

Submission ID #: 71605

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21839573>

Title: Methodological Implementation of Small Animal Irradiator Systems for Image-Guided Preclinical Studies

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

Same building, different floors. Less than half a mile apart.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 25

Number of Shots: 63

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Buck Rogers:** Our research uses a small animal irradiator to deliver precise, clinically relevant radiation doses to rodents and investigate the resulting radiation effects.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.1.2*

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Buck Rogers:** This protocol can be used for cancer research, normal tissue radiation toxicity studies, radiation biology, as well as other radiation effects.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Guilherme Rosa Ferreira:** The protocol shows researchers how to deliver radiation to small animals in the most precise way possible.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Guilherme Rosa Ferreira:** The key considerations while performing this protocol are verifying the correct filter and properly positioning the isoflurane tube in the animal's mouth to ensure reliable experiments.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Buck Rogers:** Following rodent irradiation, additional analyses can include monitoring rodents for radiation effects and harvesting tissue samples for subsequent evaluation.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Washington University in St. Louis.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Performing Cone-Beam CT Imaging for Mice with Radiator System 2

Demonstrator: Guilherme Rosa Ferreira

2.1. After initializing and warming up the radiator system, open the cabinet door [1] and insert the 2-millimeter aluminum filter into the filter slot if it is not already in place [2]. Secure the couch to the platform by depressing the quick-release pin and aligning the pin with the hole in the center of the platform [3].

2.1.1. WIDE: Talent opening the cabinet door.

2.1.2. Talent inserting the 2 millimeter aluminum filter into the filter slot.

2.1.3. Talent depressing the quick-release pin and securing the couch to the platform.

2.2. ~~Next, place the rodent bed onto the couch and secure it with tape [1].~~ Next, attach the isoflurane tube to the head of the rodent bed [2] and turn the gas on to administer 1 to 3% dose of anesthesia [3]. Place the anesthetized mouse onto the bed in a prone or supine position according to the experiment protocol [4]. **NOTE: The VO is edited for the additional shot**

~~2.2.1. Talent placing the rodent bed onto the couch and securing it with tape.~~

2.2.2. Talent attaching the isoflurane tube to the head of the bed.

2.2.2.1. Talent turning the gas on. Added shot

2.2.3. Talent placing the mouse onto the rodent bed in the required position.

2.3. Then, open Software 5. Under the **Radiation delivery** category, select **CT targeting** with either the manual or treatment protocol [1].

2.3.1. SCREEN: 71605_screenshot_1.mp4: 00:00-00:12

2.4. Under **Preset selection**, choose the preset according to the target tissue type and select **Next** [1]. Verify that the appropriate filter for the planned beam quality is inserted and confirm that the displayed filter status is green [2]. Select **Start** to begin the CT scan, and the shutter will automatically open and close [3].

2.4.1. SCREEN: 71605_screenshot_1.mp4: 00:13-00:17, 00:21-00:22, 00:26-00:28, 00:37-00:43

2.4.2. SCREEN: 71605_screenshot_1.mp4: 00:47-00:57

2.4.3. SCREEN: 71605_screenshot_1.mp4: 00:58-01:02

~~2.4.4. SCREEN: Shot of the shutter closing and opening~~ NOTE: Not provided

- 2.5. Then, use the webcam to ensure that the gantry will not collide with the couch or animal as it rotates 360 degrees [1]. If a collision looks imminent, press the red emergency stop button on the console to halt the gantry [2]. Once the CT scan is complete, select **Next** [3]. Review the acquired CT scan in the coronal, sagittal, and axial panels, then zoom in and scroll through the acquired slices to adjust the views as necessary [4].

2.5.1. SCREEN: 71605_screenshot_1.mp4: 01:28-02:00

2.5.2. Talent pressing the red emergency stop button on the console.

2.5.3. SCREEN: 71605_screenshot_1.mp4: 02:47-02:50

2.5.4. SCREEN: 71605_screenshot_1.mp4: 02:50-03:00

- 2.6. Use the mouse to drag the crosshair to the target isocenter [1]. Select **Move Stage**. If necessary, select **Scan Again** to verify the target position and click **Next** [2]. On the **Export** page, select **Go** to export the DICOM (*Die-Com*) file [3].

2.6.1. SCREEN: 71605_screenshot_1.mp4: 03:25-03:30, 03:39-03:42

2.6.2. SCREEN: 71605_screenshot_1.mp4: 03:46-04:00

2.6.3. SCREEN: 71605_screenshot_1.mp4: 06:27-6:36

3. Generation and Evaluation of the Radiation Treatment Plan for System 2

Demonstrator: Lori Strong

- 3.1. Open Software 6. Select the folder containing DICOM files and select **Import Selected Data** [1].

