

# Flow of Room Air Leads to Rapid Changes in Mucociliary Transport in the Tracheal Epithelium

Susyn Joan Kelly

Fisher & Paykel Healthcare

Paul Martinsen

Blue Leaf Software Ltd

Stanislav Tatkov (✉ [Stanislav.Tatkov@fphcare.co.nz](mailto:Stanislav.Tatkov@fphcare.co.nz))

Fisher & Paykel Healthcare <https://orcid.org/0000-0002-2064-0481>

---

## Research

**Keywords:** mucociliary transport, cilia, mucus, video-microscopy, airway epithelium, humidity

**Posted Date:** October 7th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-81509/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published on March 17th, 2021. See the published version at <https://doi.org/10.1186/s40635-021-00374-y>.

# Abstract

**BACKGROUND:** Inspired air is heated and humidified in the nose before it reaches lower airways. This mechanism is bypassed during tracheostomy, directly exposing the lower airways to colder and drier air from the environment, which is known to have negative effects on mucociliary transport; however, little is known about how quickly mucociliary transport deteriorates. The purpose of this study was to determine the short-term effect of flowing room air on mucociliary transport in the trachea. In an ovine perfused *in vitro* tracheal model (N=7) the epithelium was exposed to 25 L/min of flow, heated to lamb body temperature (38 °C) and fully saturated with water vapor as the control, followed by room air (22 °C and 50% relative humidity) for a short duration, until mucociliary transport had visually stopped. Mucus transport velocity (MTV) and cilia beat frequency (CBF), as well as the area of the surface with beating cilia, were continuously measured with video-microscopy.

**RESULTS:** Exposing the tracheal epithelium to air heated to body temperature and fully humidified resulted in stable MTV  $9.5 \pm 1.1$  mm/min and CBF  $13.4 \pm 0.6$  Hz. When exposed to the flow of room air, MTV slowed down to  $0.1 \pm 0.1$  mm/min in  $2.0 \pm 0.4$  seconds followed by a decrease in CBF to  $6.7 \pm 1.9$  Hz, after  $2.3 \pm 0.8$  second. Both MTV and CBF recovered to their initial state when heated and humidified air-flow was re-introduced.

**CONCLUSIONS:** This study demonstrates mucociliary transport can deteriorate within seconds of exposing the tracheal epithelium to flowing room air. The reduction in MTV precedes slowing of CBF. Their relationship is non-linear and a minimum CBF of approximately 6 Hz is required for MTV > 0. Clinically these findings indicate a potential rapid detrimental effect of breathing with non-humidified air via bypassed upper airways.

## Background

During nasal breathing, room air is heated and humidified when passing through the upper airways [1] and becomes fully saturated with water vapor just below the carina [2, 3]. In spite of variable inspired air temperatures, humidities, and flows, the upper airways are able to heat and humidify incoming air due to the extensive vascular system in the nasal mucosa, which is capable of producing large and varying quantities of secretions [4]. Inadequate humidification of inspired air leads to impaired mucociliary transport in the tracheobronchial tree [5]. In clinical practice, patient with tracheostomy are at a greater risk of inadequate humidification due to the bypassed upper airways. When nasal breathing, most heat and moisture exchanges between the air and airway surface occur in the nose; the tracheobronchial mucosa only adds 20% of the total water required for the relative humidity (RH) to reach 100% in the main bronchi [6]. In contrast, patients breathing through the stoma in the trachea forces the tracheobronchial mucosa to take over much of the air conditioning, causing the mucosa to have a greater water loss as it is now required to add 85% of the total water required to achieve RH 100% in the main bronchi [6]. The increased water demand from tracheobronchial epithelium is not well compensated, which results in thick secretions and impaired mucociliary transport [7–11]. These symptoms are

commonly found in patients with tracheostomy unless careful attention is given to heating and humidifying inspired air.

Williams et al. [12] proposed a model for the relationship between airway mucosal dysfunction and the temperature and humidity of the inspired gas. The authors suggest that respiratory gases at body temperature and RH 100% are optimal for mucociliary transport and even small changes to the temperature or humidity result in mucociliary transport dysfunction. Subsequent publications have supported this model, showing a deterioration in mucus transport velocity (MTV) and cilia beat frequency (CBF) after prolonged exposure (2 to 3 hours) to air temperature and humidity below the conditioning provided by the upper airways [5, 13].

Exposing the airways to cold, dry air is known to influence mucociliary transport, but how quickly changes begin to occur is not fully understood [5, 13]. In this study, we tested the hypothesis that exposing the airway epithelium to the flow of room air, that occurs during spontaneous breathing, will have a negative effect on mucociliary transport. Publications to date have measured the effect of air temperature and humidity on either MTV or CBF over hourly time intervals [5, 13]. However, there are no reports of MTV and CBF measurements over shorter time periods. In this experiment, we used a perfused *in vitro* tracheal model to continuously measure MTV and CBF, second-by-second, using video-microscopy to monitor changes in mucociliary transport when the tracheal epithelium was exposed to the flow of room air.

## Methods

### *In vitro* ovine model

As described previously [13, 14] and briefly here, lamb tracheas (ca. 160 mm in length) were collected from a local abattoir immediately after slaughter and were transported to the laboratory. The tracheas were opened longitudinally along the ventral mid-line and fixed flat with the epithelium positioned upward in a custom-made bath placed on a vibration-proof table (Fig. 1). The opposite side of the trachea was bathed in recirculating Krebs-Henseleit solution (flow 50 mL/min, 2 L reservoir) at 38 °C that was oxygenated with carbogen gas. A flow generator was connected to a three-way valve, which allowed the air path to switch between a membrane humidifier or a bypass path before connecting to the tracheal bath. The flow generator was set to 25 L/min to create unidirectional air-flow over the tracheal epithelium surface with an estimated velocity across the surface of ca. 0.4 m/s. The air was conditioned to 38 °C and RH 100%, passing through the Nafion™ (DuPont, USA) membrane humidifier (FC-125-240-5PP, Perma Pure, New Jersey, USA) with perfused and temperature-controlled deionized water for precise control of temperature and humidity of incoming air. Delivery of room air (22 °C and RH 50%) to the tracheal bath was achieved by switching the three-way valve to the bypassed path. The top cover of the bath included a heated optical window with an anti-reflecting coating for video-microscopy.

### Video-microscopy

A video microscope (VMU-V, Mitutoyo, Japan) with a long working-distance lens (M Plan ApoNIR 5x, Mitutoyo, Japan) and in-line coaxial illumination was connected to a monochrome digital camera (Lm075, Lumenera, Canada), which recorded mucociliary transport at 60 frames per second over an area of 1.0 mm × 0.7 mm (640 × 480 pixels). The experiments were performed 1 hour after the tissues were mounted to allow dissipation of any released mediators [13].

