

## Background

- The CFTR ion channel defect in airway epithelial cells reduces airway surface liquid (ASL) depth and impairs mucociliary transport (MCT).
- We have created non-invasive, synchrotron imaging-based methods for quantifying ASL and MCT as direct measures of airway health.
- Our *in vivo* MCT quantification method relies on tracking the motion of deposited high refractive index (HRI) beads that have uniform size, shape and surface properties.
- Previous studies have suggested that MCT rate is dependent on particle material, but the effects of particle size were unclear.
- The aim of this study was to determine whether particle size affects MCT rate and behaviour.

## Methods

- Intubated and ventilated C57Bl/6 mice (n=14) were imaged on the BL20XU beamline at SPring-8 Synchrotron in Japan (See Fig 1).
- A mix of two differently-sized HRI marker particles (10  $\mu\text{m}$  and 33  $\mu\text{m}$  dia, Corpustar, USA) were delivered to the airway.
- Images were captured using a scintillator and high-resolution camera.
- After baseline imaging (room-air respiration) an Aeroneb nebuliser was used to deliver aerosol (n=8 hypertonic & n=6 isotonic saline) for 15 msec on each inspiration (120 br/min) for 15 min.
- Images were collected at 5 Hz in 30 second blocks, every minute.
- Custom semi-automated image tracking software was used to tag particles and calculate individual and bulk transit rates.



## Results

- Most HRI particles remained stationary throughout the imaging period. This effect was much more pronounced than with other particles that we have examined previously (quarry dust, fibreglass, asbestos, lead, etc).
- Moving particles were only detected in 6 mice treated with hypertonic saline, and 2 treated with isotonic saline.
- Most bead motion was detected in the lower airway.
- When data was analysed for MCT rate between the large and small particle groups (using a t-test), insufficient moving particles were detected at each individual time-point for statistical analysis.

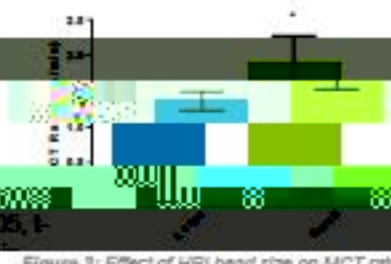


Figure 2: Effect of HRI bead size on MCT rate

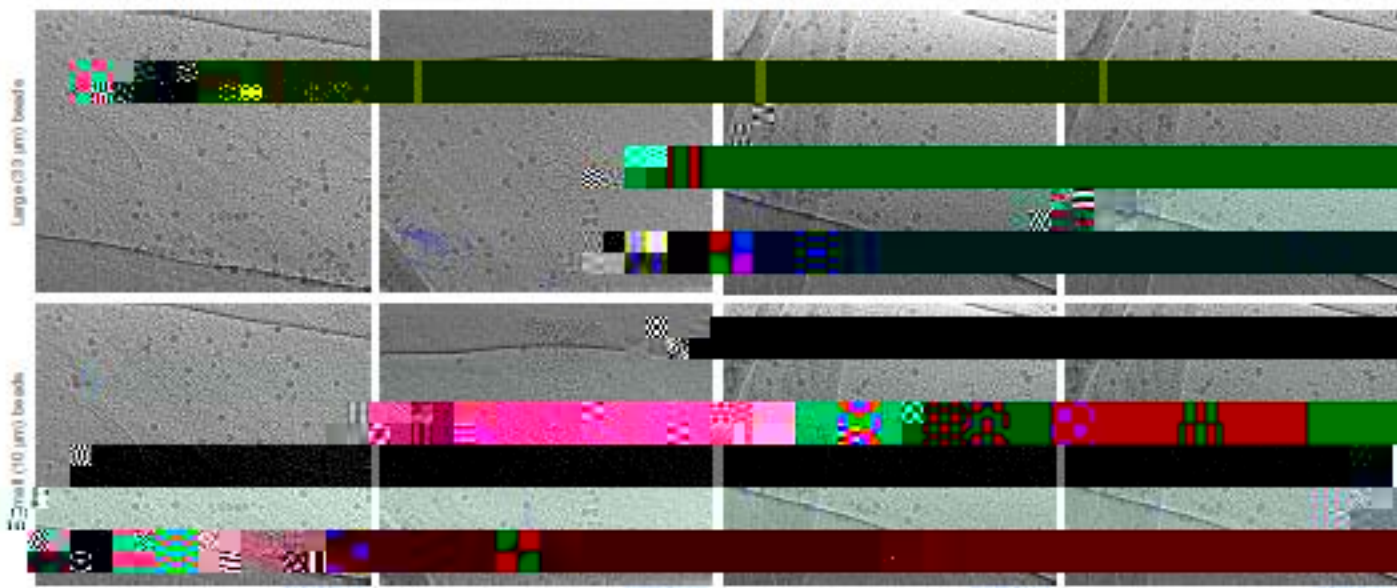


Figure 3: High magnification images of large (33  $\mu\text{m}$ ) beads, and the small (10  $\mu\text{m}$ ) beads, in the trachea. Each moving particle is identified with a red marker in the first frame and tracked with blue markers in subsequent frames. Stationary particles are not marked. Images are 1.4 x 1.2 mm.

## Conclusion

- MCT rate in live mouse trachea for these glass beads appears to be dependent on particle size, but surprisingly few of the introduced beads moved.
- We speculate that sub-optimal airway surface hydration and/or bead surface properties may have influenced the ability of most particles to move during the imaging period.
- Future studies (Nov 2015) will examine the effect of changing inhaled air humidity and particle surface coating.

## Acknowledgements

- SPring-8 proposal 2014A1576.
- Experiment Funding: NHMRC, ARC, WCH and Cure4CF Foundations.
- Experiment Travel: Australian Synchrotron ISAP.
- Conference Travel: Robinson Research Institute.