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Title: Small animal monitoring for live spectral imaging for non-invasive quantification of multiple biomarkers in different mouse models
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Introduction:

MARS Spectral CT has shown remarkable results in clinical application studies, including; imaging with functionalised gold nanoparticles to assess plaque vulnerability; soft tissue quantification with multiple contrast agents; tissue and bone engineering and other various research areas. Such studies have all used *in vitro* models, that is, the rodents being imaged are dead or the specimen has been excised. Future studies will require *in vivo* models, where the imaged small animals are living, giving a validation in using the methods and techniques that were successful in *in vitro* studies in human models. Measurement of physiological data such as respiration rates, temperature and pulse oximetry (heart rate and oxygen saturation) are necessary to ensure the welfare of the studied animal, but can also help improve the image quality e.g respiratory and cardiac image gating to reduce motion blur.

Aims:

To setup small animal monitoring equipment in the MARS scanner and provide recommendations for future integration of the simultaneously acquired physiological data. The experiment will be considered successful if physiological data, including respiration, temperature and pulse oximetry, are obtained for an anaesthetised mouse.

Methods:

12 mice were used in this experiment, 6 to attempt simultaneous physiological data acquisition, and 6 to test temperature stability over the duration of the tail vein procedure. Temperature stability was tested by measuring the rectal temperature change in the mice over a 10 minute period; 3 of the mice were heated via a heating pad set to 37°C, the other 3 were unheated.

Live mice were monitored as part of a tail vein injection procedure. Inhalant isoflurane was administered to the mice to keep them anaesthetised during the experiment. Once the mice were in the surgical plane of anesthesia (non-responsive to pain), saline solution was injected into both tail veins of the mice. After both injections were complete, the monitoring equipment was attached to the mice; respiration was measured via a pressure sensitive pillow placed under the mouse, temperature was measured via a fibre optic rectal temperature probe, and pulse oximetry sensor was tried on both the ankle and tail, via a special-purpose ankle/tail holder and a general-purpose clip.

Retrospective respiratory gating was used to reduce the effects of motion blur in the reconstructed CT image. A simple logic gate was implemented in a Matlab code that would determine if a circular projection was captured while the mouse was breathing. Projections captured when the mouse was not breathing could then be reconstructed to produce an image less affected by motion blur. The program developed would only be a proof of concept, as a fully functional version could only be developed with a truly simultaneous MARS CT image data set and respiration data set available.

Results:

Small animal monitoring equipment uses sensitive electronics so it posed some challenges. The biggest difficulty in simultaneous measurement of physiological data came from the pulse oximeter placement. Of the 6 mice, only 1 was successfully monitored fully when the pulse oximeter was attached to the ankle with the special-purpose holder. Success came about by taping the paw to a piece of thread to pull through the holder, where the tape ensured that the toes could not get caught.

Measurement of the respiration data proved useful as an indicator of the level of anaesthetisation of the mouse. If the respiration dropped too low, ≤50 breaths per minute, anaesthetic dosage needed to be reduced to prevent harm to the mouse from oxygen deprivation. If the respiration rate got too high, ~100 breaths per minute, then the mouse became responsive to pain, and the anaesthetic dosage needed to be increased. From the respiration data, a retrospective gating program was developed in Matlab, which could reject x-ray projections captured when the mouse is breathing, and in principle, will reduce motion blur in the images. Testing the program on a previously acquired MARS spectral CT dataset, using the respiration data from this experiment showed that 55%-60% of circular projections are retained with the simple gating program.

Mice that were heated via a heating pad had much better temperature stability than the unheated mice. Mice that were supplied heat dropped by an average temperature of 0.63 °C, whereas the unheated mice dropped on average 2.8 °C in 10 minutes.

Conclusion:

Small animal monitoring equipment was set up and used to monitor several anesthetized mice, as part of a procedure preparing them for scanning with MARS CT. Several issues were identified with the experiment, such as the difficulty in attaching the pulse oximeter sensor and the need for careful control of the temperature of the mice. Respiration rate, temperature, heart rate and oxygen saturation was successfully acquired from 1 of the 6 mice, and the respiration data from that mouse was used to develop a retrospective image gating program.

While the monitoring of live mouse was successful in this experiment, the monitoring equipment was not set up in the MARS scanner. A method for getting the anaesthetic line and monitoring leads to the mouse, which will be immobilized by a holder in the temperature controlled imaging chamber, must be determined before a live mouse can be scanned. Scanning of a live mouse with MARS CT, while simultaneously measuring respiration data, is needed to show if the gated image is higher quality than one that is blurred by respiratory motion of the mouse. Results of this experiment will influence the design of both the new scanner models and the imaging bed, so that the monitoring equipment can be used in future studies requiring imaging of live mice.