## Temperature and humidity settings

The effect of a change in the air temperature and humidity on mucociliary transport was observed in 7 tracheas. Video-microscopy recordings were made for the duration of each experiment. The experiments started with the air temperature and humidity set to body temperature of lambs (38 °C) and RH 100% before intermittent exposure to 25 L/min flow of room air at 22 °C and RH 50% until the movement of particles across the video-microscope field of view had stopped, after which the humidified air-flow was returned.

## Video-microscopy analysis

MTV was determined from the recordings by tracking the natural visible particles on the surface of the mucus as they moved across the field of view. CBF was determined using Fourier analysis of the video-microscopy recordings with a rolling 128 frame window and curve fitting to locate frequency peaks using Matlab (MathWorks, USA, see Additional files). CBF was measured in ten analysis regions: one region covering most of the field of view and nine smaller regions, arranged in a 3-by-3 grid (Fig. 2). These smaller regions were used to measure variation in CBF over the field of view. The relative area, within the field of view, where cilia activity was visible during mucociliary transport and after mucociliary transport had ceased was calculated from the recordings.

## Data Analysis

Measurements are presented as the mean ± standard deviation and were compiled and analyzed in Excel (Microsoft, USA) and GraphPad Prism 5 software (Prism, USA). Differences were considered statistically significant when a two-tailed t-test produced a  $p < 0.05$ .

## Results

Video-microscopy recordings of the tracheal epithelium show mucociliary transport moving particles across the field of view, from which MTV was obtained, and beating cilia as a background flicker, from which CBF was obtained (Video 1). A notable reduction in the particle speed and the background flicker is observed when the tracheal epithelium was exposed to the flow of room air. Short room air exposure times were sufficient to stop mucus transport but not long enough to cause any irreversible damage to

mucociliary transport, seen as the MTV and CBF recovering to initial values when air was returned to body temperature (38 °C) and RH 100% (Fig. 3).

When the air temperature and humidity were decreased by exposing the tracheal epithelium to room air, the MTV changed almost immediately, while the CBF started changing after a  $2.3 \pm 0.8$  second delay and did not stop completely before heated and humidified air was restored (Fig. 4). The changes in MTV and CBF caused by the exposure to room air resulted in significant decreases in MTV, which dropped from  $9.5 \pm 1.1$  mm/min to  $0.1 \pm 0.1$  mm/min ( $p < 0.05$ ) in  $2.0 \pm 0.4$  seconds and in CBF, which dropped from  $13.4 \pm 0.6$  Hz to  $6.7 \pm 1.9$  Hz ( $p < 0.05$ ) in  $3.7 \pm 0.6$  seconds (Table 1). The changes in MTV and CBF were highly correlated ( $R^2 = 0.87$ , Fig. 5) using a second-order polynomial model.

Table 1. Mean mucus transport velocity (MTV) and cilia beat frequency (CBF) measurements under body temperature and fully saturated with water vapor (38 °C and relative humidity (RH)100%) and room air (22 °C and RH 50 %) from each analyzed video. Results are presented as the mean  $\pm$  standard deviation. Time measurements represent the time the MTV and CBF took to change from measurements made at 38 °C and 100% RH to measurements made at 22 °C and RH 100%. The delay is the difference between the MTV and the CBF times taken for change.

Sample	Mucus Transport Velocity (mm/min)			Cilia Beat Frequency (Hz)			Delay (s)
	38 °C and RH 100%	22 °C and RH 50%	Time (s)	38 °C and RH 100%	22 °C and RH 50%	Time (s)	
1	$7.6 \pm 0.1$	$0.2 \pm 0.1$	2.0	$11.9 \pm 0.2$	$6.3 \pm 0.0$	3.6	1.6
2	$9.2 \pm 0.2$	$4.8 \pm 0.7$	2.2	$11.7 \pm 0.1$	$8.0 \pm 0.2$	4.9	2.7
3	$12.2 \pm 0.1$	$1.3 \pm 0.3$	1.9	$17.1 \pm 0.2$	$10.3 \pm 0.2$	3.8	1.9
4	$10.6 \pm 0.4$	$0.7 \pm 0.5$	1.4	$11.4 \pm 0.1$	$3.8 \pm 0.0$	2.7	1.3
5	$9.8 \pm 0.7$	$0.2 \pm 0.1$	2.6	$16.7 \pm 0.5$	$6.9 \pm 0.0$	3.5	0.9
6	$10.2 \pm 0.5$	$0.5 \pm 0.4$	2.4	$15.3 \pm 0.1$	$4.7 \pm 0.1$	4.1	1.7
7	$9.6 \pm 0.3$	$1.3 \pm 0.8$	1.7	$13.6 \pm 0.2$	$6.7 \pm 0.0$	3.6	1.9

Variability in CBF was observed visually and measured in the nine regions spread across the field of view (Fig. 6). This showed that the greatest change in CBF (> 80%) in regions closest to the air-flow inlet (left side of the field of view) when air temperature and humidity were changed.

Cilia activity was observed in approximately 60% of the field of view when mucociliary transport had ceased (MTV = 0), though cilia activity continued in as much as 93% in one sample and was as low as 28% in another. The regions where cilia remained active were typically longitudinally aligned with the

perfused tracheal tissue (Fig. 7). Cilia activity was visible in 89–100% of the video frame when the cilia were exposed to heated and humidified air. The remaining parts of the video were too dark to discern if cilia activity was present, possible because of the contours of the tissue reducing reflection of light during a coaxial illumination.

## Discussion

Analysis of video-microscopy recordings made of the tracheal epithelium surface enabled simultaneous measurements of MTV and CBF and the ability to observe their rapid changes when exposed to a drop in air temperature and humidity. The presented results show both MTV and CBF decrease quickly when the epithelium is exposed to room air at 25 L/min, a flow comparable to that observed during normal breathing [15]. These changes were reversible when the epithelium was exposed to air heated to body temperature and fully saturated with water vapor. Irreversible changes to mucociliary transport on the tracheal epithelium have been reported after 180 min of exposed to air cooler than body temperature and fully saturated with water (30°C, RH 100% and 34°C RH 100%) [13] and during ventilation *in vivo* with air at 23°C and RH < 10% [5]. The strong correlation between MTV and CBF when the trachea was exposed to heated and humidified air is consistent with a causal relationship, but the delayed change in CBF compared to MTV when exposed to room air (22 °C and RH 60%) suggests that the slowing of MTV was not initiated by a reduction of CBF, but rather by changes to the mucus properties.

