

HIGH-RESOLUTION SYNCHROTRON X-RAY IMAGING OF LIVE MOUSE AIRWAYS: OVERCOMING CHALLENGES IN PHYSIOLOGICAL ASSESSMENT



Government of South Australia

Martin Donnelley¹, Kaye Morgan⁵, Karen Siu^{5,8}, David Parsons^{1,2,3,4}

1. Respiratory and Sleep Medicine, Women's and Children's Hospital, Adelaide, South Australia
2. Women's and Children's Health Research Institute, Adelaide, South Australia
3. Centre for Stem Cell Research, 4. School of Paediatrics and Reproductive Health, University of Adelaide, South Australia
5. School of Physics, 6. Monash Centre for Synchrotron Science, Monash University, Melbourne, Victoria



BACKGROUND: Small animal endoscopes are useful for studying respiratory diseases such as CF. However, the complexity of physiological studies is increased when imaging live animal airways using high-resolution synchrotron phase contrast (PCXI). We have developed techniques at the Japanese Synchrotron to achieve reliable visualisation of mouse airways using high-resolution 2D imaging. Here we describe effective approaches and continuing challenges.

SETTING: PCXI is performed on the SPring-8 BL20XU undulator beamline using 25keV monochromatic X-rays. Imaging is confined to a specialised hutch, a lead-lined room attached to the end of a synchrotron beamline. When imaging live animals it is necessary to perform remote animal monitoring, maintain stable anaesthesia and remotely deliver any test substances or pharmaceuticals.

STRAIN: Fur can produce strong PCXI image artifacts. Using *Foxn1tm / HOS:HR-1* allows us to acquire images without fur artifacts, but compared to normal mice these strains may exhibit other physiological differences that may affect our respiratory studies. Using only hairless strains also precludes imaging other useful strains such as transgenic CF mice. We remove fur from the imaging area (the trachea) of normal C57BL/6 mice using depilatory cream, producing images free from fur artifacts.

AIRWAY ACCESS: Airway access via tracheotomy and intubation facilitates mechanical ventilation, respiratory function testing and pharmaceutical delivery. Tracheotomy is a relatively slow and invasive procedure and can be complicated by biology, including allowing blood to enter the airway.

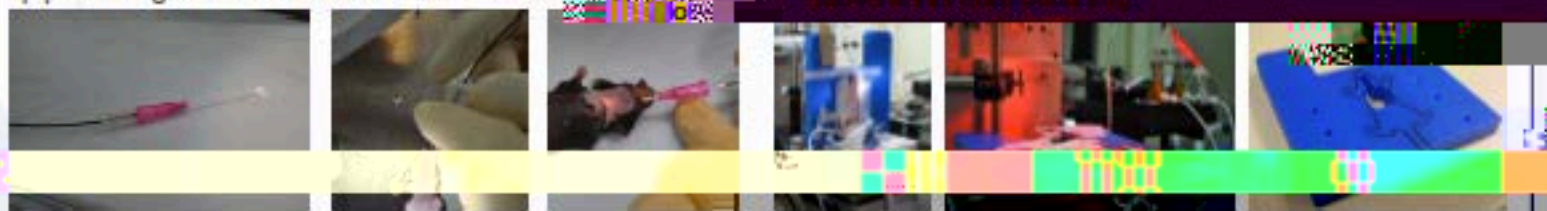
tracheal intubations are now performed via the mouth since they can be rapid, minimally invasive and readily repeatable. We use a 0.5mm plastic fiber optic guide as an introducer, and a 20Ga i.v. catheter as the endotracheal (ET) tube. The end of the fiber is attached to a bright light source so that the tip provides good direct illumination to visualize the

ANAESTHESIA: Due to Japanese government regulations only oxygen (passively humoured) are available at SPring-8. Pentobarbital is limited by the induction of unpredictable leg "kick" movements despite deep anaesthesia and the potential for overdose. Isoflurane anaesthesia is preferred as it can be easily adjusted from outside the hutch, does not produce leg

VENTILATION: Mice are ventilated using a flexiVent mouse ventilator, which allows respiratory system mechanics to be measured, coordinated delivery of aerosols, pharmaceuticals or test substances, and respiratory-gated image acquisition to minimize respiratory movements. In some studies a length of heat-shrinkable tubing is run through the wall of the inspiratory tube to the tip of the ET tube to allow test substances to be delivered to the trachea or lung airways.

ANIMAL POSITIONING: The fixed X-ray beam location and animal positioning on the imaging board using surgical table to minimize movement. For

high or supine. Despite being well anaesthetized, after approximately 25-30 minutes of imaging some mice mounted head-high appeared unsettled and displayed uncontrollable and unpredictable respiratory excursions, which reduced image quality, limiting usable imaging time to less than 30 minutes. Using a supine imaging position prevents these



DISCUSSION AND CONCLUSION: Synchrotron PCXI is a valuable technique for studying live mouse airways and despite its limitations there are currently no other imaging modalities with these capabilities. Attention to animal imaging techniques will permit continued development of novel, high-resolution, live animal airway physiology imaging for use in respiratory research.

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