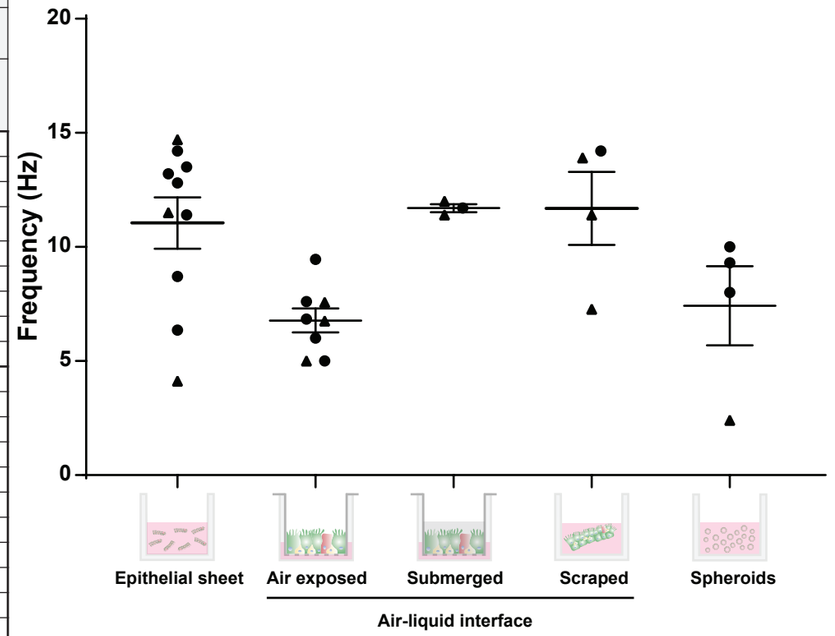
Reference	Model preparation for imaging					Imaging								Live-cell imaging environmental control unit										Sample			
	Epithelial sheet	Air-liquid interface			Spheroid	Objective				Frame rate (fps)				Chamber			Temperature (°C)			Humidity (%)		CO2 (%)		Healthy ●		Diseased Δ	
	Suspended	On permeable support		Scraped	Suspended	20×	40×	100×	N/D	120-200	300-400	500	N/D	Enclosed	Heated stage	N/D	23-26	37	N/D	85-90	N/D	5	N/D	n	CBF (Hz)	n	CBF (Hz)
		Air exposed	Submerged	Suspended																							
Coles et al. 2020 **(1)**	■							■				■					■				■		■			70	14.7
Dabrowski et al. 2021 **(2)**	■								■	■				■			■				■		■	3	14.2±0.61		
Chilvers & O'Callaghan 2000 **(3)**	■							■			■				■		■				■		■	20	13.2		
Chilvers et al. 2003 **(4)**	■							■			■				■		■				■		■	76	12.8 (Children); 11.5 (Adults)		
Nair et al. 2014 **(5)**	■								■				■		■		■				■		■	6	11.4		
Raidt et al. 2014 **(6)**	■						■			■					■		■				■		■	10	6.36		
Hirst et al. 2014 **(7)**	■							■				■		■	■		■				■		■	111	13.51	54	4.13
Hirst et al. 2010 **(8)**	■								■		■					■	■				■		■			231	11.5±1.6
Marthin et al. 2017 **(9)**	■								■	■								■			■		■	7	8.7		
Allan et al; this manuscript		■				■					■			■	■			■		■		■		3	7.61±0.11	3	6.75±0.17
Awatade et al. 2021 **(10)**		■				■					■			■	■			■		■		■		3	6.84±0.17	3	7.58±0.24
Chen et al. 2020 **(11)**		■							400×				■			■	■				■		■	18	5		
Carson et al. 2017 **(12)**		■				■							■			■			■		■		■	6	^		
Chioccioli et al. 2019 **(13)**		■					■			■				■				■		■		■		3	6	3	5
Bovard et al. 2020 **(14)**		■							4×	■				■				■			■		■	11	9.45		
Nair et al. 2014 **(5)**			■						■				■		■				■		■		■	4	11.7		
Rhee et al. 2001 **(15)**			■						■				■			■			■		■		■			28	11.4±1.3
Inui et al. 2018 **(16)**			■						■			■			■		■				■	■				N/D	12
Coles et al. 2020 **(1)**				■		■						■				■	■				■		■			70	13.9
Hirst et al. 2014 **(7)**				■				■				■		■	■		■				■		■	111	14.21	54	7.28
Hirst et al. 2010 **(8)**				■					■		■					■	■				■		■			187	11.4±2
Castillon et al. 2002 **(17)**					■		■						■			■			■		■		■	30	10±4		
Marthin et al. 2017 **(9)**					■				■	■						■	■				■		■	7	9.3	8	2.4
Jorissen & Bessems 1995 **(18)**					■		■						■			■					■		■	10	8		

