JOURNAL OF SYNCHROTRON RADIATION

ISSN 1600-5775

Design of a mouse restraint for synchrotron-based computed tomography imaging

Kenneth B. Gagnon,[a] Sally Caine,[a] Nazanin Samadi,[b] Mercedes Martinson,[c] Melanie van der Loop,[d] Jane Alcorn,[d,e] L. Dean Chapman,[a] George Belev[f] and Helen Nichol[a]*

[a]Anatomy and Cell Biology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada, [b]Biomedical Engineering, University of Saskatchewan, Saskatoon, SK, Canada, [c]Physics and Engineering Physics, University of Saskatchewan, Saskatoon, SK, Canada, [d]Research Services and Ethics Office, University of Saskatchewan, Saskatoon, SK, Canada, [e]Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada, and [f]Biomedical Imaging and Therapy Beamlines, Canadian Light Source, Saskatoon, SK, Canada. *Correspondence e-mail: h.nichol@usask.ca

Received 26 March 2015
Accepted 29 May 2015

Edited by R. W. Strange, University of Liverpool, UK

Keywords: three-dimensional printing; computer-aided design; remote physiological monitoring; anaesthesia; X-ray imaging.

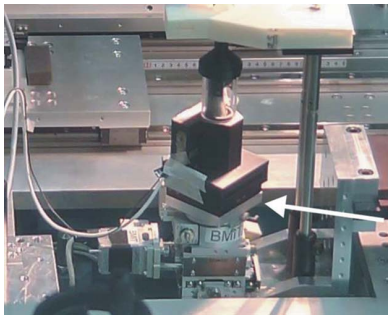
Supporting information: this article has supporting information at journals.iucr.org/s

High-resolution computed tomography (CT) imaging of a live animal within a lead-lined synchrotron light hutch presents several unique challenges. In order to confirm that the animal is under a stable plane of anaesthesia, several physiological parameters (*e.g.* heart rate, arterial oxygen saturation, core body temperature and respiratory rate) must be remotely monitored from outside the imaging hutch. In addition, to properly scan the thoracic region using CT, the animal needs to be held in a vertical position perpendicular to the fixed angle of the X-ray beam and free to rotate 180°–360°. A new X-ray transparent mouse restraint designed and fabricated using computer-aided design software and three-dimensional rapid prototype printing has been successfully tested at the Biomedical Imaging and Therapy bending-magnet (BMIT-BM) beamline at the Canadian Light Source.

1. Introduction

Non-invasive *in vivo* imaging of primary and metastatic sites of cancer has become extremely important for both early diagnosis and determining the effectiveness of treatment (Adiseshaiah *et al.*, 2014). Hard X-rays are capable of passing through large animals that are opaque to visible light with a low absorbed radiation dose. This overcomes both the depth limitations of fluorescent technologies (Chen *et al.*, 2012) and the resolution limitations of bioluminescent technologies (Contag *et al.*, 1997). The low radiation dose also permits sequential imaging of the same animal over time to monitor tumor growth and/or response to treatment with novel chemotherapeutics (Foster & Ford, 2011). As such, maintaining the animal's health status during sequential imaging events is a key goal to reducing the overall number of animals required while enhancing the statistical power of these types of studies.

Live animal imaging using synchrotron radiation presents several unique challenges. First, the synchrotron beamline has a lead-lined experimental hutch that requires remote monitoring of a stably anaesthetized animal during imaging. Second, the fixed angle and direction of the X-ray beam limits the positioning and flexibility of the animal restraint device. Third, the length of time required for the set up and the computed tomography (CT) scan increases anaesthesia duration and, therefore, the need to closely monitor animal health.



To overcome these challenges, a mouse restraint device was designed using Google Sketch-up 2014 (http://www.sketchup.com/download), and fabricated using a MakerBot Replicator 2X Desktop three-dimensional printer (https://store.makerbot.com/replicator2.html). See the supporting information for further details. Here, we present a simple and cost-effective device that restrains the mouse in an upright position, allows remote visual and physiological monitoring and provides stable anaesthesia.

