Direct x-ray measurement of airway surface health in animal models: an update on the state-of-the-art

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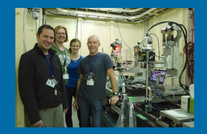

Background

- Maintenance of adequate airway surface liquid (ASL) and proper functioning of the mucociliary transit (MCT) system in the pathophysiology of CF airway disease is well understood, but until recently these parameters have been challenging or impossible to measure in live animals.
- Our goal is to non-invasively assess ASL and MCT as outcome measures for developing genetic and pharmaceutical CF airway treatments, so we have developed synchrotron phase contrast X-ray imaging (PCXI) methods to enable monitoring of these measures of airway surface health.

Methods

- All methods were developed in rodents at the SPring-8 Synchrotron in Japan.
- The tracheae of a range of *in vivo* models were imaged, including normal and β-ENaC mice.
- After anaesthesia and intubation, MCT tracking particles (10-30 μm diameter) were insufflated into the airways (via an endotracheal tube) prior to imaging.
- Rodents were attached to an animal holder and placed supine in the X-ray beam. Image acquisition was triggered from a small animal ventilator, and vital signs were monitored from outside the imaging hutch.
- The effects of aerosolised drug delivery on airway hydration (e.g. hypertonic saline compared to isotonic saline) have been assessed using an Aeroneb vibrating mesh nebuliser in the ventilator inspiratory line.

Imaging setup

- A phase-grid was placed immediately before the trachea, and arranged to cover half the field-of-view (Fig 3), in order to simultaneously perform propagation-based (PB) and single-grid (SG) PCXI.

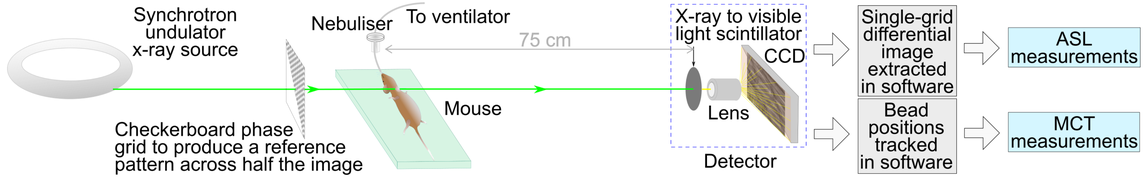

Figure 1: *The SPring-8 Synchrotron PCXI setup, where the sample distorts the grid image with propagation*

- The SG-PCXI setup allows an airway surface image to be reconstructed from the sample-induced distortions of the grid observed 75 cm downstream (Fig 2), enabling the ASL depth to be visualised (Fig 3).

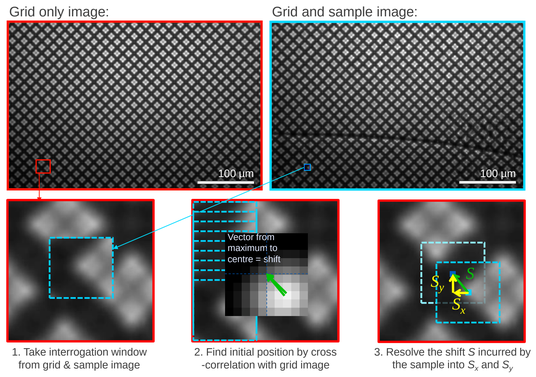

Figure 2: *Sample-induced grid distortions are determined by cross-correlation, and used to visualise ASL depth.*

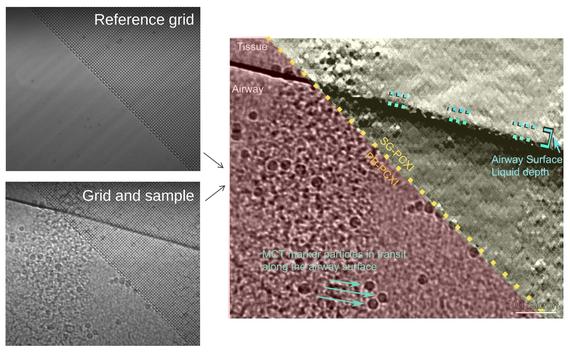

Figure 3: *Combined PB-PCXI and SG-PCXI setup allows simultaneous MCT and ASL assessment.*

- MCT behaviour was determined using a tracking program that allows the location of moving MCT marker particles to be manually tracked across multiple image frames.
- ASL measurements were conducted by manually tracing the airway-liquid interface and the liquid-tissue interfaces. The distance between these traces was calculated in software and averaged over the length of visible airway surface.