Although our data shows MTV and CBF to be highly correlated under stable conditions, MTV was found to change more quickly than CBF when the epithelium was exposed to room air. Kilgour et al. 2004 [13] reported a similar result when the tracheal epithelium was exposed to low air temperatures for a prolonged period; the CBF had a longer survival time compared to the MTV. Cold, dry air changes mucus properties, such as viscosity, and causes changes to cilia structure and coordination [11, 16, 17]. The initial decrease in MTV reported in the study could be attributed to changes in mucus properties and the subsequent slowing of CBF. Mucus properties are known to be important for maintaining effective mucus-cilia interactions, required for MTV to clear debris from the airway [18, 19]. Mucus is a hydrogel and acts as a protective layer where it maintains hydration and prevents desiccation of the ciliated epithelium cells [20]. The mucus layer is in direct contact with air passing through the conducting airways and is the first to respond to changes in air conditions. When inspired air is colder and dryer, the thermal imbalance forces heat and moisture transfers from the mucus surface into the air. This removes water from the mucus layer by evaporation, which in turn lowers the surface temperature, and ultimately causes the mucus to become more viscous [21, 22]. Mucus with greater viscosity may interfere with the cilia's ability to propel it along the surface, which can result in mucus accumulation and the need for suctioning in patients with tracheostomy [7]. With prolonged exposure, the protective function of the mucus layer lessens and thermal changes would begin to affect the beating cilia underneath, causing cilia activity to decline [13]. Cilia are known to be affected by temperature [23] and their discoordination affects mucociliary transport [24]. The thermodynamic balance between latent and sensible heat transfer during normal inspiration and exhalation, which infers water movement in and out of the mucus as a result of different temperature and water vapor content in air, needs to be investigated *in vivo*.

In the time scales considered in this study, the decrease in MTV appears to precede the slowing of the CBF and this effect is also expected to occur outside of the recorded field of view. As viscosity increases, the cilia's combined force is no longer sufficient to propel the mucus layer. Mucus is a non-Newtonian fluid [25] and its viscosity decreases under shear forces introduced by the beating cilia. It is also possible that, due to water losses, the mucus becomes more viscous, leading to a reduction in CBF that slows MTV. A visual inspection of the video-microscopy recordings revealed the debris on the top of the mucus layer went out of focus when MTV slowed down. This change in the recording's focus suggested the airway surface liquid, including the periciliary layer, receded, caused by the evaporation of water when the tracheal epithelium was exposed to flowing room air.

Another potential mechanism that may reduce CBF is from the cooling of the airway epithelium. The lower temperature of room air and the evaporation of water from the epithelium cause the heat losses which decrease the temperature of ciliated epithelium, slowing biochemical reactions in microtubules of the motile cilia and result in reduced CBF. Jones et al. [26] estimate that evaporation from the mucus surface leads to a 2 to 3 °C change of surface temperature in the nasopharynx during quiet breathing with room air. In addition, Smith et al. [27] showed that nasal cilia continue to beat with a normal pattern at temperatures as low as 2 °C. Although the authors did not measure the surface temperature of the tracheal epithelium, and the above mentioned reports were measured in the nose, known to tolerate different air temperatures [4], it is unlikely that the cilia in the tracheal epithelium measured in this study were cooled below 2 °C. This suggests that a change in physical properties of the airway surface liquid, from rapid dehydration and an increase in viscosity, caused CBF to slow down.

Regional variation in CBF could be caused by the variability in mucus thickness and non-homogeneous mucus properties which occur naturally on the trachea's surface [28]. Variability in mucus properties result from proximity to secretory cells and surface contours which creates streams and plaques of mucus movement [29]. The evaporation of water and the subsequent changes of mucus viscosity and MTV are likely to be slower in regions where the mucus layer is thicker, or partly protected by contours in the tissue, allowing patches of cilia activity to continue. This can be seen in the video-microscopy recordings as areas where cilia continue to beat, after exposure to room air, while appearing stationary in others.

Limitations of this study include a lack of humidity measurements of the air above the trachea and the temperature on the surface of the tracheal epithelium. While this information would be useful for demonstrating how quickly room air begins to affect mucociliary transport on the tracheal surface thermodynamically, the intent of these experiments was to present changes in tracheal mucociliary transport when exposed to the flow of room air (25 L/min) that could occur during breathing through a tracheostomy or an endotracheal tube, without humidified inspired air. The flow profiles usually found in normal breathing show that the maximum inspiratory flow is only reached over a short period; however, in this experiment design the authors used a constant flow, comparable to this maximum only experienced intermittently during normal breathing, to observe the effects on mucociliary transport. In addition, the flow in the experiment design was unidirectional, preventing the heat and moisture recovery during

exhalation in tidal breathing. This design enabled the authors to assess mucociliary transport for analyses of the transition period and it negated any confounding effects from ventilation with varying respiratory rates and tidal volumes, that may induce shear stress, which is known to influence mucociliary transport [14, 30].

Many clinical questions related to time of exposure of the epithelium to respiratory gases with different levels of humidification need to be assessed during tidal breathing. We did not study physical properties of mucus because of the short duration of exposure of the epithelium to room air and the complexity of collection of mucus in very small quantities. Also, due to the limited resolution of the video-microscopy recordings the authors were unable to look closely into the cilia coordination on very short time scales. The chamber used to house the perfused trachea had a rectangular cross-section for the air path when it passed over the epithelium, which may have produced a different velocity profile to that found in the cylindrical trachea. This difference in flow speed, mixing and eddy could also have an effect on the heat and moisture exchange between the epithelium and air.

It is unlikely that the circulating Krebs-Henseleit solutions on the outer surface of the trachea could substitute blood microcirculation, which maintains the heat and moisture on the epithelial surface *in vivo*. Therefore, extrapolating the presented results to the clinical condition should be performed with caution as the effects reported in the study could represent an extreme. However, these *in vitro* experiments demonstrate high sensitivity of the tracheal epithelium to changes in temperature and humidity induced by flow of room air over the tracheal epithelium over few seconds. The long-term effects of cold and dry air on mucociliary transport and the airway epithelium require an *in vivo* setting to reproduce the complex thermodynamic and physiological mechanisms that maintain water content in the airway surface liquid during breathing.

## Conclusion

This study demonstrates that mucociliary transport can deteriorate within seconds of exposure to the flow of room air. The reduction in MTV precedes the slowing of the CBF, which suggests that, at least initially, it is a change in physical properties of the airway surface liquid that affects the mucus-cilia interactions. These findings suggest that exposing conducting airways to the flow of room air can rapidly impair mucociliary transport, which could potentially result in mucostasis with a risk of airway obstruction. Patients with an indwelling endotracheal tube or tracheostomy would be at greatest risk as the as the conditioning upper airways is bypassed.

## List Of Abbreviations

CBF - cilia beat frequency

MTV - mucus transport velocity

RH - relative humidity

# Declarations

## Ethics approval and consent to participate.

Not applicable: no human subjects.

## Consent for publication.

All authors consented.

## Availability of data and material.

Data available on request.

## Competing interests.

SK and ST are employee of Fisher & Paykel Healthcare, PM is a consultant of Fisher & Paykel Healthcare.

## Funding.

The study was funded by Fisher & Paykel Healthcare Limited.

## Authors' contributions.

Literature search by SK, Data collection by ST, Study design by ST, Analysis of data by SK and PM, Manuscript preparation by SK, Review of manuscript by PM and ST.

