Received:
31 May 2018

Revised:
27 September 2018

Accepted:
01 October 2018

<https://doi.org/10.1259/bjr.20180487>

Cite this article as:

Walb MC, Carlson BL, Sarkaria JN, Tryggestad EJ. Quantifying the setup uncertainty of a stereotactic murine micro-image guided radiation therapy system. *Br J Radiol* 2019; **92**: 20180487.

SMALL ANIMAL IGRT SPECIAL FEATURE: FULL PAPER

Quantifying the setup uncertainty of a stereotactic murine micro-image guided radiation therapy system

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Objective: Investigate the reproducibility of murine cranial positioning using solely a stereotactic stage, and quantify the potential improvements from the on-board image guidance of the X-RAD SmART irradiator.

Methods: For intermouse reproducibility, athymic nude mice ($N = 5$, $\times 4$ groups) were cranially fixed on a stereotactic stage. Each mouse was imaged via cone-beam CT (CBCT). A virtual isocenter target was placed in the brain, the stage shifted to that target, and the couch positions recorded. The mouse was removed from the stage and this process repeated twice ($N=60$ measurements). The first acquired CBCT coordinates (within each group of five mice) were used to define “stereotactic couch coordinates.” CBCT shifts were calculated to quantify the accuracy of setup based on couch coordinates alone. For intramouse reproducibility, C57BL/6 mice ($N=4$) were imaged daily for 7 days. Each mouse

had individual stereotactic coordinates defined from their first day of CBCT localization, and positional shifts required on the six subsequent days of imaging were quantified ($N = 24$ measurements).

Results: The mean vector shift between stereotactic setup and CBCT alignment for inter and intramouse analysis was 0.78 ± 0.27 mm and 0.82 ± 0.34 mm, respectively.

Conclusion: Cranial irradiation that can permit positional uncertainties on the order of a millimeter can rely solely on stereotactic coordinates derived from a single daily CBCT. Irradiations of subregions requiring submillimeter accuracy require daily image guidance for each mouse.

Advances in knowledge: This is the first investigation of stereotactic reproducibility using the X-RAD SmART and it suggests a method for increased efficiency in high-throughput experiments.

INTRODUCTION

Developments in small animal irradiators are critical tools for translational research in radiation oncology. These modern irradiators offer numerous advantages over the previous generation of technologies, including image guidance systems, which provide the potential for increased accuracy in target localization, and advanced radiation delivery techniques, which may better model the paradigms utilized in clinical radiation treatments.¹ However, with these advancements still comes a need to balance enhanced radiation delivery accuracy with the fundamental requirement for efficiency in high-throughput animal experiments. The researchers at our institution are accustomed to designing experiments with cesium irradiators with batch irradiations of multiple animals simultaneously with minimal setup time and no image guidance; they are in a transitional period as they decide how to best adapt their experimental protocols to this modern technology.

Across the field of radiation research, many groups performing small animal experiments utilize single- or hypo-fractionated radiation delivery as an approach to improve efficiency.^{2–6} While this does significantly reduce the time spent on irradiations, it comes at a cost of pre-clinical translatability. Although hypofractionation techniques are becoming more common, the majority of clinical radiation paradigms involve approximately 2 Gy delivered repeatedly over 20–35 fractions. In order to better approximate this clinical scenario, intracranial orthotopic tumor experiments at our institution commonly involve 20 to 60 mice irradiated for 5–10+ fractions.⁷ For these experiments, the idealized need for high experimental throughput must be balanced against associated labor costs. The X-RAD SmART irradiator (Precision X-ray, North Branford, CT) has advanced image guidance capabilities in the form of cone-beam CT (CBCT) that can provide target localization accuracy within 0.2 mm, but each CBCT can add minutes to the irradiation of a single animal.⁸ This study seeks to investigate the reproducibility of murine cranial positioning

Figure 1. A photograph of a mouse immobilized on the X-RAD SmART irradiator. The commercially available stereotactic stage (brown) is mounted onto the white platform which attaches to the three degrees-of-freedom irradiator stage, and includes the nose cone accessory for anesthesia delivery. Inset view from above showing the ear bars.

using solely a stereotactic stage for mouse setup, and to quantify the potential improvements or margin reductions that could be gained from using the on-board image guidance of the X-RAD SmART irradiator.