Results

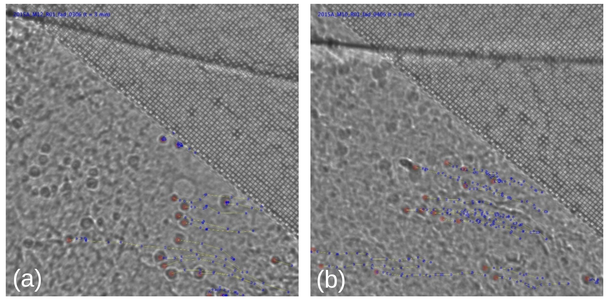

Figure 4: *Example **MCT** marker particle tracking in (a) β-ENaC and (b) littermate mice treated with hypertonic saline aerosol. Initial particle position marked in red, with subsequent positions in blue. Only moving particles are marked.*

Figure 5: *The **ASL** depth is revealed in the differential contrast reconstructed in the top-right half of the image, observed (a) before and (b) 15 minutes after delivery of aerosolised hypertonic saline to normal mice. Note that the number of MCT marker particles has decreased as many have been cleared during imaging.*

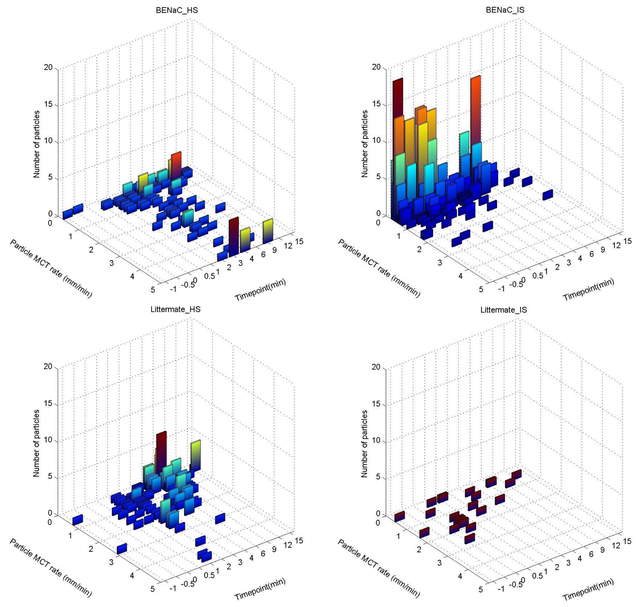

Figure 6: *MCT histograms showing the number of particles detected moving at different rates at each time point within each group of animals.*

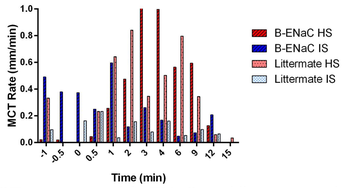

Figure 7: *Mean MCT response of β -ENaC and littermate mice following hypertonic or isotonic saline aerosol.*

- Increases in MCT activity (Fig. 6, 7) and ASL depth (qualitative impressions, analysis underway) were detected in mice following administration of aerosolised hypertonic or isotonic saline, but hypertonic saline produced greater rate increases and lasted longer. This agrees with cell culture studies assessed by optical microscopy.

Discussion

- High spatial resolution synchrotron phase-contrast X-ray imaging can reveal the depth of the ASL layer and be used for tracking MCT in live mouse nasal and tracheal airways at SPring-8.
- We are now translating MCT analysis methods to application in larger animal models at the Imaging and Medical Beamline (IMBL) at the Australian Synchrotron, but the coherence and flux density at the IMBL does not allow ASL depth assessment to be performed. In pilot IMBL studies MCT was visible in live sheep and pig tracheal segments, and live pig MCT studies are underway.

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