left panel from SmART-Plan was caused by used an angular step size of 3 degree, which has been subsequently reduced to 1 degree. Second only small differences in the shape of the penumbra will result in the appearance of large deviations in the penumbra. Normally, a 2-D gamma calculation would remove the appearance of these differences, but we found these errors telling of the real agreement of SmART-Plan. Furthermonth, achieving perfect alignment between the film measurements and SmART-Plan is challenging and in this case only pixel-wise shifts between the experiments and simulations occurred, which could have likely benefitted from alignments less than the size of the pixel.

Dose calculation times for each collimator were: 76, 124, and 495 seconds for the 1, 5, and 15-mm collimator, respectively, reflecting the larger number of particles required to simulate larger beams to achieve the same statistical accuracy.

Figure 3. Comparison of calculated (top row) and RCF measured (middle row) dose distributions in a uniform solid water phantom. Also shown are the dose differences (calculated – measured) in the bottom row. At various depths the 1,5 and 15 mm beams can be observed.

The multiple beam/multiple target treatment plan delivered in the two slab phantom geometries achieved good agreement between the RCF measurements and the SmART-Dose calculations, showing maximum absolute deviations of about 2 Gy localized near the penumbra of the beams (Figure 3, A4). For the uniform solid water phantom in the regions with doses between 80-100% of the maximum dose in a slice, only 1% of the voxels exhibited a dose difference of more than 10% with respect to the maximum dose in that slice. For the heterogeneous slab phantom similar differences were reported except for the film piece at 20-mm below the surface where larger discrepancies were noted. The primary cause for this discrepancy is a misalignment in the targeting of the smallest beam (1 mm). When voxels with doses between 20-100% of the maximum dose are considered, only 4 % of the voxels show a discrepancy exceeding 10%. The total dose calculation time in SmART-Plan for both phantoms was about 550 seconds. SmART-Plan launches all the parallel dose calculations at once and is therefore only limited by the job with the longest calculation time.

DISCUSSION

We demonstrated good agreement between dose calculations in SmART-Plan and RCF measurements for simple beam arrangements and for complex multiple-beam multiple-target treatment plans to uniform, heterogeneous and realistic mouse phantoms. For the delivered plans we see agreement within 10% for the majority of the targeted dose region, with larger differences between 10-20% near the penumbra regions (Figures 3-4, A4). Depth dose curves and profiles showed better agreement for the 5 and 15-mm collimator than the 1-mm collimator, reflecting the challenge in accurately calculating and delivering a prescribed dose to a target for beam sizes approaching the dose calculation voxel size. The output correction factor for the smallest field is caused by the inability to accurately align the smallest collimator with the location of the complexly shaped focal spot⁴. Dose discrepancies between SmART-Plan and delivery can result from a number of sources;

- (i) CBCT imaging (e.g. reconstruction artifacts);
- (ii) MC-related dose calculation errors (e.g. poor tissue segmentation or use of human tissues due to poorly known animal tissue composition);
- (iii) machine-related delivery errors (e.g. collimator misalignments, gantry sag corrections);
- (iv) measurement-related errors (e.g. absolute dosimetry, RCF);
- (v) analysis-related errors (e.g. image registration)

When CBCT is used in radiotherapy patients the conversion of the Hounsfield units to electron densities, needed for accurate dose calculation, may be inaccurate¹⁰. The conversion may be problematic due to effects such as beam hardening and photon scatter. However, these effects are mostly relevant for large objects such as radiotherapy patients. For small animals photon scatter is minimal (<5 % for a 5 cm diameter water cylinder) and beam hardening is insignificant. Other issues such as streaking artifacts could lead to incorrectly assigned tissues. Therefore, more research is needed to establish the suitability of CBCT imaging for small animal dose calculations.

Figure 4. 2D CT slices containing target location and dose distribution through the PlastiMouse phantom (top row). The bottom row shows the calculated and measured dose distributions in the sagittal plane and their difference (calculated – measured).

Small field dosimetry and dose calculation is known to pose problems in radiotherapy with MV beams^{11,12} and also in kV dosimetry^{13,14}. We recently studied in detail the influence of the focal spot shape and its geometric occlusion by small collimators on x-ray output⁴. We found it is difficult to calculate absolute dose for the smallest fields (order of a mm) but that for larger fields MC simulations with a detailed model of the irradiator offer an accuracy of better than 5%. The level of detail in our simulations cannot improve much further but the mechanical robustness and reproducibility of the irradiator can be improved upon. For dosimetry we currently only recommend radiochromic film due to its ease of use, high spatial resolution and good absolute dose measuring capabilities. We use a 3-color readout scheme⁹ to avoid pre-scanning the film. We estimate the accuracy of radiochromic dosimetry for the small fields to be about 5%. Others have advocated the use of solid plastic dosimeters¹⁴ but these cannot be used to measure dose closer than 4mm to the surface. Regular quality assurance should be part of the operation of small animal irradiators.

In more advanced future versions of Smart-Plan we will add multi-modality imaging planning, faster optimized dose calculations, extended DICOM import/export capabilities, advanced methods for structure contouring, dose analysis, image registration and treatment verification with the onboard imager⁵.

CONCLUSION

A dedicated treatment planning system for small animal pre-clinical work, SmART-Plan, was presented and validated. Good agreement was achieved between measured and calculated dose distributions in various phantoms: uniform, multislabs and a realistic plasticized mouse geometry. Achieving good agreement for the smallest field in the study (1mm) is challenging, indicating that pre-clinical studies with the smallest beams may require improvements in the irradiation system. SmART-Plan is a useful tool to support pre-clinical radiotherapy studies.

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CHAPTER

7

A longitudinal evaluation of partial lung irradiation in mice using a dedicated image-guided small animal irradiator

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ABSTRACT

In lung cancer radiotherapy the dose constraints are mostly determined by healthy lung toxicity. Preclinical micro-irradiators are a new tool to evaluate treatment strategies closer to clinical irradiation devices. In this study, we quantified local changes in lung density symptomatic of radiation induced lung fibrosis (RILF) after partial lung irradiation in mice using a precision image-guided small animal irradiator integrated with micro-CT imaging. C57BL/6 adult male mice (n=76) were divided into six groups: a control group (0 Gy) and groups irradiated and treated with a single fraction irradiation of 4, 8, 12, 16 or 20 Gy using 5-mm circular parallel-opposed fields targeting the upper right lung and no irradiation. A Monte Carlo model of the small animal irradiator was used for dose calculations. Following irradiation, all mice were imaged at regular intervals over 39 weeks (10 time points total). Non-rigid deformation was used to register the initial micro-CT scan to all subsequent scans. Significant differences could be observed between the three highest (>10 Gy) and three lowest irradiation (<10 Gy) dose levels. A mean difference of 120 ± 10 HU between the 0 and 20 Gy groups was observed at week 39. RILF was found to be spatially limited to the irradiated portion of the lung. Our data suggest that the severity of RILF in partial lung irradiation compared to large field irradiation in mice for the same dose is reduced and therefore higher doses can be tolerated.

