Detection of regional lung disease in β-ENaC mice using a laboratory x-ray source

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1. Introduction

- Sensitive measures of lung structure and function are necessary to track the progression of Cystic Fibrosis (CF) lung disease, and to assess the local and global effects of genetic and pharmaceutical therapies.
- X-ray velocimetry (XV) combines CT techniques with volumetric particle image velocimetry (PIV), which tracks motion of the lung speckle produced when x-rays pass through alveoli.
- The magnitude and direction of the motion of the speckle pattern between frames allows the expansion across all areas of the lung to be determined, along with the amount of air displaced by each section.
- Areas where airflow is restricted manifest as reduced expansion.

2. Methods

- β-ENaC mice (n=17, bred on a C57Bl/6N background) were imaged along with littermate controls (n=13) [1].
- Mice were anaesthetized with domitor/ketamine and surgically intubated for mechanical ventilation (AccVent200, NHD) (PIP=12cmH_2O; PEEP=2cmH_2O). Breathing rate was set to 120 bpm with inspiration / expiration time at 150ms/350ms.
- Forced oscillation technique (FOT) was used to obtain a standard global measurement of lung health.

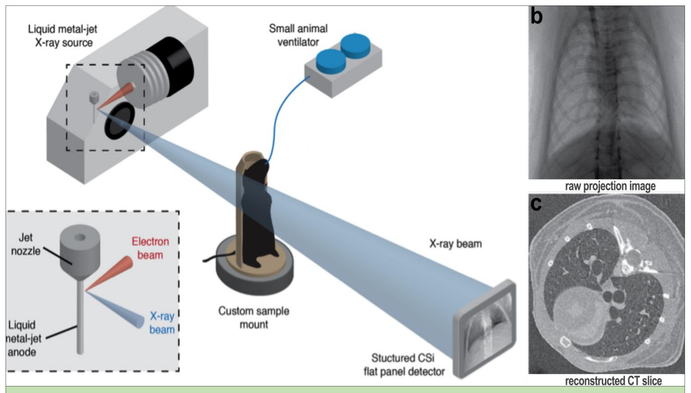

Figure 1: Experimental set-up.

- Mice were mounted on a rotary stage in front of a high-brightness liquid-metal-jet x-ray beam (Excillum AB) as shown in **Figure 1**.
- A flat-panel detector (PaxScan, Varian Medical Systems) captured images at 30 Hz with an exposure time of 15 ms as the mouse rotated through 360^0.
- Imaging was synchronized with ventilation to capture images at 15 time points throughout the breath, accumulating 400 projections per time point for 15 separate CT reconstructions [2].
- Using XV, the x-ray speckle pattern generated by the lungs was analyzed frame-by-frame over each time point to determine local tissue displacement in three dimensions [2].

[1] Mall, M et al., Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. *Nat. Med.* **10**, 487–493 (2004).
[2] Samarage, C.R. et al., Contrast free angiography of the pulmonary vasculature in live mice using a laboratory x-ray source. *Med Phys.* **43**, 6017—6023 (2016).

3. Results

- The regionality of CF-like disease can be captured in XV data.
- **Figures 2a and 2b** show regional tissue expansion across the lung of a healthy littermate and β-ENaC mouse at peak inspiration.
- **Figure 2c** shows a probability density function (PDF) of local tissue expansion for each animal. In the β-ENaC mouse, the larger standard deviation (STD) of the fitted Gaussian (broken line) combined with the multi-modal peak of the PDF reflects a range of expansion across the lung and is indicative of the typical heterogeneity of the CF-like disease.
- **Figure 2d** shows the volume-time curves for each mouse. The volume of air displaced during the breath is normalized according to individual lung volume (which is typically larger in β-ENaC mice). As expected, the expiratory time constant is larger in the sick mouse.