## Acknowledgements.

The authors would like to acknowledge Prof. Bruce Rubin and A/Prof. Michael Davis for reviewing the manuscript.

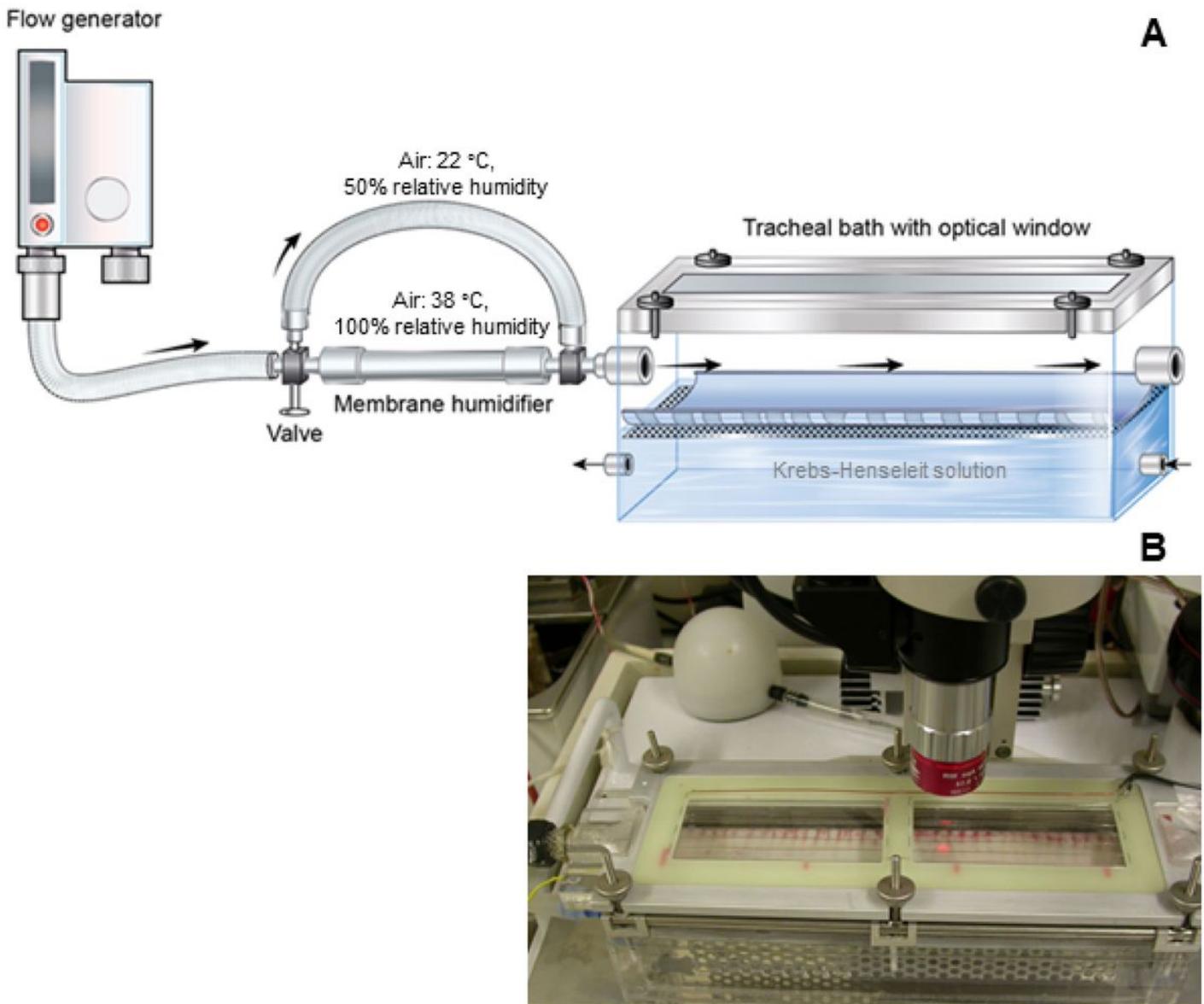
# References

1. Walker, J.E., R.E. Wells, Jr., and E.W. Merrill, *Heat and water exchange in the respiratory tract*. Am J Med, 1961. **30**: p. 259-67.
2. Déry, R., *Humidity in anaesthesiology IV: Determination of the alveolar humidity and temperature in the dog*. Canadian Anaesthetists' Society Journal, 1971. **18**(2): p. 145-151.
3. McFadden, E.R., Jr., et al., *Thermal mapping of the airways in humans*. J Appl Physiol (1985), 1985. **58**(2): p. 564-70.
4. McCutchan, J. and C.L. Taylor, *Respiratory heat exchange with varying temperature and humidity of inspired air*. J Appl Physiol, 1951. **4**(2): p. 121-35.
5. Hirsch, J.A., et al., *Effects of dry air and subsequent humidification on tracheal mucous velocity in dogs*. Journal of Applied Physiology, 1975. **39**(2): p. 242-246.

6. Toremalm, N.G., *A Heat-And-Moisture Exchanger for Post-Tracheotomy Care An Experimental Study*. Acta Oto-Laryngologica, 1960. **52**(1-6): p. 461-472.
7. Birk, R., et al., *Heated air humidification versus cold air nebulization in newly tracheostomized patients*. Head Neck, 2017. **39**(12): p. 2481-2487.
8. Alexopoulos, C., B. Jansson, and C.-E. Lindholm, *Mucus Transport and Surface Damage After Endotracheal Intubation and Tracheostomy. An Experimental Study in Pigs*. Acta Anaesthesiologica Scandinavica, 1984. **28**(1): p. 68-76.
9. Konrad, F., et al., *Mucociliary transport in ICU patients*. Chest, 1994. **105**(1): p. 237-41.
10. Boucher, R.C., *Muco-Obstructive Lung Diseases*. New England Journal of Medicine, 2019. **380**(20): p. 1941-1953.
11. Li, M., et al., *Cold temperature induces mucin hypersecretion from normal human bronchial epithelial cells through a transient receptor potential melastatin 8;mediated mechanism*. Journal of Allergy and Clinical Immunology, 2011. **128**(3): p. 626-634.e5.
12. Williams, R., et al., *Relationship between the humidity and temperature of inspired gas and the function of the airway mucosa*. Crit Care Med, 1996. **24**(11): p. 1920-9.
13. Kilgour, E., et al., *Mucociliary function deteriorates in the clinical range of inspired air temperature and humidity*. Intensive Care Medicine, 2004. **30**(7): p. 1491-4.
14. Tatkov, S. and R.J. Pack, *Symmetrical-waveform high-frequency oscillation increases artificial mucus flow without changing basal mucus transport in in vitro ovine trachea*. Respiratory Care, 2011. **56**(4): p. 435-41.
15. Elcner, J., et al., *Velocity profiles in idealized model of human respiratory tract*. EPJ Web of Conferences, 2013. **45**: p. 01025.
16. D'Amato, M., et al., *The impact of cold on the respiratory tract and its consequences to respiratory health*. Clinical and Translational Allergy, 2018. **8**(1): p. 20.
17. Davis, M.S., B. Schofield, and A.N. Freed, *Repeated Peripheral Airway Hyperpnea Causes Inflammation and Remodeling in Dogs*. Medicine & Science in Sports & Exercise, 2003. **35**(4).
18. Voynow, J.A. and B.K. Rubin, *Mucins, mucus, and sputum*. Chest, 2009. **135**(2): p. 505-512.
19. Button, B., et al., *A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia*. Science, 2012. **337**(6097): p. 937-941.
20. Crouzier, T., et al., *Modulating Mucin Hydration and Lubrication by Deglycosylation and Polyethylene Glycol Binding*. Advanced Materials Interfaces, 2015. **2**(18): p. 1500308.
21. Nachtigall, W., *Some aspects of Reynolds number effects in animals*. Mathematical Methods in the Applied Sciences, 2001. **24**(17-18): p. 1401-1408.
22. Lai, S.K., et al., *Micro-and macrorheology of mucus*. Advanced drug delivery reviews, 2009. **61**(2): p. 86-100.
23. Clary-Meinesz, C.F., et al., *Temperature effect on the ciliary beat frequency of human nasal and tracheal ciliated cells*. Biology of the Cell, 1992. **76**(3): p. 335-338.