3.1.1. SCREEN: 71605_screenshot_2.mp4: 00:02-00:07, 00:23-00:25

- 3.2. Once the DICOM file is loaded, select the **CT2MD** (*C-T-Two-M-D*) tab and slide the density bar until the rodent tissue is visible [1].

3.2.1. SCREEN: 71605_screenshot_2.mp4: 00:29-00:33, 00:39-00:49

- 3.3. Next, select the **Contouring** tab. To add contours, select **Add**, name the contour, and specify its type under the **Type** drop-down menu [1].

3.3.1. SCREEN: 71605_screenshot_2.mp4: 00:50-01:00

- 3.4. Select the desired tool, then select **Automatic** and brush or outline the desired organ [1]. Use the scrollbar to move through the frames and continue outlining the organ to increase interpolation accuracy [2]. Once the last frame of the organ is outlined, select **Interpolate** [3].

3.4.1. SCREEN: 71605_screenshot_2.mp4: 01:01-01:10

3.4.2. SCREEN: 71605_screenshot_2.mp4: 01:29-01:38

3.4.3. SCREEN: 71605_screenshot_2.mp4: 01:39-01:41

- 3.5. Review the frames to confirm that the interpolation outlined the organ as expected and correct any frames that have deviated [1]. Select the **Planning** tab and select **Add** to add an isocenter to the plan. Then align the isocenter to the desired target in the coronal and axial planes and enter the expected dose [2].

3.5.1. SCREEN: 71605_screenshot_2.mp4: 01:45-01:54,

3.5.2. SCREEN: 71605_screenshot_2.mp4: 02:16-02:28, 02:41-02:54

- 3.6. To add beams, select **Add** under the **Beams** section. [1]. Choose between static and dynamic beams, select the collimator according to the planning parameters, adjust the beam angles as desired, and select a couch angle if necessary [2]. Under **Calculation**, select **Start Calculations** [3].

3.6.1. SCREEN: 71605_screenshot_2.mp4: 02:55-02:59

3.6.2. SCREEN: 71605_screenshot_2.mp4: 03:03-03:06, 03:17-03:18, 03:28-03:38, 03:43-03:53

3.6.3. SCREEN: 71605_screenshot_2.mp4: 04:05-04:10, 04:35-04:37

- 3.7. Once the calculation is complete, review the **Volume Slice** display [1]. Select the tabs to display the Dose Volume Histogram and Dose Volume Metrics [2].

3.7.1. SCREEN: 71605_screenshot_2.mp4: 04:39-04:42

3.7.2. SCREEN: 71605_screenshot_2.mp4: 04:45-04:56

- 3.8. Under **Evaluation**, verify the beam-on time, dose to the isocenter, Dose Volume Histogram, and Dose Volume Metrics [1]. Select **Export data**, name the file, and select **Export to file** to generate an initialization file [2].

3.8.1. SCREEN: 71605_screenshot_2.mp4: 05:05-05:06, 05:11-05:13, 05:17-05:18, 05:25-05:30

3.8.2. SCREEN: 71605_screenshot_2.mp4: 05:34-05:46.

4. Radiation Treatment Using Radiator System 2

- 4.1. After opening the door, remove the 2-millimeter aluminum filter and insert the 0.3-millimeter copper filter and the collimator according to the experiment protocol [1]. ~~Navigate back to the System 2 Program [2].~~

4.1.1. Talent removing the 2 millimeter aluminum filter and inserting the 0.3 millimeter copper filter and the collimator.

~~4.1.2. SCREEN: System 2 Program being opened.~~ NOTE: Not provided

- 4.2. For a treatment protocol, in the System 2 Program, go to the **Protocol Selection** tab, select **Load Protocol**, and choose the initialization file [1]. Verify the plan name and

parameters by selecting the **Show protocol details** box. Then, select **Next [3]**.

4.2.1. SCREEN: 71605_screenshot_3.mp4: 00:00-00:06

4.2.2. SCREEN: 71605_screenshot_3.mp4: 00:07-00:26

4.3. After that, select **Start Treatment** followed by **Start Beam [1]**. If more than one beam is included, select **Start Beam** again after the previous beam is delivered to begin the next beam **[2]**. Watch the webcam to ensure that there are no collisions **[3-TXT]**.

4.3.1. SCREEN: 71605_screenshot_3.mp4: 00:30-00:40.

4.3.2. SCREEN: 71605_screenshot_3.mp4: 01:29-01:33,

4.3.3. SCREEN: 71605_screenshot_3.mp4: 01:45-01:51, 02:00-02:08 **TXT: Refer to the text protocol for the post-treatment procedure**

5. Acquisition of Cone-Beam CT Images with Radiator System 1

Demonstrator: Livia Huang

5.1. After initializing and warming up the radiator system, open the cabinet door **[1]**. Slide the CT **(C-T)** window cover up and pull the CT unit forward until it locks **[2]**. ~~Ensure that all couches are removed from the platform [3]. Remove the collimator and holder from the gantry head [4].~~ Then insert the 1-millimeter aluminum filter for imaging **[3]**.

