SUMMARY

Lungs are protected from infection by mucociliary clearance. The coordinated beating of the cilia of the mucociliary epithelium lining the airways provides the airway's first line of defense: mucus traps particles and microorganisms, and moves them away from the lungs toward the larynx, where they are then expelled from the airways. Ciliary activity, manifested by the optimal frequency of ciliary beat **frequency** (CBF) and **coordination** of ciliary motion, is subject to numerous levels of regulation, including signals associated with invasion by infectious agents.

Using new techniques of live imaging of tracheal ciliary activity that we have developed, we have recently found that CBF and the overall coordination of ciliary movement are linked, and that manipulation of CBF affects the coordination of the ciliary beat within and between ciliated cells, thus ensuring robust flow and airway clearance. We have also identified agents, including spike S1 protein of SARS-CoV-2, that affect the CBF and coordination of the ciliary beat in tracheal explants, thwarting the ability of the cilia to generate flow and expel the foreign particulates.

Ciliary beat coordination, despite its importance, has not been adequately studied, mainly because of the complexity of the analysis. To address this gap, we propose to combine the efforts of three teams of scientists with expertise in cell biology, imaging, and machine learning to develop an approach for unbiased evaluation and analysis of the coordination of ciliary motion.

We will generate an extensive library of dynamic representations of ciliary movement and fluid flow in diverse settings including various treatments, mouse mutants, ciliopathy and S1 protein. This library will be analyzed by using a computer vision pipeline to quantify the changes and automate the analysis. Furthermore, we will probe the basic mechanisms that may be responsible for the link between the beat frequency and beat coordination. We expect that our project will provide the first unbiased description of ciliary dynamics in live tissue, which can be also applied to other settings, for instance the CSF transport in the brain ventricles. Our project will advance understanding of the fundamental mechanisms underlying cooperative and coordinated ciliary motion and enable screening of compounds and treatments targeting ciliopathies and viral infections. Furthermore, the results of this cross-disciplinary effort will aid in securing external funding for the project.