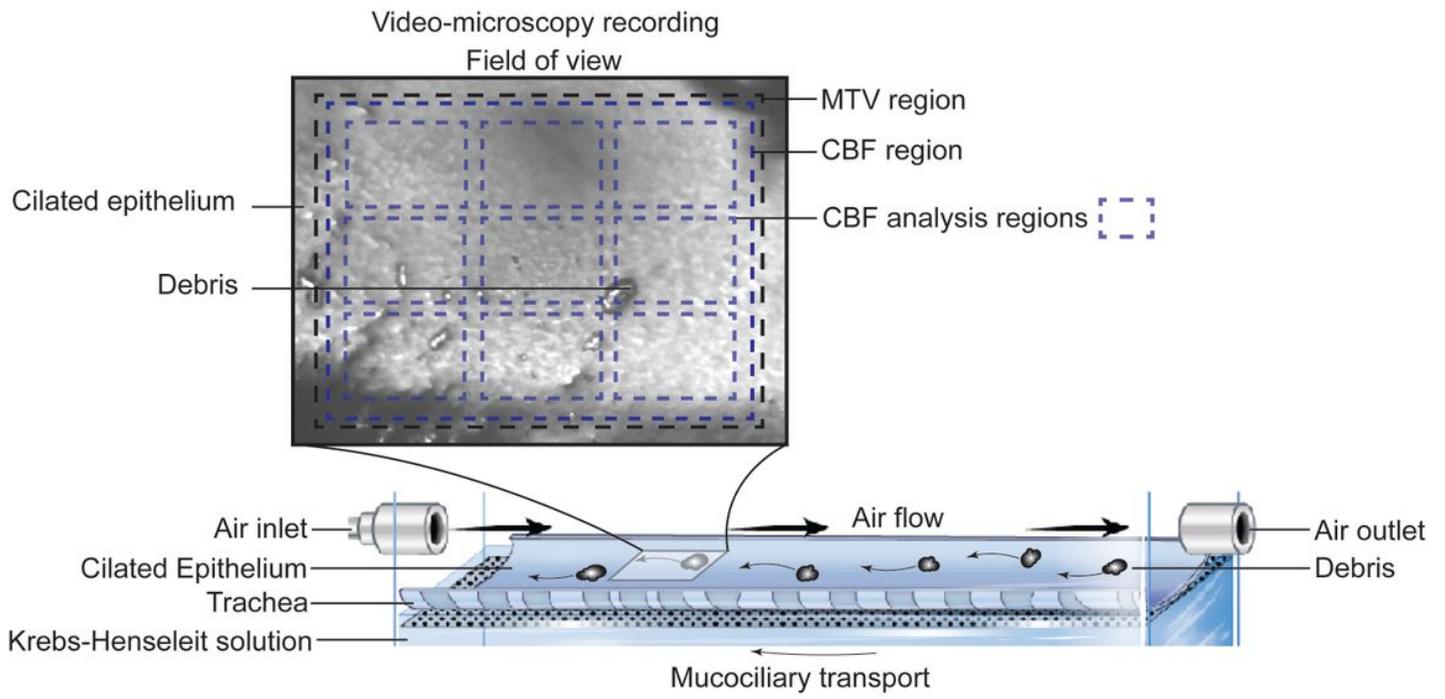
24. Möller, W., et al., *Mucociliary and long-term particle clearance in airways of patients with immotile cilia*. Respiratory Research, 2006. **7**(1): p. 10.
25. Lai, S.K., et al., *Micro- and macrorheology of mucus*. Advanced Drug Delivery Reviews, 2009. **61**(2): p. 86-100.
26. Jones, H.S., D.F. Proctor, and I.H.P. Andersen, *The Nose : upper airway physiology and the atmospheric environment*. 1982, New York: Elsevier Biomedical Press.
27. Smith, C.M., et al., *Cooling of Cilia Allows Functional Analysis of the Beat Pattern for Diagnostic Testing*. CHEST, 2011. **140**(1): p. 186-190.
28. Gerber, V., et al., *Mucus quality on horse tracheal epithelium: microscopic grading based on transparency*. Respir Physiol, 1997. **107**(1): p. 67-74.
29. Van As, A., *Pulmonary airway clearance mechanisms: a reappraisal*. Am Rev Respir Dis, 1977. **115**(5): p. 721-6.
30. McEvoy, R.D., et al., *Lung mucociliary transport during high-frequency ventilation*. Am Rev Respir Dis, 1982. **126**(3): p. 452-6.

## Figures



**Figure 1**

(A) Diagram of the experimental setup used for in vitro measurements of mucociliary transport on the tracheal epithelium of perfused lamb trachea. Air-flow was set to 25 L/min and was directed from left to right while mucociliary transport was from right to left. Video-microscopy recordings were made through the heated optical window on the top of the tracheal bath to measure mucociliary transport along the tracheal epithelial surface. (B) Photo of the in vitro experiment setup with the trachea mounted in the organ bath, with circulation of Krebs-Henseleit solution below. The microscope with coaxial illumination is shown above.



**Figure 2**

Field of view from video-microscopy recordings and the analysis region for the mucus transport velocity (MTV) and cilia beat frequency (CBF) with the 3-by-3 gridded subset of regions for more detailed analysis. Arrows indicate the direction of air-flow and mucociliary transport.