INTRODUCTION

Lung cancer remains the most common type of cancer worldwide and strategies are needed to improve local tumor control¹. The dose-limiting toxicity of radiotherapy with or without drugs is acute-stage radiation induced lung pneumonitis or late-stage radiation induced lung fibrosis (RILF). Advances in radiation dose delivery such as stereotactic body radiation therapy (SBRT) and intensity modulation radiation therapy have been shown to minimize the rate of treatment complications through radiation avoidance to normal lung tissue²⁻⁴. However, despite recognition of radiation induced lung injury (RILI) as early as the use of radiotherapy itself, treatment strategies to alleviate lung toxicity have hitherto been largely ineffective⁵.

Preclinical small animal models of RILI are a means to evaluate new treatment strategies that would be unethical to investigate on patients and allow for studies with large cohorts, transgenic animals, controlled experimental conditions and accelerated results due to the shorter lifespans. The use of computed tomography (CT) imaging to non-invasively monitor changes in lung morphology - principally changes in lung density - due to RILI has been successfully applied in both clinical⁶⁻⁹ and preclinical studies¹⁰⁻¹⁵. However, the majority of these previous preclinical studies were performed using large radiation fields often covering the whole thorax, and including relatively coarse clinical resolution CT imaging. The clinical utility of the results of these studies may be limited due to the radiation dose distributions being highly dissimilar to current radiotherapy practice.

Dedicated precision image-guided small animal irradiation (IR) devices are now available that better mimic clinical radiotherapy making them potentially better suited to study and quantify RILI¹⁶⁻¹⁸. The purpose of this study is to quantify local changes in lung density after image-guided precision lung irradiation in a large cohort of mice using micro-CT imaging up to 39 weeks post-irradiation (post-IR). Two aims of the study are to develop methods to precisely irradiate and analyze a large cohort of specimens using a dedicated small animal precision irradiator and to monitor the progression of RILF by high-resolution micro-CT imaging in fibrosis-prone C57BL/6 mice.

METHODS

Selection of subjects

C57BL/6 adult male mice (n= 76) at 12 weeks of age entered the study and are known to be fibrosis prone^{19,20}. Animal experiments were in accordance with local institutional guidelines for animal welfare and approved by the Animal Ethical Committee of the University (number 2012-006). Animals were divided into six groups, corresponding to six radiation dose levels (see further), with each group containing at least 11 animals.

Before and subsequent to irradiation, all mice were imaged using micro-CT at regular intervals for a total of 39 weeks (i.e. ten imaging time points) as illustrated in the timeline in figure 1. Animals were anesthetized using isoflurane (induction 4%, maintenance 1.5 – 2%) and permitted to breath freely during the imaging and irradiation procedure. Animals were monitored daily for signs of compromised health and were weighted at each imaging session.

Figure 1. The study design and illustration of the deformation process showing the propagation of the lung and dose contours from the initial CT image to all subsequent CT images.

Small animal micro-IR

The small animal micro-IR (X-RAD 225Cx, Precision X-ray Inc., North Branford, CT) with an integrated micro-CT has been previously described^{16,18,21}. A high-resolution 80 kVp, 2.5 mA imaging protocol filtered by 2 mm Al with an acquisition rate of 5 frames/s and a gantry rotation of 1 revolution/min was used for all imaging. Image projections were acquired and reconstructed using the Feldkamp filtered backprojection (Pilot v 1.1.18, Precision X-ray Inc.), resulting in a final reconstructed isotropic pixel spacing of 102 μ m and a standard deviation of 20 HU within a sufficiently large region-of-interest (100 voxels) in a uniform medium such as water. The total micro-CT imaging skin dose for this procedure was 39 cGy, which should have no adverse effects for the frequency and duration of the study²².

Parallel-opposed beams 5-mm in diameter were used to irradiate the upper right lung of the mice in an area of minimal displacement of the lung during breathing. Animals were irradiated with parallel-opposed x-ray beams (225 kVp, 0.3 mm Cu added filtration) at 12 mA with radiation doses of 4, 8, 12, 16, and 20 Gy, equally divided over the two beams for irradiation times of 88, 176, 264, 352 and 440 seconds. The dose prescription was defined to a point (i.e. target voxel) within the upper right lung following completion of the CT image. During treatment this location is also the mechanical isocentre of the beams. A control group received 0 Gy. To spare the heart and spine from unwanted irradiation, the beam angle with the best avoidance was chosen. To

extract CT data from a lung sub region in the control group that received no radiation to compare to groups that did receive irradiation, beam angles were chosen and dose calculations performed as if the group had received radiation.

Dose calculations

Dose calculations were run DOSXYZnrc 23 (rev. 1.47) with a validated MC source model in voxelized geometries based on the CT image data 21, containing an intrinsic experimental dose uncertainty of 4% or 0.8 Gy for the 20 Gy dose prescription. For MC dose calculations both the material (i.e. cross-section data) and density are required and are assigned on a voxel-by-voxel basis according to the CT number 16. The conversion of CT number to material type and density was performed based on a 4-material CT number threshold scheme and a density calibration curve using materials of known composition and density 24. The 4-material classes were air, lung, soft tissue, and cortical bone (ICRU report 44 25). Tissues were segmented using software that permits the user to visualize materials overlaid by CT data and adjust CT thresholds accordingly based on a sliding window visualization 26. Ten micro-CT image volumes from different mice were segmented and three threshold values were averaged. Dose calculations were run with 5×10^8 photons and energy cutoffs for the photon (PCUT) and electron (ECUT) transport of 10 and 100 keV, respectively and reached a statistical uncertainty less than 1% in the target region. Regions bounded by the 80% isodose to max lung dose, and 2% isodose and below, represented the irradiated and out-of-field regions within the irradiated lung.

Figure 2. The segmentation of the CT and dose data into regions of interest. The top row illustrates the segmentation of the axial CT slice into appropriate tissues components, and the isolation of the lungs. The middle row shows the dose deposition, the segmentation of the dose region (80% to max) and the intersection of the >80% dose region with the lung region (ROI. 1a). ROI. 1b represents the non-irradiated region of the right lung with bounds defined at < 2 % of the max lung dose. ROI. 2 is the segmented non-irradiated left lung. The bottom row shows the CT data of the regions of interest.

Deformable registration

The lung geometry of a mouse varies at different time points for several reasons. Deformable registration was required in this study to track the location of the irradiated portion of lung in the micro-CT images acquired at different imaging time points. For each mouse, 3-D contours (i.e. masks) of the left and right lung were generated using the initial micro-CT image volume in a semi-automatic manner and visually checked. Using the material segmented micro-CT images, mouse lungs were selected and isolated from their surroundings by region growing lung voxels. The isolated lungs were then segmented into the right and left lung by a plane passing through the spine

and the sternum. To reduce the sensitivity of the lung boundaries influencing the mean CT numbers of the lung, each lung contour surface was eroded by 2 voxels (i.e. 204 μm).

Figure 3. Sequential CT axial images per week of a mouse with visible changes in lung density (A) consistent with radiation induced lung fibrosis appearing in the last weeks of the study after a dose of 20 Gy (indicated by arrow), and (B) an non-irradiated mouse image series with no visible changes in lung density (B).

Deformable registration was performed on all micro-CT images using in-house developed software that allows for batch runs through command scripting²⁷. The initial micro-CT image volume was registered (i.e. non-rigidly deformed) to every subsequent micro-CT image so that only contours were propagated from the initial micro-CT image to all subsequent CT images. Downsampling by a factor of three (i.e. reducing the isotropic pixel spacing from 102 μm to 306 μm) was performed on both image volumes to achieve a smoother and continuous deformation field. Rigid followed by deformable registration were performed based on parameters outlined in the next section. The deformation fields were then re-sampled to the original isotropic spacing and deformations applied to the lung and dose contours. An illustration of the application of the deformed contours is given in figure 1.

