WHERE DOES IT GO? DYNAMICALLY TRACKING THE FATE OF FLUID FROM LUNG INSTILLATIONS IN MICE

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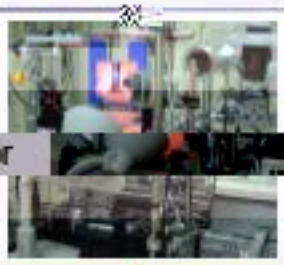
BACKGROUND: In mice the most direct lung dosing method delivers the agents directly into the trachea. For our CF gene-therapy studies we deliver fluids – an airway pre-treatment followed by a lentiviral vector – directly into the mouse trachea to target conducting airways. Despite using [illegible]ed delivery techniques we see substantial variability in the amount and location of gene-transfer. The aim of this experiment was to use synchrotron X-ray imaging at SPring-8 (F1) to track the dynamics and distribution of fluid doses delivered into live mouse trachea.

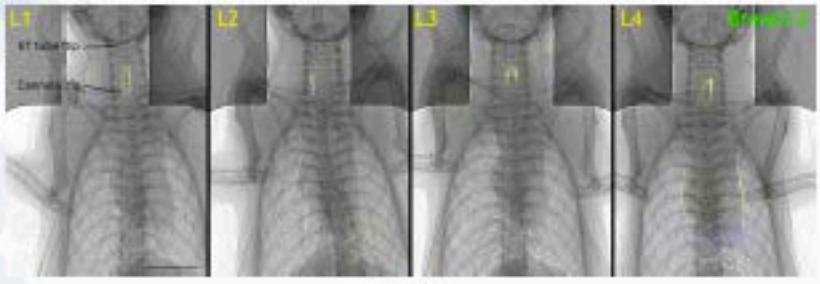
(F1)

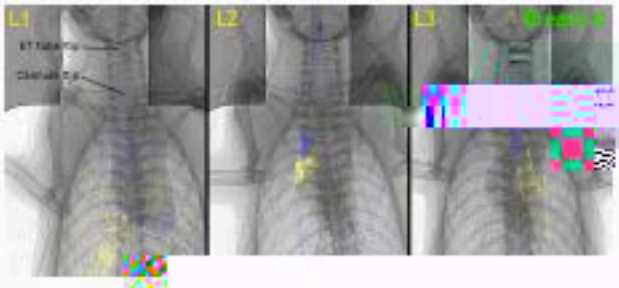
METHODS: Four nembutal anaesthetised C57Bl/6 mice were imaged on the BL20B2 beamline at the SPring-8 synchrotron in Japan. Mice were intubated and ventilated at 80 br/min with 1 image captured per breath (F2). After 1 min of baseline a 15 µl sample of iodine-based contrast fluid (airway pre-treatment or gene-vector surrogate) was delivered over 30 sec. Following 20 min of data collection an additional 15 µl bolus was delivered over 3.6 sec. Image capture continued for further 10 min. Frame differencing was used to reveal fluid motion.



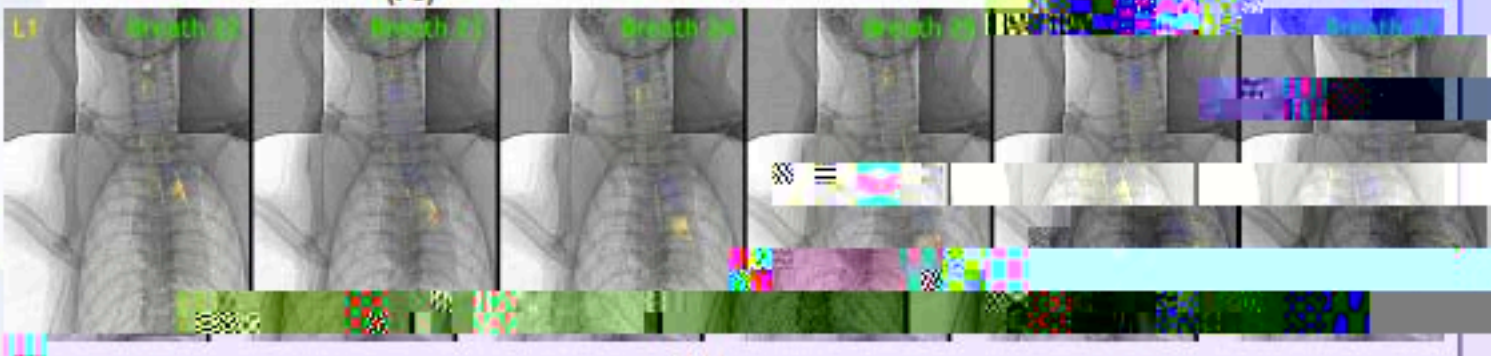
(F2)

RESULTS: Substantial dose losses may occur upon delivery into mouse trachea via immediate retrograde fluid motion (F3). The speed of bolus delivery (30 sec in F3 versus 3.6 sec in F4) into the lung may also influence dose retention. [illegible] delivery created [illegible] (F4) using this technique. However, a bolus of fluid, marked with a red X, can still clearly be seen moving down the left main bronchus (F5) of one mouse that received 15 µl of fluid over 30 seconds.





(F3)



(F5)

CONCLUSION: Our findings suggest the need for, and permit, much greater attention to dosing specifics– animal orientation, volume, speed – and enable improvements in dosing technique design.

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