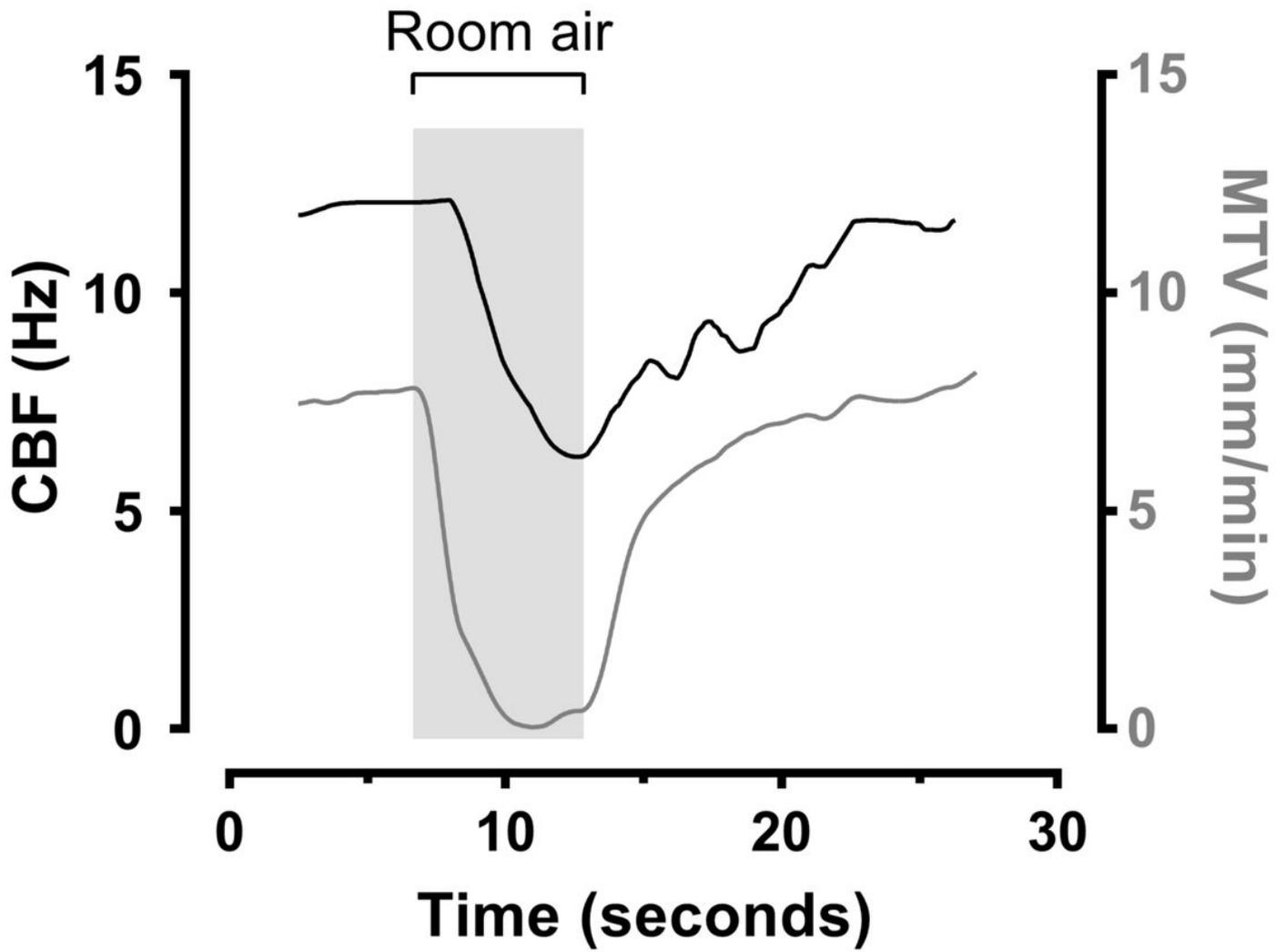
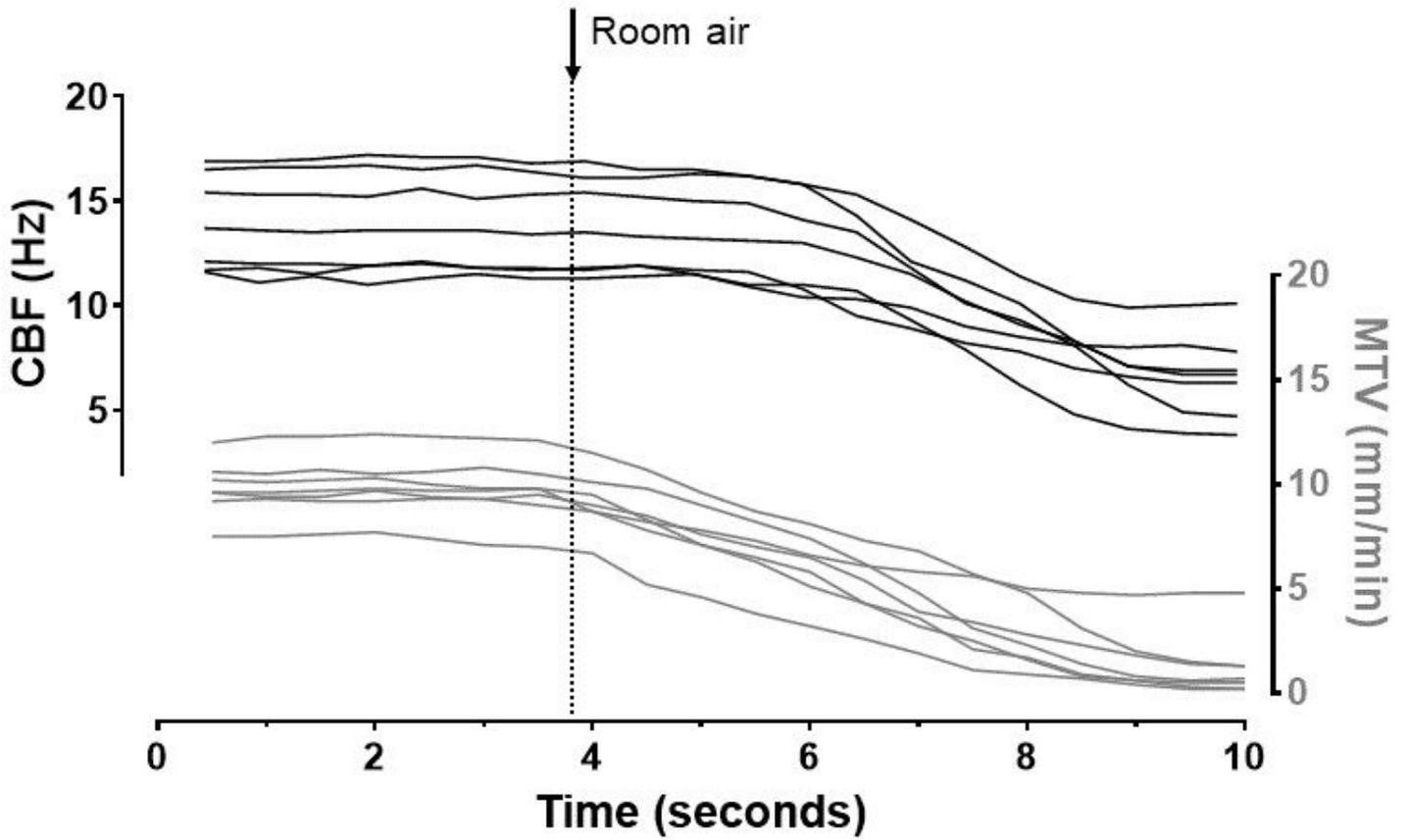