2. Methods

2.1. Anaesthesia and physiological monitoring

An adult specific-pathogen-free C57BL6 × BALB/c mouse (~25 g) donated through the University's Surplus Animal/Tissue Sharing Program was anaesthetized using isoflurane/oxygen within an acrylic induction chamber, and then moved to an insulated heated pad where the anaesthesia was maintained with a nose cone. Lacrilube (Allergen, Irvine, CA, USA) was applied over both eyes to prevent dehydration, and a rectal probe lubricated with Vaseline (petroleum jelly) was inserted to record the internal core body temperature. The PhysioSuite™ physiological monitoring unit (Kent Scientific Corp., Torrington, CT, USA) has an infrared light sensor that was attached to the hind paw to measure arterial oxygen saturation (SpO_2), heart rate (HR) and respiration rate (RR). The animal protocol was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane use of animals in research.

After verifying a stable plane of anaesthesia, the mouse was transferred from the heated pad to the restraint device (under anaesthesia) and secured with a 2 mm silicone tubing under the forelimbs to prevent the body from slumping during imaging. Dental floss looped behind the incisors and taped to the vertical upright kept the head from shifting during imaging. The fully assembled restraint was then placed onto the beamline imaging stage. A voltage rheostat outside the experimental hutch permitted small remote adjustments to the intensity of the external heat lamp regulating the air temperature around the restraint device. A closed-circuit camera connected to a computer workstation outside the hutch was focused on the PhysioSuite™ monitor to manually record SpO_2, HR, RR and core body temperature. A second closed-circuit camera was focused on the restraint to visually monitor the mouse for any signs of distress or changes in the plane of anaesthesia (*e.g.* respiration rate or unusual movement). Consistent with a previous study, the mouse showed no overt signs of distress while under isoflurane anaesthesia for two hours (Szczesny *et al.*, 2004). Since no further experiments were planned, following the CT imaging the mouse was removed from the restraint, returned to the acrylic induction chamber and euthanized with a terminal concentration of isoflurane.

3. Results

The mouse restraint consists of the six components (i–vi) as illustrated in Fig. 1(*a*) and as photographed in Fig. 1(*b*). A 7 mm gap was built into the restraint base to provide a null space for obtaining an image of the X-ray beam without any obstructions. This 'flat' image is used during processing to normalize absorption images. Two 10 mm holes were also built into the restraint base for magnets that hold the entire restraint onto an imaging stage, which rotates 360° in the z axis and translates in the z axis up to 20 cm. The BMIT-BM beamline (http://www.lightsource.ca/beamlines/bmit.php) imaging stage with the mouse restraint device, anaesthetic supply and recovery hose, and PhysioSuite™ monitoring module are shown in Fig 1(*c*).