METHODS AND MATERIALS

All experiments were performed with the approval of the Institutional Animal Care and Use Committee at Mayo Clinic. A commercially-available stereotactic stage (Model 900M, Kopf Instruments, Tujunga, CA) was modified to attach it to a platform which could be mounted to the irradiator stage. The stereotactic stage is constructed from Ultem, a thermoplastic resin with approximately 19% attenuation of 225 kVp X-rays. The ear bars were also modified to be removable, which was desirable for irradiation with lateral beams. Athymic nude mice ($N = 5$, repeated for four groups) were anesthetized under isoflurane and cranially fixed on the stereotactic stage using a bite block. Two of the groups were additionally immobilized with ear bars. An image of the stereotactic stage and the murine immobilization is shown in Figure 1. Each mouse was volumetrically imaged with CBCT (60 kVp, 0.3 mA, 2.0 mm Al filter, 256 images, $200 \mu\text{m}^3$ voxel size). This CBCT has been commissioned and calibrated by a medical physicist, with flex-maps measured quarterly (or more frequently as needed), and plans for routine imaging performance verified quarterly using the Micro-CT Quality Assurance Phantom (Shelley Medical Imaging Technologies, London, Ontario, Canada). A virtual target was placed at a reproducible location in the mouse brain using bony landmarks, the stage

shifted to that target, and the couch positions recorded. A representative example of the target placement is shown in Figure 2. The mouse was then removed from the stage and this process was repeated twice more for each animal (thus, $N = 60$ total measurements). Within each cohort of five mice, the first acquired CBCT couch coordinates for the first mouse imaged were used to define “stereotactic couch coordinates” for all the subsequent imaging performed on all of the mice in that group. From these stereotactic coordinates, the CBCT positional shifts were calculated to quantify the setup accuracy based on couch coordinates alone. This sequence of steps was intended to be an end-to-end investigation for intermouse reproducibility; to quantify how accurately a target could be irradiated if the stereotactic coordinates from a single CBCT scan of a single mouse were used to position all the mice in a given fraction.

A similar sequence of imaging was performed to probe intramouse setup reproducibility, specifically leveraging this information for the context of a forthcoming focal optic nerve (ON) irradiation experiment. A pilot ON imaging and targeting study was first performed on a euthanized mouse with a needle placed in the optic canal in order to better visualize the ON location and also identify reliable bony landmarks or surrogates for setting CBCT-based targeting. This ON virtual target is shown in Figure 3. Ongoing studies will include histological confirmation to verify the reliability of this target choice for ON damage. Subsequently, C57BL/6 mice ($N = 4$) were anesthetized under isoflurane and cranially fixed on the stereotactic stage using the bite block without ear pins. Each mouse was imaged with a higher-dose and higher-resolution CBCT protocol (40 kVp, 8.0 mA, 2.0 mm Al filter, 256 images, $100 \mu\text{m}^3$ voxel size) to facilitate the best possible visualization of the relevant anatomy, the stage shifted to the optic nerve virtual target, and the couch positions recorded. In order to restrict this study to intramouse reproducibility, each mouse was assigned individual stereotactic coordinates based on their CBCT couch coordinates from the first day of imaging. They were subsequently imaged for 6 consecutive days, and the positional shifts required from their stereotactic coordinates to the daily CBCT coordinates were quantified. The goal was to assess the accuracy of irradiating each mouse over multiple fractions relying solely on the stereotactic coordinates derived from imaging on the first fraction.