Deformable registration volume-correspondence

We tested the two frequently used deformation algorithms of CT images, Demons and Morphons²⁷. In six mice, deformed lung contours of the last imaging time point, three with apparent changes in lung morphology and three without any major changes in lung morphology were compared against lung contours delineated by an individual experienced in mouse anatomy. Two parallel runs of eight (16 combinations total) deformable non-rigid registration parameters were evaluated (supplemental table 1.) against the oncologist lung contours using a Dice overlap ratio (DR) defined as:

$$DR = \left(\frac{2 \times V_{MAN \cap DSA}}{V_{MAN} + V_{DSA}} \right)$$

where V is the volume defined by the delineated lung and the subscripts MAN, and DSA indicate the lung contours determined manually and the deformed semi-automated lung contours. The deformation parameters resulting in the best DR were used on all the acquired images for CT density analysis for all mice.

Image analysis

Once the lung and dose contours had been propagated to all subsequent micro-CT scans using deformable registration, four regions-of-interest (ROI) were established, defined as; the right and left lung (ROI.1, 2), the irradiated region of the right lung (ROI.1a), and the non-irradiated portion of the right lung (ROI.1b). An illustration of the segmentation of the CT and dose data to ROI can be seen in figure 2. The mean CT number and the standard deviation inside each of these regions were calculated for each mouse for all ten CT imaging time points. ROI.1b and ROI.2 were used as controls of changes in ROI.1a. The differences in CT number between the regions (1a vs. 1b; 1a vs. 2) within each CT image in time (normalized to the initial CT scan) was calculated for each mouse and averaged per dose group. All lung and dose volume non-rigid deformations were inspected visually; if either resulted in obviously incorrect results those measurements were excluded from the analysis.

Figure 4. Dose volume histograms in the total lungs (left and right lung) of the different mouse dose groups 4, 8, 12, 16, and 20 Gy with the dashed lines representing the standard deviation within each group demonstrating the variation in irradiated mice lung volume when using the same size irradiation 5-mm field.

RESULTS

Animals appeared healthy for the duration of the study; their body weights steadily increased over the 39-week study endpoint (see appendix Figure A). The whole cohort of mice was imaged and irradiated within 17 hours spread over two days. In the subsequent micro-CT imaging follow-up, we achieved a throughput of 6 minutes per animal per imaging session. In a subset analysis of six mice, animal breathing remained principally in a state of maximum exhalation with the diaphragm relaxed with approximately 13% of the respiratory cycle in a state of inhalation and motion. Increased lung density, consistent with the development of fibrotic tissue, could be visually observed in CT images in the late stage imaging time points for mice irradiated with higher dose levels confirming that RILF could be induced in partial lung irradiation as compared to a mouse that received no irradiation (figure 3A, 3B).

MC dose calculations resulted for the different dose groups in an average and standard deviation dose of 3.8 ± 0.1 , 7.6 ± 0.1 , 11.3 ± 0.1 , 15.0 ± 0.1 , and 19.0 ± 0.2 Gy in the 80% to max irradiated lung region; for simplicity we will refer to these irradiation doses as nominal 4, 8, 12, 16, and 20 Gy. The CT thresholds segmenting air, lung, soft tissue and cortical bone between three researchers familiar with animal pathology were found to be -655 ± 6 , -102 ± 17 , and 383 ± 15 HU, indicating they were very similar for all animals. Dose volume histograms (DVH) of the whole lung for the different irradiation groups can be seen in figure 4. The prescription dose of 4-20 Gy was reached in approximately 10 ± 3.5 % of the total lung volume of mice. As can be seen from the DVHs, the parallel-opposed fields provided for a well-defined shoulder (fall-off) and the beams were highly conformal to a localized lung region as is reflected in figure 2.

The validation and performance of the non-rigid registration between oncologist delineated lung contours compared to segmented and deformed lung contours of six mice resulted in an average DR of 0.83 ± 0.02 for the average of both lungs for all mice. The performance of the deformations was not influenced in those mice with visible signs of fibrosis. The best performing deformation algorithm was the Demons algorithm; for the specific deformation parameters see appendix table 1. The total calculation time for the deformable registration was about 80 minutes per animal (i.e 9 deformations in total).

Figure 5. Change in CT number between the irradiated and non-irradiated portion of the right lung (ROI.1a, ROI.1b.) compared to the non-irradiated left lung (ROI. 2) at the beginning and at the end of the 39-week study for the 12, 20 Gy, and zero dose level groups.

Visual inspection of all deformations led to the discovery of occasionally unsuccessful deformations resulting in contours that were outside of the lung. Typically this occurred when there were significant changes in the physiology of the irradiated right lung as seen in the CT images (e.g. pleural infusions²⁸), but these events were usually transient and not apparent in the subsequent CT scan. In total, 3% of the CT data from the irradiated region (ROI.1a) was excluded from analysis due to obvious errors in the deformation.

The change in CT number over time between the irradiated right lung region (ROI.1a in figure 2) and the left lung (ROI.2 in figure 2) resulted in significant differences between the high dose group compared to the group that received no irradiation between the initial CT scan and the last scan. However, it was found that for 25% of the left lung (ROI.2) significant morphological changes (e.g. compressed, collapsed, or fluid filled) did not permit an adequate comparison and therefore reduced the statistical power of the groups. Instead, the non-irradiated region (ROI.1b) of the irradiated lung was found to be a good surrogate. Figure 5 shows CT number differences between the two regions (ROI.1a vs. 1b) of the irradiated right lung compared to the remaining valid data of the left lung for dose groups 12 Gy, 20 Gy and no irradiation. Principal changes in lung density were contained to the irradiated region, therefore ROI.1b could be used as a control. The change in CT number between ROI.1a and ROI.1b is illustrated in figure 6 for all dose groups and time points. At the 39th week end point there was a difference of 120 ± 20 HU between the 20 Gy and 0 Gy groups, which was significant ($p < 0.001$). From 7.5 weeks post-irradiation there is a clear incremental change in CT number for the 20 Gy dose group.

Figure 6. Change in CT number over time for the six levels of irradiation between the right irradiated lung region (ROI.1a) and the region of the right lung that was primarily outside the field (ROI.1b).

DISCUSSION

This study demonstrates the feasibility of large animal cohort radiation studies that are more representative of clinical irradiation schemes. Following the 7th week post irradiation, C57/BL6 male mice irradiated with 5-mm parallel opposed beams of prescription doses greater than 10 Gy exhibited a steady increase in lung density most pronounced for the 20 Gy group having a maximum difference of 120 HU at the 39th week end point as compared to the zero dose group. We have shown that partial lung irradiation can induce symptoms characteristic of RILF that are discernable as early as 10 weeks post irradiation when compared to the non-irradiated group. While the manifestation of RILF may only be evident visually in CT images at late stages, analysis of CT data can show early on (10 weeks post-IR) the development RILF.