Figure 3

An example of the effect of intermittent exposure of room air on the cilia beat frequency (CBF) and mucus transport velocity (MTV) from a video-microscopy recording shown in the Additional files (Video 1). The gray region represents room air and white regions represent air at body temperature and fully saturated with water vapor.



**Figure 4**

Cilia beat frequency (CBF) (black) and mucus transport velocity (MTV) (gray) measured over time. The dotted vertical line marks the change in air passing above the surface from body temperature and fully saturated with water vapor (38 °C and 100% relative humidity) to room air (22 °C and 50% relative humidity).

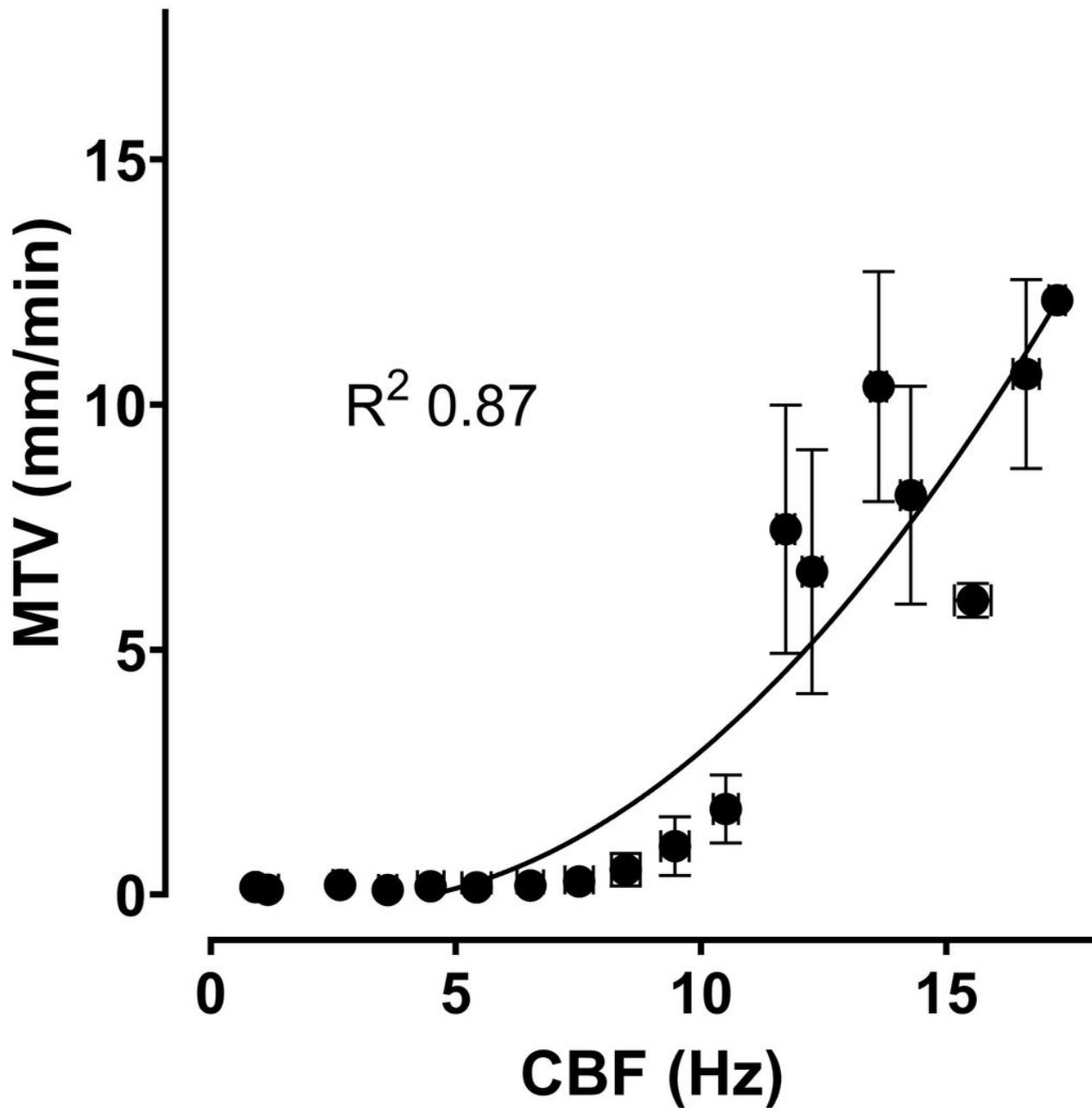
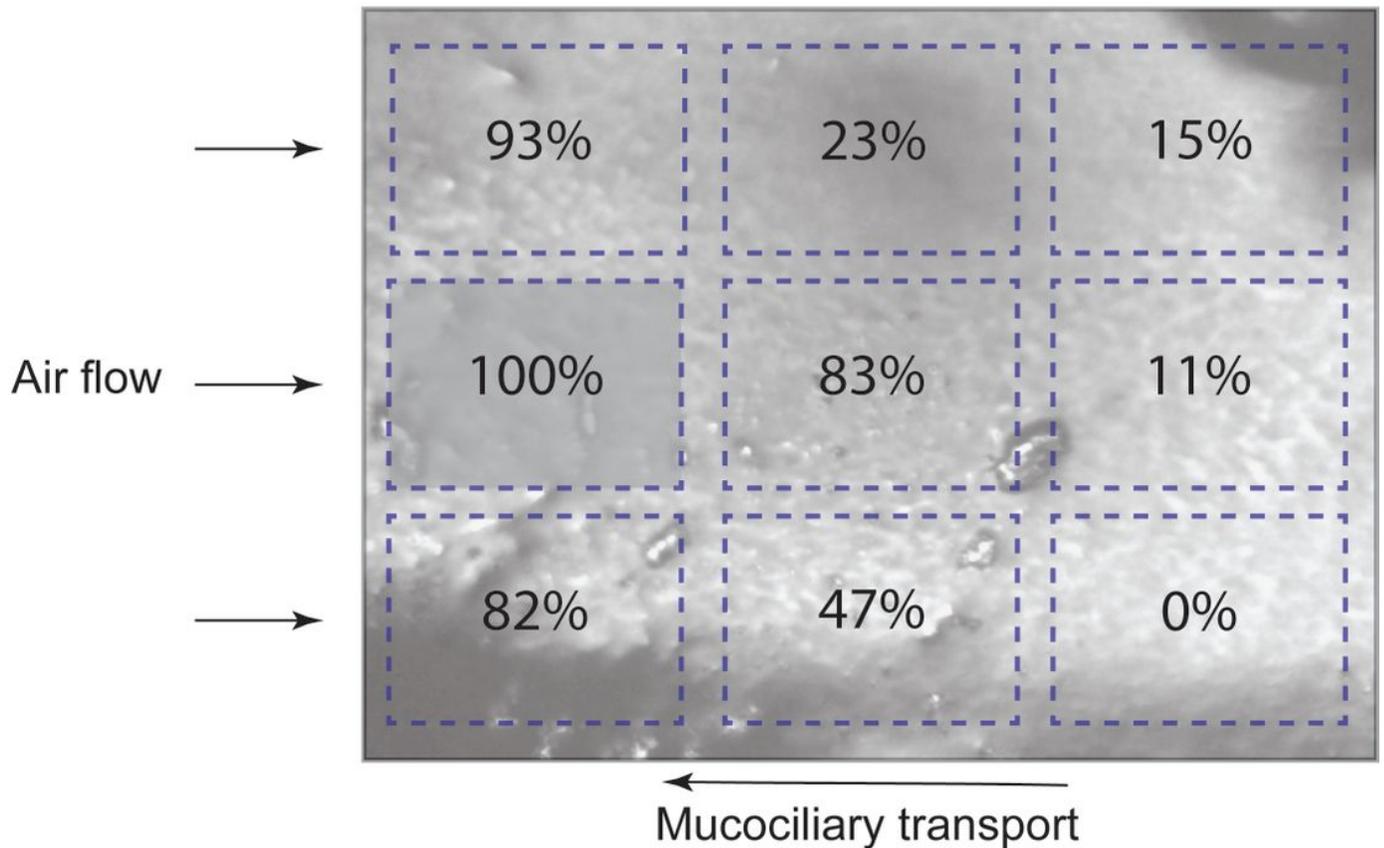