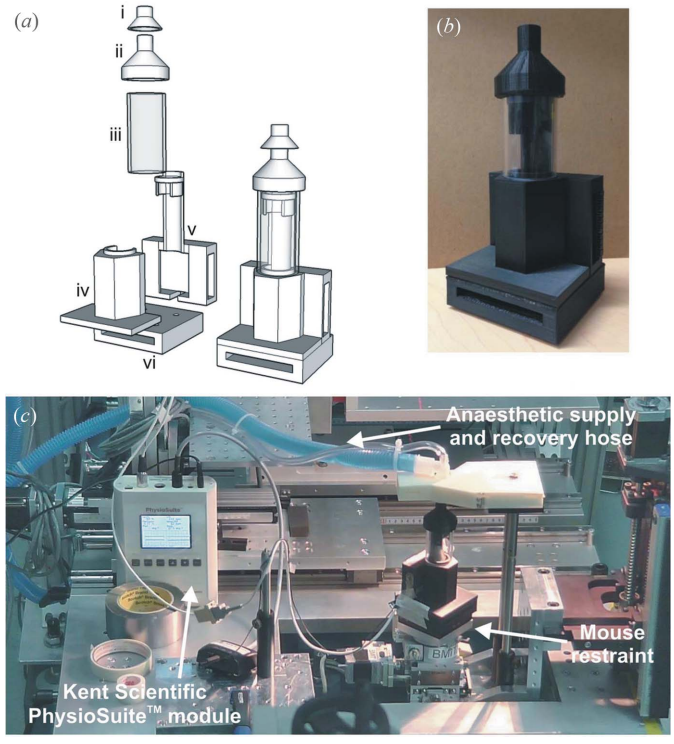


Figure 1
Mouse restraint device. (*a*) Schematic of the mouse restraint illustrating the anaesthetic supply cone (i) which directly connects to an anaesthetic supply and recovery hose. The restraint cone (ii) freely rotates under the anaesthetic supply cone and focuses the flow of anaesthesia onto the mouse's nose. A clear acrylic tube (iii) contains the mouse and allows for visual monitoring of respiration rate. The front half of the restraint (iv) was designed to house physiological infrared sensors. The back half of the restraint (v) was designed with an upper portion for the mouse and a lower portion for a temperature probe. The restraint base (vi) locks together both pieces of the restraint and magnetically attaches to the beamline imaging stage. (*b*) Photograph of fully assembled three-dimensional-printed mouse restraint in black polylactic acid. (*c*) Closed-circuit camera photograph showing the setup at the CLS BMIT-BM beamline. The anaesthetic supply (clear tube) and recovery hose (blue tube) are shown fixed above the rotating restraint. The Kent Scientific PhysioSuite™ monitoring module with wires connected to the mouse restraint device to measure HR, SpO_2, RR and core body temperature sits to the left of the rotating imaging stage.

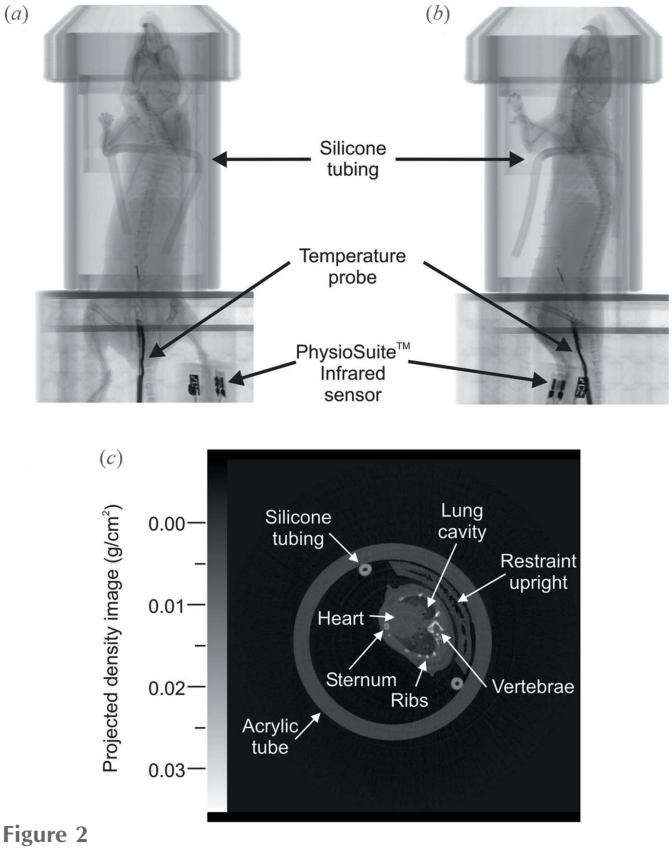


Figure 2
Spectral-KES water density projection and CT images. Dorsal–ventral (*a*) and lateral (*b*) X-ray images of an anaesthetized mouse held vertically in the restraint device. Note the silicone tubing under the forelimbs, the temperature probe and PhysioSuite™ infrared sensor attached to hind paw. (*c*) Reconstruction of 600 individual water density CT slices as the mouse restraint was rotated 180°. Note that the silicone tubing, clear acrylic tube and restraint upright do not interfere with the quality of the CT images. The denser structures such as sternum, ribs and vertebrae have greater image intensities (g cm^{-2}) than the soft tissues (*e.g.* heart) and air spaces (*i.e.* lung cavities).

Projected water density images of the mouse in dorsal–ventral (Fig. 2*a*) and lateral views (Fig. 2*b*) were obtained using a spectral *K*-edge subtraction (spectral-KES) (Zhu *et al.*, 2014) imaging setup with a Hamamatsu C9252DK-14 flat-panel detector with 100 µm pixel size. With the mouse/restraint rotating 180°, 600 projections were captured at equal radial intervals with a 30 ms exposure time for each projection (Fig. 2*c*). It is important to mention that the clear acrylic tube, silicone tubing and restraint upright did not interfere with either projection or CT imaging. It should be noted, however, that rapid-prototype printed materials may increase phase contrast and so this material may not be suitable for phase contrast imaging.