5.1.1. WIDE: Talent opening the System 1 cabinet door.

5.1.2. Talent sliding the CT window cover upward and pulling it forward until it locks.

~~5.1.3. Talent checking that all couches have been removed from the platform.~~ **NOTE: 5.1.3 and 5.1.4 are not filmed**

~~5.1.4. Talent removing the collimator and holder from the gantry head.~~

5.1.5. Talent inserting the 1 millimeter aluminum filter into the gantry head.

5.2. Once the detector calibration is completed, place the couch onto the platform and secure it by tightening the screw **[2]**. **NOTE: The VO is edited for the deleted shot**

~~5.2.1. WIDE: Talent opening the cabinet door.~~ **NOTE: Not filmed**

5.2.2. Talent placing the couch onto the platform and tightening the screw.

5.3. Position the mouse onto the bed in a prone or supine position as demonstrated earlier **[3]**.

5.3.1. Talent placing the mouse onto the rodent bed in the required position.

5.4. In Software 1, under **Imaging and Robotics Control**, select **CBCT Acquisition (C-B-C-T)**

Acquisition) for the principal investigator and study [1]. Name the scan, select the desired reconstruction protocol, set the number of 2-dimensional projections and the couch speed, then select **OK** [2]. Watch the webcam on the software screen to ensure there are no collisions. If a collision looks imminent, press **Stop** to halt the gantry [3].

5.4.1. SCREEN: 71605_screenshot_4.mp4: 00:16-00:33

5.4.2. SCREEN: 71605_screenshot_4.mp4: : 00:32-00:38, 00:56-01:00, 01:04-01:15

5.4.3. SCREEN: 71605_screenshot_4.mp4: 01:54-02:00

6. Execution of the Radiation Treatment Using System 1

6.1. Once the segmentation threshold is adjusted, select **Treatment Planning**. To create a new plan, under **Targets**, press the green plus sign for each desired isocenter and move the isocenter to the target [1-TXT].

6.1.1. SCREEN: ~~Treatment three views.Planning being selected. Isocenters being moved to the center of the target organ and adjusted in all~~

6.1.2. SCREEN: 71605_screenshot_5.mp4: 00:00-00:11 **TXT: Refer to the text protocol for the segmentation threshold workflow**

6.2. For each target, set the dose by double-clicking the **Dose** field [1]. Under **Beams**, press the green plus sign with a **B** to add a static beam [2]. Set the beam weight, collimator, and gantry angle for each beam [3]. To add a beam for a different isocenter, select the desired isocenter under **Targets** so that it is highlighted in purple, and then add the beam. [4]. Under **Dose**, select the desired **Dose Engine** using **Superposition Convolution** or **Monte Carlo**, then select **Compute Dose** [5].

6.2.1. SCREEN: 71605_screenshot_5.mp4: 00:12-00:15.

6.2.2. SCREEN: 71605_screenshot_5.mp4: 00:16-00:18

6.2.3. SCREEN: 71605_screenshot_5.mp4: 00:19-00:38

6.2.4. SCREEN: 71605_screenshot_5.mp4: 00:42-00:52, 00:58-01:16

6.2.5. SCREEN: 71605_screenshot_5.mp4: 01:17-01:36

6.3. Select **Verify**, then click **Compute Isodose** and **Compute DVH** (*Compute-Dose-Volume-Histogram*) to ensure the plan and dose-volume histograms accurately deliver the desired dose to the targets and organs at risk [1].

6.3.1. SCREEN: 71605_screenshot_5.mp4: 01:38-01:41, 03:17-03:20, 04:09-04:20

6.4. After opening the cabinet door, remove the aluminum filter and insert the copper filter [1]. Pull the CT window cover down and push it back until it locks [2]. Insert the collimator into the holder and slide the unit back into the gantry head [3]. Select **Execute** and **Execute Beam** [4].

6.4.1. Talent removing the aluminum filter and inserting the copper filter.

6.4.3 Talent pulling the computed tomography window cover down and pushing it back until it locks. **NOTE: 6.4.3 and 6.4.2. are swapped**

6.4.2 Talent inserting the collimator into the holder and sliding the unit back into the gantry head.

6.4.4 SCREEN: 71605_screenshot_5.mp4: 04:28-04:37

Video editor: please use title card to show the following information

- Remove the anesthetized mouse from the machine after treatment.
- Observe until fully awake and recovered from anesthesia.
- Monitor once daily for 3 days according to protocol.