We have also demonstrated the use of deformable registration for longitudinal animal studies to accurately deform mice lung contours from an initial CT scan to subsequent follow-up CT scans, without which the study would be susceptible to operator specific lung contour delineation. The use of deformable registration also significantly speeds up the data analysis and allows for processing large datasets in reasonable times. We

found no significant difference with the experienced person familiar with animal anatomy contouring in the performance of the deformation for the three mice with visible signs of RILF compared to mice without any sign of RILF.

The data shown in figure 6 demonstrates the progression of the disease for partial lung irradiation that has not been seen previously at such a temporal resolution. Interestingly, an increase of CT number present in all the high dose groups (12, 16, 20 Gy) was found at the second time point 3.5 weeks post-IR potentially attributed to radiation-induced lung pneumonitis. Despite significant differences between the low and high dose group, a clear differentiation for all other irradiations in between 0 and 20 Gy is absent for a single time point but evident when considering all the time points greater than 10 weeks (so as to avoid potential confusion with any pneumonitis event) as seen in the dose response curve of the summed CT difference (see appendix Figure B).

When we evaluate the prescription dose of our study to other similar studies the onset of lung density changes appears to happen later, and the severity of those changes appears to be lower in our study. In Plathow *et al.* CT density changes of greater than 200 HU are observed 21 weeks post-irradiation, while in our study we see only differences of approximately 60 HU for the same prescription and observation time point; neither do we observe any deaths as in whole thorax irradiation studies of the same prescription dose¹³. Travis *et al.* also saw significant morbidity for similar levels of irradiation between 14 and 20 Gy in larger-volume partial lung irradiations of mice²⁹. The lower severity of the partial volume irradiation in this study (~15% total lung volume) is not surprising given that previous histopathological analysis of RILF studies have previously shown that whole body mouse irradiations of 12-15 Gy resulted in the presence of fibrosis in week 20, while thorax-limited irradiations of the same dose prolonged the onset of fibrosis to week 24³⁰. The ability to target precisely and increase the prescription dose to murine lungs is an attractive feature of these micro-IR devices that offers opportunities to better understand radiation-induced lung toxicity in SBRT³¹.

We found surprisingly that the use of the left lung as a control for the change in CT number of the irradiated region was unreliable in a significant portion of mice (~25%), which is likely due to the free positioning of the animals on their abdomen combined with location of the heart. Fortunately, RILF was limited to the irradiated region of the lung alone, allowing the use of the right irradiated lung region outside of the field to be used as a control, as shown demonstrated in patients^{32,33}. However, future studies may wish to investigate the use of conformal beds or forced breath-hold techniques to reduce animal set-up variability³⁴, though the added benefit of these techniques will have to be weighted against cost and time constraints.

At present, we are faced with a wealth of new compounds that may be combined with radiotherapy; nearly none of the novel drugs have been investigated in animals with regard to normal tissue damage. Indeed, results from preclinical animal experiments cannot be translated directly to humans, but at least an indication of the side effects as well as the genetic or mechanistic basis for the interaction may arise. In that context,

in our view, normal tissue work may be more readily extrapolated from mice to humans than from xenografts to patients where the predictive value of tumor work has been increasingly challenged. The new high-precision animal irradiation platforms may thus allow testing several compounds and combinations of medication and radiation in normal tissue models in mice with different genetic backgrounds and with specific diseases. This may allow clinicians to assess the best strategy to widen the therapeutic window when designing clinical studies. Our study employed a radiation field size that is proportionally more realistic to clinical experience than previously published semi- or full-thorax irradiations, which has been used frequently in mice. We hope that future pre-clinical studies may benefit from this study given that there is a growing community of users, in particular for normal tissue toxicity studies or for more realistic models of disease like new orthotopic tumor models for e.g. non-small cell lung cancer.

Figure 7. Dose response curve for the total integral change in CT number for all imaging times between the irradiated right lung region (Reg.1a) and the region of the right lung that was primarily outside the field (Reg.1b) vs. the prescribed dose.

APPENDIX

Case number	Deformation parameters	DICE ratio	Ave. calc. time per image vol. (s)
1a	(D,d,5, 1.5,[10 8 6 4 2])	0.81	250
1b	(D,d,5, 1.1,[10 8 6 4 2])	0.83	270
2a	(D,ws,5, 1.5,[10 8 6 4 2])	0.76	210
2b	(D,ws,5, 1.1,[10 8 6 4 2])	0.79	230
3a	(M,d,5, 1.5,[10 8 6 4 2])	0.78	880
3b	(D,d,5, 1.25,[10 8 6 4 2])	0.82	270
4a	(M,ws,5, 1.5,[10 8 6 4 2])	0.76	830
4b	(D,ws,5, 1.25,[10 8 6 4 2])	0.78	230
5a	(M,d,5, 1.5,[10 8 6 4 2])	0.80	1350
5b	(D,d,5, 1.25,[10 10 10 10 10])	0.83	300
6a	(M,ws,5, 1.5,[10 8 6 4 2])	0.77	1300
6b	(D,ws,5, 1.25,[10 10 10 10 10])	0.79	250
7a	(M,d,3, 1.5,[10 6 2])	0.69	1300
7b	(D,d,3, 1.25,[10 10 10])	0.82	290
8a	(M,ws,3, 1.5,[10 6 2])	0.66	1240
8b	(D,ws,3, 1.25, [10 10 10])	0.77	240

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CHAPTER

8

General discussion and future perspectives

Patrick V. Granton

Emergence of precision small animal radiotherapy

The emergence of dedicated precision image-guided small animal irradiators represents an important advance for pre-clinical radiation research. These devices will permit targeted irradiation studies to be performed in controlled laboratory environments and help to avoid the often-inappropriate use of linear accelerators to conduct small animal irradiations studies. The use of these image-guided micro-IRs represent a leap forward in targeting accuracy, and the acquisition of image data will better allow comparison of the 3-D dose data between studies. In downscaling the energies used for small animal radiotherapy, treatment plans akin to their clinical counterparts can be created, which better facilitates a promising research finding to be more easily translated into clinical trials and ultimately reach the average patient.

When Frank, Erik, and I wrote the first review on small animal radiotherapy platforms three years earlier we had calculated that there were 11 dedicated precision image-guided small animal devices in use throughout research institutes; today, we estimate that number to have increased to approximately 40 devices. The rapid growth of this particular application of radiation research highlights the desire to conduct translational medicine and its use has been further promoted in a recent editorial in the *International journal of radiation oncology biology physics* ¹. Given that this type of radiation research is a relatively new field of expertise, improved collaboration between institutes who have already purchased or developed in-house one of these small animal precision irradiators is something that should be actively encouraged and maintained.

To some extent this is developing, our research department established and hosted the first ever conference on precision small animal irradiation in March 2013 that was well received and attended by more than 100 attendees (see figure 1). A second conference has already been announced in Vancouver for August 2014, again organized by our department. These efforts exemplify our pioneering role in the field. It is hoped that from these conferences a research community can be formed between users and an exchange of information can better occur between institutes. A user's group could assist in identifying relevant papers to the community as many related publications are currently spread over a wide range of journals. In addition, electronic files such as treatment planning files or dose calculation input files could be shared to accelerate study development, ensure quality control, or enhance collaboration between institutions.