RESULTS

The mean vector shift $\left(r = \sqrt{x^2 + y^2 + z^2} \right)$ between stereotactic setup and CBCT alignment for the intermouse reproducibility study was 0.78 ± 0.27 mm ($\pm$ standard deviation, SD). The results for each cohort of mice are summarized in Table 1. Groups #1 and #2 included the ear bars as part of the immobilization, while Groups #3 and #4 exclusively utilized the bite block. As the first two groups were not significantly different than the last two groups, all four groups were combined to report a mean vector shift.

The mean vector shift $\pm$ SD between stereotactic setup and CBCT alignment for the intramouse reproducibility study was 0.82 ± 0.34 mm. The results for each mouse are summarized in Table 2.

Figure 2. (A, B) The target for Groups #1 and #2 was placed at the midline vertex of the mouse skull, here shown in axial (A) and sagittal (B) views, as an arbitrary cranial location to test positioning reproducibility. (C,D) As we began to focus specifically on whole-brain irradiation studies, the target for Groups #3 and #4 was placed at a location potentially suitable for brain irradiation with oral cavity sparing. Both images are sagittal views depicting the anterior (C) and inferior (D) landmarks (gold lines) that were used to reproducibly place the target.

DISCUSSION

To our knowledge, this is the first analysis of setup reproducibility on the X-RAD SmART system using a commercially available stereotactic device. The data collected here has guided the development of pre-clinical intracranial imaging and irradiation protocols at our institution. A conservative margin to ensure target coverage can be calculated by the summation of the mean vector shift to the standard deviation and the

0.2 mm intrinsic targeting accuracy of the X-RAD SmART, which results in a recommended beam margin of approximately 1.3 mm if relying on stereotactic coordinates derived from a single daily CBCT.⁸ If the advanced tracking feature (an online correction which accounts for intracollimator variability) is not utilized, this margin could increase by 0.2 mm.⁸ A margin recipe formalism could also be applied, requiring a separate analysis of systematic and random components, which could potentially

Figure 3. (A) A needle placed in the optic canal to better visualize the ON location. (B) Bony landmarks (gold lines) were identified for target placement in the coronal plane. (C) Once the lateral and longitudinal coordinates were set using the bones in the top of the skull, the vertical coordinate was dropped down to the base of skull to facilitate an isocentric ON treatment. ON, optic nerve.

Table 1. The mean positional shifts ($\pm$ SD) from the stereotactic couch coordinates to the coordinates dictated from the target set on CBCT (x-lateral, y-vertical, z-longitudinal). Each group contains five mice, each imaged three times, and the stereotactic coordinates within each group were defined from the first scan of the first mouse in that group

Mean shifts from stereotactic coordinates to CBCT coordinates				
Group ID	$\Delta X(\text{mm})$	$\Delta Y(\text{mm})$	$\Delta Z(\text{mm})$	$\Delta r(\text{mm})$
#1	0.31 ± 0.23	0.44 ± 0.26	0.31 ± 0.25	0.69 ± 0.32
#2	0.22 ± 0.19	0.62 ± 0.39	0.48 ± 0.30	0.84 ± 0.48
#3	0.30 ± 0.27	0.34 ± 0.28	0.41 ± 0.23	0.70 ± 0.32
#4	0.23 ± 0.10	0.66 ± 0.43	0.38 ± 0.30	0.89 ± 0.36
Average	0.26 ± 0.20	0.52 ± 0.32	0.40 ± 0.24	0.78 ± 0.27

CBCT, cone-beam CT; SD, standard deviation;

slightly reduce this margin.⁹ These results inform the recommendation for daily image guidance, depending on the target and organ-at-risk requirements of each specific study. Two examples of these studies are highlighted in the applications section below. If a target can permit a planning volume expansion by this margin without compromising organs-at-risk, the frequency of daily imaging can be significantly reduced. These magnitudes of the average vector shifts from Tables 1 and 2 show high agreement to published data. A similar study, which used a different irradiator, methodology, and immobilization device, reported an average vector shift of 0.80 ± 0.49 mm.¹⁰