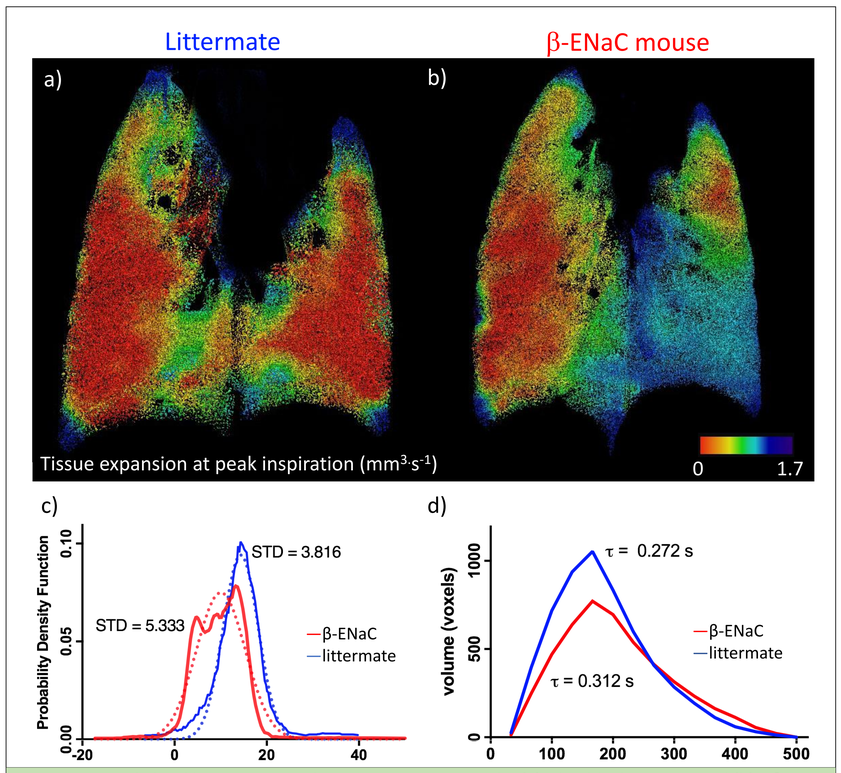

Figure 2: Example local lung expansion from XV measurements for (a) a healthy littermate and (b) a β-ENaC mouse. Panel (c) shows the spread of local displacement and (d) the change in volume during inhalation for both the β-ENaC mouse (red) and the healthy littermate (blue).

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4. Analysis and Discussion

- **Figure 3** shows a statistically significant difference in the combined expiratory time constant between the β-ENaC and littermate animals.
- **Figure 4** shows the lung hysterisivity (η) from the FOT measurements, a global measure that indicates disease heterogeneity, although the location of the functional deficits cannot be determined.

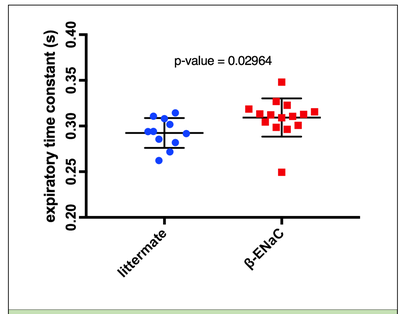

Figure 3: Expiratory time constant from XV

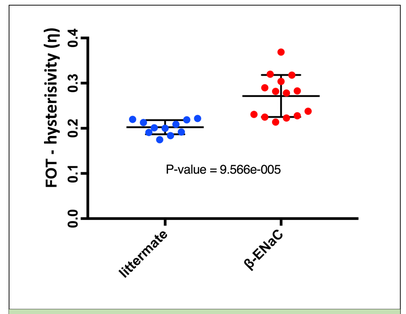

Figure 4: Global FOT measurement

- **Figure 5** compares these whole-lung XV and FOT measures, with a clear separation between three groups. The separation of the blue and black groups (see below) is only possible through the XV analysis, which captures and visualises regional differences across the lung.
- Interestingly, in half of the littermates we observed a pattern of highly bimodal PDF peaks in the XV data, which we traced back to uncharacteristic "dark" areas in the lung expansion volumes, always on the same side of the lung. An example is shown with in **Figure 5b** (c.f. Fig. 2 a). We hypothesize this is due to uneven ventilation as a results of a deep single-lung intubation.

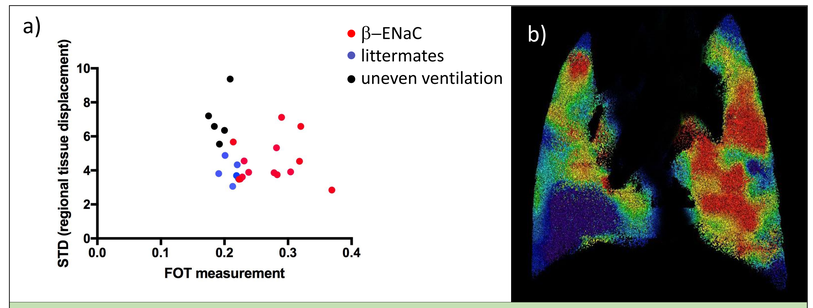

Figure 5: (a) FOT data (η) vs XV data for β-ENaC (red) mice, littermates (blue) and those littermates where we suspect uneven ventilation (black), due to (b) sharply defined low displacement areas in the displacement map of those littermates (same colorbar as Fig. 2).

5. Conclusion

- This non-invasive method is a novel technique for assessment of disease and treatment in CF lung disease.
- The variability seen in β-ENaC mice is an accurate reflection of the variability of this disease.
- Importantly, unlike the global measures available from FOT, XV allows the location of the disease to be determined.