NOTE: This script does not have results

1. Radiator

Pronunciation link: <https://www.merriam-webster.com/dictionary/radiator>

IPA: /ˈreɪdi,etər/

Phonetic Spelling: RAY·dee·ay·ter

2. Aluminum

Pronunciation link: <https://www.merriam-webster.com/dictionary/aluminum>

IPA: /əˈlu:mənəm/

Phonetic Spelling: uh·LOO·muh·nuhm

3. Isoflurane

Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>

IPA: /ˌaɪsoʊˈflʊr,ɛɪn/

Phonetic Spelling: eye·soh·FLUR·ayn

4. Anesthesia

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>

IPA: /ˌænəsˈθiːzə/

Phonetic Spelling: an·uhs·THEE·zhuh

5. Prone

Pronunciation link: <https://www.merriam-webster.com/dictionary/prone>

IPA: /ˈproʊn/

Phonetic Spelling: PROHN

6. Supine

Pronunciation link: <https://www.merriam-webster.com/dictionary/supine>

IPA: /suːˈpaɪn/

Phonetic Spelling: soo·PYN

7. Coronal

Pronunciation link: <https://www.merriam-webster.com/medical/coronal>

IPA: /kəˈroʊnəl/

Phonetic Spelling: kuh·ROH·nuhl

8. Sagittal

Pronunciation link: <https://www.merriam-webster.com/dictionary/sagittal>

IPA: /ˈsædʒətəl/

Phonetic Spelling: SAJ·uh·tuhl

9. Axial

Pronunciation link: <https://www.merriam-webster.com/dictionary/axial>

IPA: /ˈæksɪəl/

Phonetic Spelling: AK·see·uhl

10. Isocenter

Pronunciation link: No confirmed link found

IPA: /ˈaɪsoʊˌsɛntər/

Phonetic Spelling: EYE·soh·sen·ter

11. DICOM

Pronunciation link: No confirmed link found

IPA: /ˈdaɪkɑːm/

Phonetic Spelling: DYE·kom

12. Contouring

Pronunciation link: <https://www.merriam-webster.com/dictionary/contour>

IPA: /ˈkɑːnˌtʊrɪŋ/

Phonetic Spelling: KON·toor·ing

13. Interpolation

Pronunciation link: <https://www.merriam-webster.com/dictionary/interpolation>

IPA: /ɪnˌtɜːpəˈleɪʃən/

Phonetic Spelling: in·ter·puh·LAY·shuhn

14. Collimator

Pronunciation link: <https://www.merriam-webster.com/dictionary/collimator>

IPA: /'kɑ:ləˌmeɪtər/

Phonetic Spelling: KOL·uh·may·ter

15. Dose Volume Histogram

Pronunciation link: No confirmed link found

IPA: /dɒs 'vɔ:lju:m 'hɪstəˌgræm/

Phonetic Spelling: DOHS VOL·yoom HIS·tuh·gram

16. Initialization

Pronunciation link: <https://www.merriam-webster.com/dictionary/initialize>

IPA: /ɪˌnɪʃələˈzeɪʃən/

Phonetic Spelling: ih·NISH·uh·luh·ZAY·shuhn

17. Copper

Pronunciation link: <https://www.merriam-webster.com/dictionary/copper>

IPA: /'kɑ:pər/

Phonetic Spelling: KOP·er

18. Cone-beam

Pronunciation link: No confirmed link found

IPA: /'kɒnˌbi:m/

Phonetic Spelling: KOHN·beem

19. Acquisition

Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>

IPA: /ˌækwəˈziʃən/

Phonetic Spelling: ak·wuh·ZISH·uhn

20. Segmentation

Pronunciation link: <https://www.merriam-webster.com/dictionary/segmentation>

IPA: /ˌsɛgmənˈteɪʃən/

Phonetic Spelling: seg·muhn·TAY·shuhn

21. Isodose

Pronunciation link: No confirmed link found

IPA: /'aɪsɒˌdɒs/

Phonetic Spelling: EYE·soh·dohs

22. Superposition

Pronunciation link: <https://www.merriam-webster.com/dictionary/superposition>

IPA: /ˌsu:pərpəˈziʃən/

Phonetic Spelling: soo·per·puh·ZISH·uhn

23. Convolution

Pronunciation link: <https://www.merriam-webster.com/dictionary/convolution>

IPA: /ˌkɒ:nvəˈlu:ʃən/

Phonetic Spelling: kon·vuh·LOO·shuhn

24. Monte Carlo

Pronunciation link: <https://www.merriam-webster.com/dictionary/Monte%20Carlo>

IPA: /ˌmɑːnti ˈkɑːrləʊ/

Phonetic Spelling: MON·tee KAR·loh