Contributions to precision small animal radiotherapy

This thesis outlines research efforts that will contribute knowledge to the further understanding and development of small animal precision irradiation work based on publications and continual developments at Maastricht clinic. In chapter 2, the requirements for small animal precision image-guided irradiators to replicate modern clinical radiotherapy in small animals were outlined; some of the stated capabilities in the

commercially developed micro-IRs have been achieved such as the dose rate, and smallest beam size, but other areas have not, including the required imaging resolution, frame rate, and targeting for the laboratory mouse, which future developments should strive to achieve.

Figure 1. *The participants of the first conference on precision image-guided small animal irradiation.*

In chapter 3, a multi-institutional study was performed between three independent institutions that had the identical model of the small animal irradiator. This investigation involved a thorough investigation of the commissioning of these devices such that a dose could be described to an animal within 5% uncertainty. The study was challenging because each institution was inaccessible for regular visits and access for the same instruments varied across institutes. Nevertheless, two years later, a lot was learned, and our report was published, which presented recommendations for future commissioning of micro-IRs. What was clearly identified from within that study was – not surprising – that each micro-IR requires individual commissioning. Between our three institutes and for the same model irradiator and x-ray tube we characterized significantly different dose rates and beam profiles, resulting from small manufacturing differences and installation differences between machines. We were also able to quantify the out-of-field dose and identify influence of scatter for conditions deviating from full scatter conditions.

In chapter 4, a Monte Carlo model of the micro-IR was developed to allow forward 3-D dose calculations of animal irradiations. The ultimate goal was to enable treatment verification using the on-board imaging detector by modeling the whole irradiation process from the x-ray to the imaging detector. Essentially, this work consisted of three great efforts; the first challenge was to model the output of the x-ray tube; the second challenge, involved correctly implementing the appropriate radiation transport physics in the animal; while the third challenge was to characterize the flat-panel imaging detector. Each effort revealed new insights. The model of the x-ray tube demonstrated the importance of accurately modeling the electron target and focal spot, highlighting the sensitivity of the collimator alignment with the source to achieve flat and symmetric beam profiles. The model of the flat-panel imager revealed that

there is a non-negligible component of scattered irradiation from within the imagers, which contributes signal to the final acquired portal image. All things considered, we were able to demonstrate a proof-of-principle to use the onboard imager to be able to compare a planned irradiation plan against the experimentally measured irradiation plan. For the smallest 1-2 mm field sizes however, the model required fine-tuning the electron target using experimental data.

This unsatisfactory result of failing to achieve ideal agreement of the smallest collimators meant the introduction of correction factors based on measurements. Chapter 5, further explored the causes of the disagreement with the micro-IR model by attempting to more rapidly generate the irradiation field at the exit of the collimators using an analytic model, as this step in MC calculations is significantly computationally intensive. The use of the focal spot image was incorporated into the model to improve the size, shape, and intensity distribution of the focal spot of this specific micro-IR we were modeling. The incorporation of the actual focal spot intensity distribution improved the agreement between our model and experimental measurements; however, it was also found that the output of the smallest beams is extremely sensitive to the correct alignment of the collimator with the focal spot source. Moreover, even with perfect alignment of the collimator and the source for the same model x-ray tube one can achieve wildly different fluence outputs as seen in figure 2. The result of this work has identified improvements in collimator design to reduce the sensitivity of the collimators to alignment, which have been implemented.

One interesting application to eliminate these correction factors for the smallest fields could be to include the work performed in chapter 4, and use the imaging panel to generate a reconstructed fluence at the exit of the collimators prior to treatment. In clinical radiotherapy, we refer to this type of improvement in the dose calculations as Dose Guided Radiotherapy (DGRT) and specifically the imaging of the treatment plan prior to treatment as “pre-treatment” verification. This type of application could improve day-to-day variations in the field output and shape due to collimator misalignment. It could also in principal ensure an accurate assignment of materials within the animal by also generating full field simulated images around the animal, use standard CT reconstruction techniques and compare the resulting CT image with the measurement CT image. Currently, this process would be a time consuming but given improvements in hardware capabilities not out of reach.

Based on the three earlier chapters a significant amount of the work conducted within this thesis has focused on improved understanding and modeling of the smallest collimated fields. Small field dosimetry has been a well-discussed challenge in clinical routine where smaller and smaller fields sizes are increasingly being used in stereotactic radiotherapy². The two principal challenges in the applications of small field radiotherapy are to accurately predict the dose delivered as previously described, and to ensure that the beam remains on target. The latter challenge requires that all collimator motion, and machine flex are well described so that fine table correction can be made to keep the beam on target and is further described in the following section.

Figure 2. From left to right, 10 source intensities (i.e. focal spot images), and the resulting beam fluences for 1-mm collimated beams with their respective 2-D beam profiles.

Evidently, a major work that has resulted through the efforts undertaken throughout this thesis has been the development of our dedicated *Small Animal Treatment Planning* software SmART-Plan. The manufacturer of the micro-IR PXI located in Branford, Connecticut, has supported this software development. The company has also recognized our efforts in the development of the treatment planning software that we have been designated a centre of excellence for their product. With the help of PXI we have been able to commercialize the use of SmART-Plan. As of February 13, 2014, there have been five commercial purchases of SmART-Plan. Further development of SmART-Plan is planned for the foreseeable future.

Finally in chapter 7, we outline a normal tissue toxicity study involving radiation induced lung fibrosis in mice. This study could not have been accomplished without the precision targeting, high-resolution imaging and an accurate modeling of the micro-IR irradiator to perform dose calculations. The study characterizes the changes in lung density at several imaging time points using validated deformable registration for several prescription doses. In this normal tissue toxicity study, when compared to previous studies the severity and onset of lung density changes in our mice were

lessened and delayed, which we attribute to a volume effect of the partial lung irradiation as when compared to whole-lung or hemi-thorax irradiation. It is hoped that by well characterizing changes in the lung density in mice, normal tissue complication probabilities can be refined and explored when the use of a radioprotector is used.

Remaining challenges in precision small animal radiotherapy

Collimator flex

Despite the significant progress that has been achieved in precision small animal irradiation over my 4-year tenure as a Ph.D. candidate there are still a number of lingering challenges that ought to be resolved. To start with, the PXI 225 Cx still exhibits a significant amount of collimator play that is not adequately accounted for within the system. Targeted radiotherapy using the smallest 1-mm beam is susceptible to targeting errors on the order of the beam size itself. Recently, these errors were highlighted in end-to-end testing using SmART-Plan, as well as a paper published by Ritman et al that showed targeting errors of ± 2 mm, which were reduced after recalibration of the system to ± 0.8 mm^{3,4}.

The PXI 225Cx small animal irradiator attempts to minimize these deviations by applying real time stage corrections per gantry angle. The remaining observed targeting errors are the result of the fact that a single collimator motion is applied to the full range of collimators. This collimator motion is derived from a Winston-Lutz test using a 1-cm ball bearing (BB) and a larger size collimator to derive motion corrections. This test can be applied for smaller collimators but due to the broad penumbra for the fields < 2.5 mm and the finite size of the pixels at the detector this method would fail for small fields. In addition, the stage corrections are loaded on to the memory of the stage controller, which is limited in memory to store only a few collimator-specific Winston-Lutz stage corrections. We are told that addressing these remaining targeting errors are a priority of the commercial PXI distribution through improved collimator design and stability, as well as through additional software.