Figure 5

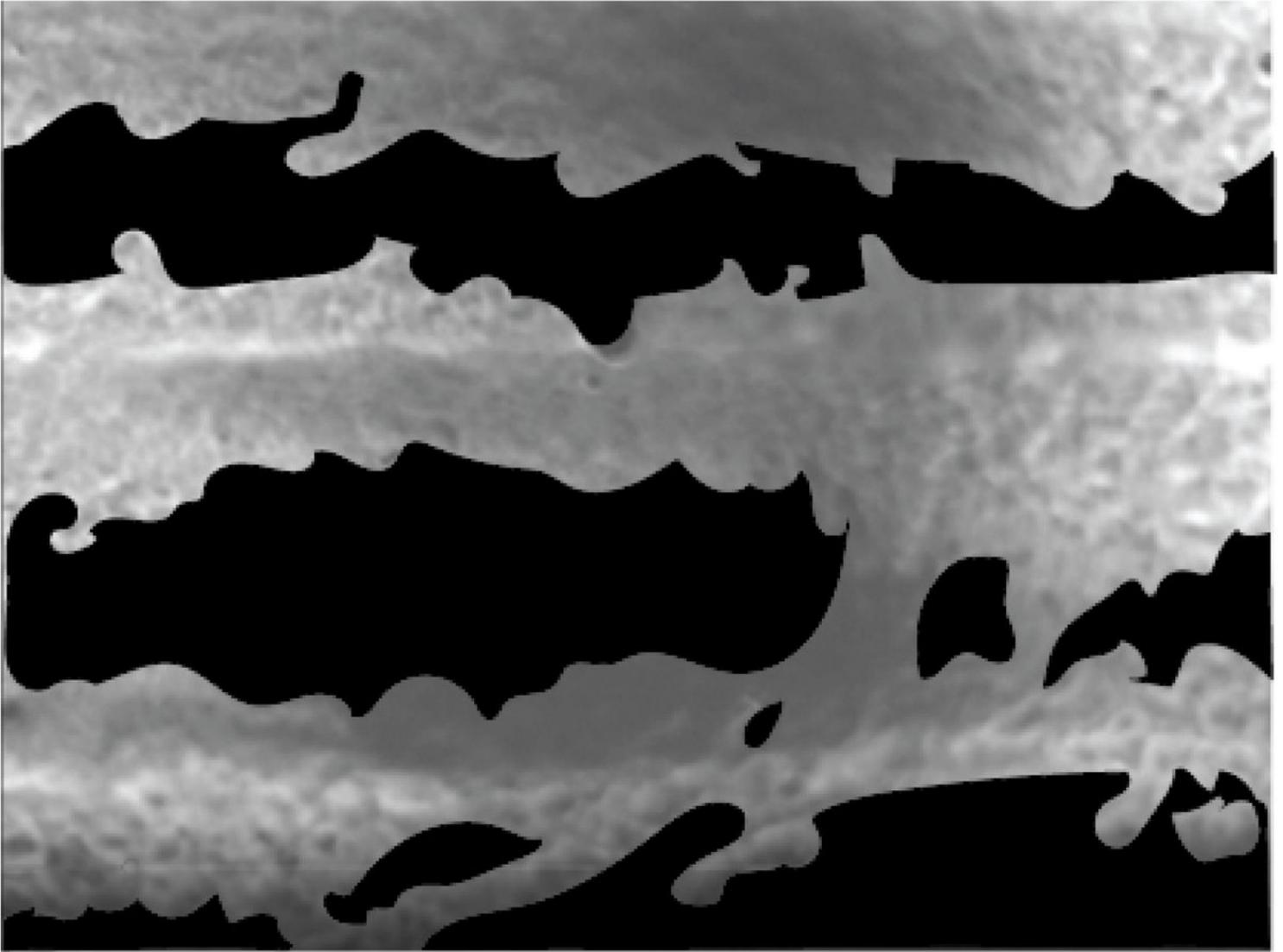
Correlation between overall mean mucus transport velocity (MTV) and cilia beat frequency (CBF) measurements under different air temperatures and humidities. Non-linear regression give an  $R^2$  of 0.87 and suggests that a minimum CBF of ca. 6 Hz is required for  $MTV > 0$ .

## Video-microscopy recording field of view



**Figure 6**

The average percentage change in cilia beat frequency, from all video-microscopy recordings, made when the tracheal epithelium was exposed to room air (22 °C and 60% relative humidity) where 100% represents the largest percentage change.



**Figure 7**

Representative image showing regions (white) where cilia activity continued (63% of the area) after mucociliary transport had ceased shown in Video 1.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile.docx](#)
- [AdditionalfileVideo1.mp4](#)