Both the inter and intramouse reproducibility studies returned setup variabilities of similar magnitudes. This implies that the dominant source of variability is from the inconsistency in setup and not from anatomical differences among the mice. Looking at Table 2 in more detail, the lateral shift (Δx) is the dominant quantity in the vector shift. Noticeably, the ear bars were not utilized for that intramouse portion of the study, which could have had an effect on lateral reproducibility. However, if the ear bars are entirely to blame for the lateral variance, one would expect the Δx variance for Groups #3 and #4 in Table 1 (where ear bars were not used) to differ significantly from Groups #1 and #2 (which did use ear bars), which is not the case. Most likely the increased Δx variance is primarily due to inherent inaccuracies arising partially from our in-house solution. Specifically, the connection between the platform on which the Kopf stereotactic frame is mounted and the X-RAD robotic stage is a single *t*-pin which results in the lateral direction allowing for noticeable

“wiggle.” As we have gained more experience with the system we note anecdotally that the x stereotactic coordinate is that which has tended to show systematic bias.

Our group has moved away from using the ear bars for two reasons. For many of our previously-conceived experiments, an opposed lateral beam arrangement is the most relevant treatment geometry. The base for the ear bars are constructed of Ultem, a thermoplastic resin with significant attenuation of orthovoltage X-ray beams, which introduces disadvantageous inhomogeneities into the dose distribution. Even if the removal of the ear bars causes a minor increased lateral variance in the setup position, this will be dosimetrically compensated for through the use of opposed lateral beams (*e.g.* one beam will be slightly closer to the target, and the complimentary beam will be slightly farther away). Another reason to not utilize the ear bars for immobilization is because the pair of ear bars tend to make contact with the mouse skull at slightly differently planes, which results in a lateral roll to the immobilized mouse skull (not explicitly quantified above). This is particularly undesirable for the proposed ON irradiation experiment, involving a single superior (*i.e.* vertex) beam. The associated ON target is set using bony landmarks which are more superior to the optic nerve, and if there is a significant roll to the mouse skull, these superior landmarks will no longer be directly above the optic canal, potentially leading to a partial geometric miss. Lastly, the time spent adjusting the ear bars and rescanning the mice was found to be overly detrimental to efficiency. Nevertheless, the lateral variance remains a subject for future research. One possibility could be to design a U-shaped foam neck cradle,

Table 2. The mean positional shifts ($\pm$ SD) from the stereotactic couch coordinates (determined on day 1) to the subsequent CBCT coordinates obtained on days 2–7

Mean shifts from stereotactic coordinates to CBCT coordinates				
Mouse ID	$\Delta X(\text{mm})$	$\Delta Y(\text{mm})$	$\Delta Z(\text{mm})$	$\Delta r(\text{mm})$
#1	0.75 ± 0.55	0.15 ± 0.10	0.13 ± 0.08	0.81 ± 0.52
#2	0.57 ± 0.31	0.22 ± 0.19	0.30 ± 0.17	0.72 ± 0.31
#3	0.83 ± 0.27	0.22 ± 0.16	0.15 ± 0.14	0.90 ± 0.23
#4	0.73 ± 0.37	0.22 ± 0.10	0.20 ± 0.20	0.83 ± 0.30
Average	0.72 ± 0.38	0.20 ± 0.14	0.20 ± 0.16	0.82 ± 0.34

CBCT, cone-beam CT; SD, standard deviation;

Figure 4. (A) A sagittal beam's-eye-view showing the visualization of field edges for a 10 mm diameter circular collimator. The inferior edge of the field is 1.5 mm below the base of skull and the anterior edge of the field covers the entire olfactory bulb. (B) An axial view of the opposed lateral fields. (C) A T_2 MR image fused with the planning CT in order to determine the extent of the tumor cells and to verify that the radiation beam covers the tumor with ≥ 1.3 mm margin. The T_2 hyperintense region (green) indicates the region of high tumor cell probability. The dashed red lines are representative of the 10 mm diameter field edge, which provide a sufficient margin on the target.

which could enhance the lateral immobilization without introducing inhomogeneities into the beam path.