Tissue identification and the use of dual-energy computed tomography

One unique aspect of conformal small animal irradiation compared to clinical radiation therapy is the photon energies employed. Typically, mice and rats are irradiated with x-ray beams generated at tube potentials of 225 kVp, heavily filtered by copper, producing treatment beams with mean energies around 80 keV, whereas clinical energies are in the megavoltage range. The use of kV energies compared to clinical MV provides greater conformity of the target but requires accurate knowledge of the tissues to perform accurate dose calculations. Because the photoelectric effect plays a much more significant role at energies less than 80 keV, dose is deposited not only based on electron density but strongly influenced by the effective atomic number. The sensitivity of dose calculations to tissue compositions in small animal radiotherapy has

been recently investigated^{5,6}. However, the major limitation of all these studies is the presumption that animal tissues are like human tissues. Information on the atomic compositions of human tissues is scarce but available, those for animal tissues are very poorly known. This is partly because traditionally biologists are not interested in the effective atomic number of animal tissues but rather in the relative abundance of proteins within tissues; moreover, methods to determine the effective atomic number of tissues such as mass spectrometry are not widely available in radiotherapy research and mass spectrometry for high atomic elements and on wet samples is particularly challenging and requires specific expertise and equipment.

Figure 3. Mini phantom (3.5 cm in diameter) machined and developed at Maastric clinic containing materials with known material composition for use to verify dual-energy computer tomography material decomposition algorithms.

In assessing what may be the dosimetric impact of assigning human tissue compositions to animal tissues, one can look at the intrinsic variability of human tissues and their constituent elements as an estimation of the inter-biological uncertainty, which is likely also present in animals as the minimal dosimetric uncertainty in tissue assignment. For example, commonly, a 4-component model is used for soft tissue composing of H, C, N, O, with mass fractions 0.10, 0.11, 0.03, and 0.76, respectively that results in an effective atomic number of 7.32, where effective atomic is calculated with an exponent of 3.3. When trace elements commonly found in tissues are included, such as Na, P, S, Cl, and K in mass fraction quantities 0.001, 0.002, 0.001, and 0.001, respectively, the effective atomic number raises to 7.47. As a first order approximation, and without invoking Monte Carlo methods, if we consider the photoelectric effect as the dominant contribution to Dose, the cross-section of the photoelectric effect is proportional to $Z^{3.3}$, and substituting the two effective atomic numbers results in a ratio of

0.935 or in otherwords, could lead to a dose difference of around 7%. However, if tissues are missassigned incorrectly, for instance between water and adipose, for a 225 kVp, the spectral-averaged Uen ratio difference is even larger at around 22%. Making tissue assignment a crucial step in treatment planning.

One promising technique that would be available more broadly to research institutes is the use of dual-energy computed tomography (DECT) ⁷. DECT is essentially a CT device that can acquire two CT image volumes at different energies that are ideally registered so that two image volumes can be decomposed from effective mass attenuation (i.e. CT number) into effective atomic number and electron density, thereby providing the required information to initiate accurate dose calculations. Currently, there is ongoing dual-energy work at our lab attempting trying to address two questions; one, how similar are animal tissues to human tissue, and two, what is the influence of accurate knowledge of animal tissues on dose calculations. Figure 3 shows a mini phantom machined in our lab to contain materials with accurately known material composition that we can assess and optimize different DECT decomposition algorithms.

Dose-to-water vs. Dose-to-medium

Historically, dose has been primarily measured in water or using water-equivalent phantoms and thereby most clinical dose-effect observations with radiation has been reported as dose-to-water, even if not explicitly stated. The distinction between reporting dose-to-water versus dose-to-medium in soft tissues for traditional high energy external beam radiotherapy energies is relatively small (1- 2%) ⁸. Even though these difference may be up to 15 % different in cortical bone ⁹, tumors are more water-like than bone-like such that reporting either quantity has not been terribly an issue for radiotherapy. Traditionally, radiotherapy departments have not even had the capability to report dose-to-medium as most treatment planning systems were hither-to commissioned as dose-to-water (though in recent years clinical TPS can now report both radiation quantities).

Table 1. Potential errors in converting dose-to-medium to dose-to-water in medium for two difference spectra, illustrating the need to perform Dwm calculation during the dose calculation.

Location	225 kVp beam Mean energy (keV)	Uen (spectral weighted) tissue ratio		
		Muscle/Water	Adipose/Water	Cortical bone/water
Surface	83.6	1.017	0.820	4.005
4-cm depth	86.0	1.016	0.833	3.818
Percent difference (%)		0.1	1.5	4.8

However, for small animal radiotherapy applications the distinction between dose-to-water versus dose-to-medium is much greater even for soft tissues because of the lower photon energies used. There are several concerns when considering the implication of large differences between dose calculations in water and those in medium. The first is the ability to convert from one dose calculation medium to another. Since generally not all model based dose calculation engines offer the choice to report dose-to-water in medium, this conversion must be applied post dose-calculation and requires knowledge of the photon spectrum. Preliminary studies have revealed that these assumptions can be up to 5 % in adipose tissue, meaning that truly accurate interpretations of dose-to-water in medium will require calculations on the fly (Table 1). Secondly, the dose difference between dose-to-medium and dose-to-water in some cases such as bone can be ~2.5 times greater, such that previous dose constraints for organs-at-risk can not be achieved. These differences are even greater for irradiation studies using energies less than 225 kVp. The issue of adequate dose constraints for organs-at-risk (OAR) at energies used for small animal radiotherapy is even more complicated by a lack of available data that is both comprehensive and systematic. There has been no similar systematic review like the Quantec study for animals and efforts to report even on a single organ require a great deal of effort¹⁰. Discussion to make these efforts through crowd sourcing online may greatly speed up these efforts.

General discussion on small animal irradiators and research

Multi-leaf collimators/Jaws and wedges Vs. dose painting

One observation from the efforts of small animal treatment planning software SmART-Plan (Chapter 6) has accentuated the limitations of static fields. Although, several size collimators can be used to cover a target and avoid organs-at-risk, those field sizes remain static. The consequence of these static fields means that typical constraints or prescription objectives like the D90 (the dose to 90% of the delineated target volume) have to be relaxed. However, as more and more orthotopic animal models become routine in laboratories improved conformity of the radiation fields will be necessary. Obviously, efforts to develop a multi-leaf collimator for use in pre-clinical micro-IR represents a significant amount of engineering work that needs adequate resources to be allocated and justified.

To my knowledge there have been four different approaches to develop dynamic fields. One group at Madison University has attempted to design and build a micro multi-leaf collimator¹¹; Oxford University has built dynamic jaws to fit with the small animal research radiation platform (SARRP)¹²; Two other groups, one at Stanford university, and at Western University in London Canada both modified a micro-CT device, the former using an iris and the latter using two cylinders with sliding slit window^{13,14}. Other institutions are attempting a different “dose painting” approach; rather than change the field shape, using a single field and scanning the treatment area (i.e. “painting dose”) by keeping the collimator positions fixed while moving the

stage – an approach that would not work for clinical patients but could for small animals¹⁵. Either option between a dynamic field or a dynamic stage appear equally feasible, however, the merits of one approach over the other is the subject of investigation.