The goal was to develop a restraint device that would allow remote visual and physiological monitoring of the mouse while under anaesthesia. The rectal temperature probe and an infrared light sensor connected to the PhysioSuite™ monitoring module accurately recorded core body temperature, SpO_2, HR and RR (Fig. 3). Over the course of two hours of anaesthesia, the animal's RR averaged 92 breaths per minute, HR averaged 490 beats per minutes, SpO_2 averaged 90% and

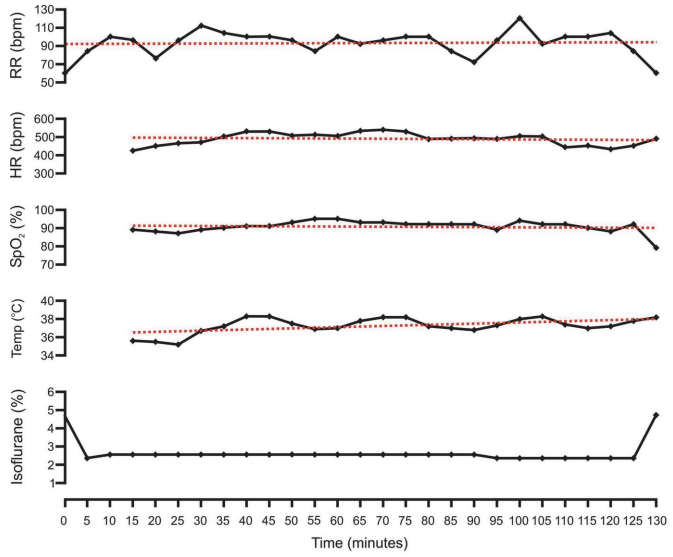


Figure 3
Physiological properties of the mouse in the restraint device. Respiration rate (RR, breaths per minute), heart rate (HR, beats per minute), arterial oxygen saturation (SpO_2, percentage), core body temperature (Temp, °C) and isoflurane concentration (percentage) plotted against duration of anaesthesia (Time, minutes). Dashed red lines indicate the mean trend line for the entire recording period.

core body temperature averaged 37°C, indicating that being held vertically for 2 h did not produce distress. This is consistent with a previous study that investigated the influence of an upright body position on the cardiophysiology of the mouse (Wiesmann *et al.*, 2001).

4. Discussion

CT imaging the thoracic region of a live anaesthetized animal with synchrotron light presented several unique challenges. We designed and fabricated a novel restraint device that held a mouse in a vertical position perpendicular to the fixed angle of the synchrotron X-ray beam and free to rotate 180°–360°. We provide files and instructions to make this inexpensive device using a three-dimensional printer (see the supporting information). Successful testing of the restraint at the Canadian Light Source (CLS) BMIT-BM beamline showed consistent and accurate monitoring of HR, SpO_2, RR and core body temperature throughout projection and CT X-ray imaging. The flexibility of CAD and three-dimensional printing enables researchers to inexpensively design and fabricate restraints that meet their research needs.

Acknowledgements

The College of Medicine, University of Saskatchewan, supplied the MakerBot Replicator 2X Desktop three-dimensional printer. This work was supported by a Saskatchewan Health Research Foundation grant to the GEMS Research Group. KG, SC, NS and MM are Fellows in the Canadian Institutes of Health Research Training grant in Health Research Using Synchrotron Techniques (CIHR-THRUST).

Research was performed at the Canadian Light Source, which is funded by the Canada Foundation for Innovation, the Natural Sciences and Engineering Research Council of Canada, the National Research Council Canada, the CIHR, the Government of Saskatchewan, Western Economic Diversification Canada, and the University of Saskatchewan.

References

Adiseshaiah, P. P., Patel, N. L., Ileva, L. V., Kalen, J. D., Haines, D. C. & McNeil, S. E. (2014). *Int. J. Mol. Imaging*, **2014**, 1–13.

Chen, Z., Wei, L., Zhu, X. & Min, W. (2012). *Opt. Express*, **20**, 18525–18536.

Contag, C. H., Spilman, S. D., Contag, P. R., Oshiro, M., Eames, B., Dennery, P., Stevenson, D. K. & Benaron, D. A. (1997). *Photochem. Photobiol.* **66**, 523–531.

Foster, W. K. & Ford, N. L. (2011). *Phys. Med. Biol.* **56**, 315–326.

Szczesny, G., Veihelmann, A., Massberg, S., Nolte, D. & Messmer, K. (2004). *Lab. Anim.* **38**, 64–69.

Wiesmann, F., Neubauer, S., Haase, A. & Hein, L. (2001). *J. Cardiovasc. Magn. Reson.* **3**, 311–315.

Zhu, Y., Samadi, N., Martinson, M., Bassey, B., Wei, Z., Belev, G. & Chapman, D. (2014). *Phys. Med. Biol.* **59**, 2485–2503.