Initially, fluoroscopy was considered as a potential compromise between setup accuracy and imaging time. However, the acquisition of two orthogonal images, coupled with an increased difficulty in accurate virtual isocenter placement, was found to still require a significant amount of time with minimal benefits vs CBCT. Fluoroscopy also has the disadvantage of delivering an inconsistent amount of dose with each imaging procedure, dependent on the amount of beam-on time. The scope of this investigation was thus limited to CBCT, although fluoroscopy remains a potential area for future study, particularly in treatment sites that only require a single beam.

APPLICATIONS

These results have shaped the design of our radiation experiments and set the requirements for imaging frequency. Two examples for how this data has guided our irradiation protocols are discussed below.

Whole-brain radiotherapy

In this animal model, glioblastoma tumor cells are implanted intracranially into athymic nude mice. T_2 MR sequences are periodically obtained to visualize the representative location of the implanted tumor cells. The beam geometry from the planning system (SmART-ATP, SmART Scientific Solutions B.V., Maastricht, Netherlands) is shown in (Figure 4). Parallel opposed lateral beams, tipped to match divergence at the base of skull, were chosen to provide a homogenous dose distribution while sparing as much mucosa in the oral cavity as possible. The inferior edge of the fields is placed 1.5 mm below the base of skull, and the anterior edge of the fields is placed flush with the superior border of the olfactory bulb. These field edges ensure that there is ≥ 1.3 mm margin around the target tumor cells. Thus, the results from Table 1 can be applied, and only a single CBCT is acquired on the first mouse treated for a given day within an experiment. This single CBCT is used to verify or modify the target location, and then the remaining mice (20–40) for that day are irradiated using those stereotactic coordinates without further imaging. As the CBCT acquisition and virtual targeting

process take approximately 3 min/mouse, this has led to a significant improvement in overall time. The images shown in Figure 4 are for a 10 mm diameter circular collimator. In the future, a 15 mm collimator will likely be utilized in order to improve the posterior/inferior brain coverage.

Optic Nerve damage

This animal model is being developed to investigate optic nerve injury. The goal of this radiation protocol is to irradiate the

Figure 5. (A) Axial view with visualization of the vertex optic nerve beam. (B) Coronal beam's-eye-view showing the 2.5 mm circular collimator target. The medial edge is placed slightly lateral of midline, and the superior edge is flushed to the inferior edge of the globe, leaving negligible margins for organs-at-risk.

ipsilateral optic nerve to ablative doses while sparing the ipsilateral retina, contralateral optic nerve, and optic chiasm. Figure 5 shows the isocenter placement for the 2.5 mm diameter circular collimator, sandwiched tightly between midline (to avoid the chiasm and contralateral optic nerve) and flush up behind the ipsilateral eye. Since any setup variability would almost certainly lead to undesirable irradiation of organs-at-risk or a geometric miss, a CBCT must be acquired daily for each mouse.

CONCLUSION

Cranial setup reproducibility was evaluated using a commercially available stereotactic stage and the on-board image guidance of the X-RAD SmART irradiator. From this data, margin requirements were developed for image guidance recommendations. For irradiations of small targets that require submillimeter accuracy, the on-board imaging should be used to ensure accurate target coverage and organ sparing. However, for irradiations of larger cranial targets that can reasonably permit a planning target volume expansion of approximately 1.3 mm, stereotactic coordinates derived from a single daily CBCT can be used to irradiate all the mice for a given fraction. For most of

our experiments to date, the primary time-limiting step has been the CBCT acquisition; consequently this methodology has the potential for significant time savings.

ACKNOWLEDGMENT

The authors would like to thank Stephen Corner & the Division of Engineering for their work modifying the stereotactic stage, Sonja Dragojevic for her contributions to the optic nerve targeting, and Bianca-Maria Marin for her contributions on the MR imaging.