Optical imaging

Molecular imaging is a new and promising field of non-invasive 2-D imaging that can visualize, characterize, and quantify biological processes at the cellular and molecular level without perturbing the biological system. Optical imaging is a molecular imaging modality that has previously been extensively used in microscopy. Recently, optical imaging has advanced to where noncontact imaging can be used to record weak light sources originating within tissues up to several centimeters deep^{16,17}.

Small animal *in vivo* optical imaging detection in light-tight boxes may occur either following an enzymatic reaction with the luciferase enzyme (i.e. the firefly), known as bioluminescent imaging (BLI), or following the fluorescent excitation from an external light beam source, known as fluorescent imaging. In fluorescent imaging, an externally applied light source (typically a solid state laser) is used to excite fluorescent proteins that are almost immediately re-emitted as a longer-wavelength, lower energy photon. BLI requires that the luciferase enzyme to react with a substrate (e.g. luciferin) under the presence with oxygen and adenosine triphosphate (ATP). One advantage in BLI is there is no requirement for an external light source. Since BLI relies solely on the endogenous production of light there is virtual no background signal due to autofluorescence, which means that BLI has a high signal-to-noise ratio and is sensitive enough to detect biological process which produce minimal signal *in vivo*. It also means that you can simultaneously acquire whole-animal BLI rather than an external exited small region of interest. Typical spatial resolutions and acquisition times of BLI have been suggested to be similar to micro-PET at around 1-2 mm¹⁸, though are likely to be larger when considering deep-seeded tumors like in the brain or lung. BLI does have favorable acquisition times, though between 1-5 minutes acquisition¹⁹⁻²¹.

Figure 4. Shows the first bioluminescent image acquired at our lab of luciferase expressing cells in concentrations from left to right 160, 80, 40, 20, and 10 uL in the top row with equal injections of luciferin. The bottom row contains equal 200 uL concentrations of luciferase expressing cells and equal concentration of luciferin.

There have been recent efforts to convert 2-D planar optical images of whole animal specimens to 3-D image volumes²². Nonetheless, 2-D optical imaging is increasingly becoming an attractive technology for molecular imaging in pre-clinical small animal models. It is safe, fast, cost-effective, and many optical biomarkers have already been developed^{18,19}. Recently, the two commercial micro-IR products 225Cx (PXI, CT) and the SARRP (Xstrahl, Camberley, UK) have incorporated optical imaging into their own respective platforms (the PXI unit mounting the camera on the gantry while the Xstrahl unit has a separate removable stage holder for external BLI) and some initial studies have been performed and published^{23,24}. Maastric Clinic have recently acquired one of these optical BLI cameras. Figure 4 shows preliminary results from imaging luciferase-expressing cells and the signal linearity to increasing amounts of cell in difference wells.

There are many attractive features of combined BLI/micro-CT and irradiations and a host of research studies that can be performed to reveal new insights into potential new applications of BLI. I believe that BLI will be a supplementary imaging modality to PET imaging, and therefore eliminating the need for radioactive isotopes should drastically increase the number of studies that can more easily be performed.

The limited use of animal studies

Even given the most ideal capabilities and well-controlled environment to treat small animals with radiation indistinguishably from clinical radiation therapy, study outcomes may not be clinically translatable. It's an inescapable fact that small animals are not humans and so it should be no surprise that the regulation of the basic pathophysiology of small animals may not be replicated in humans.

A recent publication by Seok *et al.* that demonstrates just how different some animal models of disease are from the human counterpart in a simple study involving the inflammatory response and gene changes following burns, trauma and endotoxin²⁵. In that study, they “observed highly consistent genomic response in patients between trauma and burns in contrast to the lack of correlations in the murine models”. This challenges just how translational molecular results from animal models may mimic human disease. Given the rising interest in immunotherapy and success for some cancers such as melanoma²⁶, an important question to address is to what similarity is the immune response between patients and small animals when exposed to radiation.

As earlier discussed in the introduction, orthotopic small animal models of disease are more clinically representative than xenograft tumor models, however even these models do not reflect clinical experience and often patient care is complicated by comorbidity that is to say that patients suffer from a multitude of diseases. For example; chronic obstructive pulmonary disease or diabetes to name a few of the most common. In other words animal models might be overly optimistic in predicting drug response in patients.

Future radiation research questions

There are a host of potential research questions that can and should be addressed with these highly sophisticated micro-IR devices. However, where does one start, and how does one prioritize which studies should be performed first and in what order? It’s a difficult question to address and will depend on the available resources on hand. In my opinion however, those research questions that can be directly translatable should be given the higher priority. Specifically, these questions should include radiobiological models of tumor control probability, and where possible conduct animal studies in parallel with clinical trials so that complimentary information can be gleaned. Improved modeling of normal tissue complication probability should also be explored with these research platforms given that preventing normal tissue toxicity is the limiting factor when treating patients.

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Summary

External beam radiotherapy is a heavily used treatment modality for therapeutic and palliative patients burdened by cancer. The clinical outcome of some cancers treated with radiotherapy is to date unsatisfactory, resulting in poor survival times, debilitating toxicities, or late secondary tumors. Preclinical radiotherapy represents one approach to try and identify promising new radiotherapy treatment regimes. In this thesis, a thorough examination of the dosimetric aspects of an image-guided small animal irradiator has been examined and a preclinical research study has been undertaken.

A general introduction into cancer and the need and use for preclinical research is outlined in **Chapter 1**. A literature review is followed in **Chapter 2**, in which early conformal animal radiation work is identified. In this review, the ideal operating parameters for small animal irradiation that would best replicated clinical radiotherapy are outlined including beam size, image quality, and targeting accuracy. Currently, the best small animal image guided irradiators do not meet the ideal resolution of 30 μm , beam diameter 0.5 mm, or a required targeting accuracy 0.1 mm, though they do closely approach these capabilities. This review also outlines the need to reduce the treatment energy from 6 MV to orthovoltage energies. The review also identifies all the existing centers that possess one of these image-guided small animal irradiators; four years ago these numbered 11, and now exceed 40 institutions and growing. Critically, this review article also acknowledges the lack of a treatment planning system for small animals, which became a major development within our research group.

In **Chapter 3**, together with two other institutions we explore the considerations when commissioning small animal irradiators. What we found was that for the same device, model x-ray tube, dose rates can vary ($\pm 10\%$) and that independent machine-specific commissioning is required. We also found that backscatter is a significant contribution to the dose at the target and strongly dependent on the beam size, and not on object size so long as the object is as big as the field. The out-of-field dose was found to be less than 1%. Differences in the manufacturing of the collimator dimensions were identified between institutions as well as system flex that requires routine calibration.

In **Chapter 4**, full Monte Carlo modeling of the x-ray tube, specimen and the flat-panel, of an image-guided small animal micro-IR is performed and compared to measurements. The results were very promising but did reveal the complexity to modeling the smallest collimated fields below 3mm, which are focal spot occluded. Significant drops in output ($\sim 30\%$ drop) were also observed for the smallest 1- and 2-mm beam. We were also to successfully model the sensitivity response of the imaging panel with good agreement to experimental measurements. We managed to quantify the contribution of scatter-glare of the within the panel. There was also very good agreement between imaging situation and the experimentally measured projection images, which we now recognize as a potential method to validate tissue assignment for small animals the compositions of which are not well known. We also found the penumbra of larger fields can be sensitivity to positions in alignment of the collimator and the source. Finally, this work provided the basis as proof of principle work for performing electron-

ic portal imaging dosimetry, or dose guided radiation therapy, though image lag remains still a technical challenge.