N/D: not described; n: sample size; CBF: cilia bet frequency; ^reported as fold change

1. Coles, J. L. et al. A Revised Protocol for Culture of Airway Epithelial Cells as a Diagnostic Tool for Primary Ciliary Dyskinesia. Journal of Clinical Medicine. 9 (11), (2020).2. Dabrowski, M., Bukowy-Bierylło, Z., Jackson, C. L. & Zietkiewicz, E. Properties of Non-Aminoglycoside Compounds Used to Stimulate Translational Readthrough of PTC Mutations in Primary Ciliary Dyskinesia. International Journal of Molecular Sciences. 22 (9), (2021).3.Chilvers, M. A. & O'Callaghan, C. Analysis of ciliary beat pattern and beat frequency using digital high speed imaging: comparison with the photomultiplier and photodiode methods. Thorax. 55 (4), 314-317, (2000). 4.Chilvers, M. A., Rutman, A. & O'Callaghan, C. Functional analysis of cilia and ciliated epithelial ultrastructure in healthy children and young adults. Thorax. 58 (4), 333-338, (2003). 5. Nair, C. et al. Cyanide levels found in infected cystic fibrosis sputum inhibit airway ciliary function. European Respiratory Journal. 44 (5), 1253-1261, (2014). 6. Raidt, J. et al. Ciliary beat pattern and frequency in genetic variants of primary ciliary dyskinesia. European Respiratory Journal. 44 (6), 1579-1588, (2014). 7. Hirst, R. A. et al. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. PloS One. 9 (2), e89675, (2014). 8. Hirst, R. A., Rutman, A., Williams, G. & O'Callaghan, C. Ciliated air-liquid cultures as an aid to diagnostic testing of primary ciliary dyskinesia. Chest. 138 (6), 1441-1447, (2010). 9. Marthin, J. K., Stevens, E. M., Larsen, L. A., Christensen, S. T. & Nielsen, K. G. Patient-specific three-dimensional explant spheroids derived from human nasal airway epithelium: a simple methodological approach for ex vivo studies of primary ciliary dyskinesia. Cilia. 6 3, (2017). 10. Awatade, N. T. et al. Significant functional differences in differentiated Conditionally Reprogrammed (CRC)- and Feeder-free Dual SMAD inhibited-expanded human nasal epithelial cells. Journal of Cystic Fibrosis. 20 (2), 364-371, (2021). 11. Chen, Q. et al. Host Antiviral Response Suppresses Ciliogenesis and Motile Ciliary Functions in the Nasal Epithelium. Frontiers in Cell and Developmental Biology. 8 581340, (2020). 12. Carson, J. L. et al. Temporal structure/function variation in cultured differentiated human nasal epithelium associated with acute single exposure to tobacco smoke or E-cigarette vapor. Inhalation Toxicology. 29 (3), 137-144, (2017).13. Chioccioli, M., Feriani, L., Kotar, J., Bratcher, P. E. & Ciciuta, P. Phenotyping ciliary dynamics and coordination in response to CFTR-modulators in Cystic Fibrosis respiratory epithelial cells. Nature Communications. 10 (1), 1763, (2019). 14. Bovard, D. et al. Comparison of the basic morphology and function of 3D lung epithelial cultures derived from several donors. Current Research in Toxicology. 1 56-69, (2020). 15. Rhee, C. S. et al. Ciliary beat frequency in cultured human nasal epithelial cells. Annals of Otology, Rhinology and Laryngology. 110 (11), 1011-1016, (2001). 16. Inui, T. A. et al. Daidzein-Stimulated Increase in the Ciliary Beating Amplitude via an [Cl(-)] Decrease in Ciliated Human Nasal Epithelial Cells. International Journal of Molecular Sciences. 19 (12), (2018). 17. Castillon, N. et al. Polarized expression of cystic fibrosis transmembrane conductance regulator and associated epithelial proteins during the regeneration of human airway surface epithelium in three-dimensional culture. Laboratory Investigation. 82 (8), 989-998, (2002). 18. Jorissen, M. & Bessems, A. Normal ciliary beat frequency after ciliogenesis in nasal epithelial cells cultured sequentially as monolayer and in suspension. Acta Oto-Laryngologica. 115 (1), 66-70, (1995).



Supplementary File 1. Summary of 18 publications showing diversity of culture and live-cell imaging parameters used to quantify cilia beat frequency in organotypic models of the airway epithelium.