IACUC APPROVAL

All research on animal subjects was carried out in accordance with the latest addition of the National Institutes of Health guide for the care and use of Laboratory animals. All research procedures were performed with the approval of the Institutional Animal Care and Use Committee at Mayo Clinic.

FUNDING

This work was funded by the Mayo SPORE in Brain Cancer CA108961 and Mayo Clinic.

REFERENCES

- Verhaegen F, Dubois L, Gianolini S, Hill MA, Karger CP, Lauber K, et al. ESTRO ACROP: Technology for precision small animal radiotherapy research: optimal use and challenges. *Radiother Oncol* 2018; **126**: 471–8. doi: <https://doi.org/10.1016/j.radonc.2017.11.016>
- Li W, Liu Y, Yang W, Han X, Li S, Liu H, et al. MicroRNA-378 enhances radiation response in ectopic and orthotopic implantation models of glioblastoma. *J Neurooncol* 2018; **136**: 63–71. doi: <https://doi.org/10.1007/s11060-017-2646-y>
- Alexander TC, Butcher H, Krager K, Kiffer F, Groves T, Wang J, et al. Behavioral effects of focal irradiation in a juvenile murine model. *Radiat Res* 2018; **189**: 605–17. doi: <https://doi.org/10.1667/RR14847.1>
- Dunne V, Ghita M, Small DM, Coffey CBM, Weldon S, Taggart CC, et al. Inhibition of ataxia telangiectasia related-3 (ATR) improves therapeutic index in preclinical models of non-small cell lung cancer (NSCLC) radiotherapy. *Radiother Oncol* 2017; **124**: 475–81. doi: <https://doi.org/10.1016/j.radonc.2017.06.025>
- Kimura M, Zodda AR, Mahmood J, Das SK, Nguyen GB, Jackson IL, et al. Pilot study evaluating a rat model of radiation-induced erectile dysfunction using an image-guided microirradiator. *Urology* 2015; **85**: 1214.e1–e6. doi: <https://doi.org/10.1016/j.urology.2014.12.020>
- Blaes J, Thomé CM, Pfenning PN, Rübmann P, Sahm F, Wick A, et al. Inhibition of CD95/CD95L (FAS/FASLG) signaling with APG101 prevents invasion and enhances radiation therapy for glioblastoma. *Mol Cancer Res* 2018; **16**: 767–76. doi: <https://doi.org/10.1158/1541-7786.MCR-17-0563>
- Carlson BL, Grogan PT, Mladek AC, Schroeder MA, Kitange GJ, Decker PA, et al. Radiosensitizing effects of temozolomide observed in vivo only in a subset of O6-methylguanine-DNA methyltransferase methylated glioblastoma multiforme xenografts. *Int J Radiat Oncol Biol Phys* 2009; **75**: 212–9. doi: <https://doi.org/10.1016/j.ijrobp.2009.04.026>
- Stewart JM, Ansell S, Lindsay PE, Jaffray DA. Online virtual isocenter based radiation field targeting for high performance small animal microirradiation. *Phys Med Biol* 2015; **60**: 9031–46. doi: <https://doi.org/10.1088/0031-9155/60/23/9031>
- van Herk M, Remeijer P, Rasch C, Lebesque JV. The probability of correct target dosage: dose-population histograms for deriving treatment margins in radiotherapy. *Int J Radiat Oncol Biol Phys* 2000; **47**: 1121–35. doi: [https://doi.org/10.1016/S0360-3016\(00\)00518-6](https://doi.org/10.1016/S0360-3016(00)00518-6)
- Armour M, Ford E, Iordachita I, Wong J. CT guidance is needed to achieve reproducible positioning of the mouse head for repeat precision cranial irradiation. *Radiat Res* 2010; **173**: 119–23. doi: <https://doi.org/10.1667/RR1845.1>