Chapter 5 is a study that was initiated based on the observed finding in the previous chapter that source modeling is an important aspect of modeling these small animal irradiators. We wanted to be able to quantify the tolerance of the collimators to small shifts in alignment, which necessitated modeling the source distribution and finding a method to create faster calculations as tracking electrons hitting the anode of an x-ray tube is a rather computationally intensive step. In the end, we were able to create an analytical source model that was 1200 times faster than using Monte Carlo methods to perform dose calculations. The analytical model was used to investigate the fluence of the focal spot, which showed the effect of different focal spot sizes and shapes on output.

Chapter 6 outlines the development of *SmART-Plan*, our small animal treatment planning software – the first commercially developed software available for preclinical small animal image-guided irradiators. *SmART-Plan* was developed in Matlab and interacts with the EGSnrc code *Dosxyznrc* to perform dose calculations. *SmART-Plan* uses pre-calculated phase-space files, which rely on the analytical model described in the previous chapter to rapidly produce large phase-space files. In this article, we outline the requirements of a small animal treatment planning system and emphasize the workflow differences between preclinical and clinical treatment planning systems. We demonstrate the accuracy in uniform and heterogeneous phantoms, which have not been shown previously by any competing calculation method. We also demonstrate the feasibility of a multi-target treatment planning system based on MC calculations.

In **Chapter 7**, a large small animal research study is performed based on all of the work from the previous chapters. The large-scale research study was used to investigate normal tissue toxicity in the lungs of mice and to assess the progression of radiation induced lung fibrosis through the use of micro-CT. In this study we used and validated deformable registration methods of mice lungs. We demonstrated the progression of intensity increases for conformal lung irradiation in a common breed of mice and show that when compared to literature the onset and the severity of lung changes is less severe for larger irradiation of the same dose.

In **Chapter 8**, a general discussion again summarizes the findings within this thesis, reports the progress image-guided small animal irradiators as well as outlines current challenges in delivery and tissue assignment in mice. A small portion of this chapter is dedicated to the ongoing research efforts of small animal irradiation as well as future developments of small animal irradiation including the use of bioluminescent imaging – a promising new area of preclinical radiation research.

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Curriculum Vitae

Patrick V. Granton was born on January 12th 1981 in Ajax Canada and attended high school at St. Mary's Catholic Secondary School in neighbouring Pickering. He completed a Bachelor of Science with Honors Specialization in Astrophysics at the University of Western Ontario. Shortly thereafter, he enrolled in a Master's program under the supervisor of David W. Holdsworth at Robarts Research Institute and received his M.Sc. in 2008 in material classification schemes using cone-beam micro-CT department of Physics and Astronomy and University of Western Ontario. After graduating he engaged in research activities at the London Regional Cancer Centre investigating scatter in optical cone-beam CT for 3-D Gel dosimetry under the supervision of Kevin Jordan and Jerry Battista. In 2010, Patrick relocated to Maastricht in the Netherlands to commence a Ph.D. track under the supervision of Frank Verhaegen in the development of preclinical small animal radiation therapy. Patrick has received numerous awards and accolades in particular a prestigious National sciences and engineering research council post-graduate award.

List of publications

PUBLICATIONS

Published

1. F. Verhaegen, S. J. van Hoof, **P.V. Granton**, Daniela Trani
“A review of treatment planning for precision image-guided photon beam pre-clinical animal radiation studies.” Accepted for publication in Zeitschrift fur Medizinische Physik, February, 2014
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In-preparation

1. S. Yahyanejad, **P.V. Granton**, A. Chalmers, F. Verhaegen, M. Vooijs. "Combined BLI and CT to measure brain tumor growth." Target journal: Nuclear Medicine
2. **P.V. Granton**, K. Jordan, J. Battista "Methods to reduce stray light in an optical cone-beam scanner." Target journal: Physics in Medicine and Biology
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1. F Verhaegen, P.V. Granton, S van Hoof, D Trani, L Dubois. F Verhaegen, P.V. Granton, S van Hoof, D Trani, L Dubois. A dedicated small animal radiotherapy research platform. 59th Radiation Research Society Annual Meeting, New Orleans, Sept 15-18, 2013.
2. F Verhaegen, P.V. Granton, S van Hoof, D Trani, L Dubois. A dedicated small animal radiotherapy research platform. ECCO meeting, Amsterdam, Sept 27-Oct 1, 2013.
3. P.V. Granton, and F. Verhaegen. “Focal spot issues for dose calculations in image-guided small animal radiotherapy” Presented at 1st Symposium on precision small animal radiotherapy, Maastricht, March 2013.
4. P.V. Granton, Stefan Van Hoof, Stefan Nijst, M. Podesta, and F. Verhaegen. “A dose calculation and verification method for a conformal small animal irradiator based on a MC dose engine and on-board imaging” Presented at ICTR-PHE, Geneva, March 2012.
5. P.V. Granton, M. Podesta, G. Landry, G. Boostma, and F. Verhaegen. “A combined dose delivery and transmission dose verification model for a small animal precision irradiator for pre-clinical” Presented at the joint meeting of American Association of Physicists in Medicine (AAPM) and the Canadian Organization of Medical Physicists (COMP), Vancouver, July 2011.
6. P.V. Granton, M. Podesta, G. Landry, S. Nijsten, W. Van Elmpt, and F. Verhaegen. “2-D Portal Dosimetry For A Small Animal Conformal Irradiator” Presented at the European Society for Therapeutic Radiation Oncology, London, May 2011.
7. P.V. Granton, A.J. Nelson, S.N. Friedman, I.A. Cunningham, and D.W. Holdsworth. “Advanced Non-invasive Radiographic Analysis and Image Processing of an Egyptian Raptor Mummy.” Presented at London Imaging Discovery, London, Ontario, 2009.
8. P.V. Granton, C. Norley, J. Umoh, D.W. Holdsworth. “Rapid *In-vivo* Whole-body Composition Of Rats Using Micro-CT.” Presented at London Imaging Discovery, London, Ontario, 2008.
9. P.V. Granton, S.I. Pollmann, M. Drangova, and D.W. Holdsworth. “Implementation of Multi-energy Micro Computed Tomography Post-reconstruction mate-

rial decomposition.” Presented at Radiological Society of North America, Chicago, IL, USA, 2007.

INVITED PRESENTATIONS (HIGHLIGHTS)

1. P.V. Granton, F. Verhaegen “Challenges in small animal treatment planning” Presented at European Society for Therapeutic Radiation Oncology (ESTRO), March 2014.
2. P.V. Granton “Challenges in small animal treatment planning” Presented at Princess Margaret Hospital, Toronto, Canada, July 2013.
3. P.V. Granton “The State-of the-art of the Dose Guided Small Animal Irradiator (SSmART)” Presented at Catharina ziekenhuis, Eindhoven, The Netherlands, December 2011.
4. P.V. Granton “Recent advances in micro-CT small animal imaging.” Presented at Maastricht Clinic, Maastricht, The Netherlands, and March 2010.
5. P.V. Granton “Multi-Energy Micro-CT.” Presented for the Teaching support Unit, The University of Western Ontario, London, and Ontario, 2007.