Spatial reorganization of F-actin in respiratory cells as measured by Brillouin microscopy

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Brillouin microscopy has emerged as a non-invasive and label-free technique to map micro-mechanical properties of cells. Here we apply Brillouin microscopy to probe reorganization of F-actin network in respiratory cells treated with Timothy grass pollen protein extracts. The results of our measurements in conjunction with clustering data analysis confirm spatial cellular reorganization of F-actin proteins and compromised junctional integrity in treated cells as compared to controls.

Direct respiratory exposure to highly concentrated, pollen-based aeroallergens is known to stimulate asthma in susceptible population with far reaching detrimental consequences for patients' wellbeing. Timothy grass pollen is described as one of the most common allergens contributing to asthma exacerbations. It has been well-known that actin, an essential cytoskeleton protein, plays a key role in regulating ion and solute transport via interactions with cell-cell junctional proteins. Loss of actin localisation near cell-cell junctions has been associated with pathophysiological changes to respiratory cellular barrier function, integrity and the severity of lung disease [1]. In this work, we apply Brillouin microscopy to study changes in cellular micromechanical properties and the distribution of filamentous actin (F-actin) in respiratory cells.

Brillouin microscopy is a non-invasive and label-free optical method that is used to map micromechanical properties in cells, tissues and biomaterials [2]. The Brillouin frequency shift (BFS) directly measures the speed of hypersound waves that, in turn, depends on the underlying structure, density and material elastic properties (Figure 1(A)). In heterogeneous samples such as cells, the Brillouin peaks have complex line shapes composed by overlapping lines of individual cellular components (cytoskeleton, cytoplasm, membrane, and nucleus). Numerical algorithms such as supervised and unsupervised clustering may be helpful in separating spectral components, improving analysis and interpretation of hyperspectral images. Here, we applied unsupervised clustering to separate raw measurement data into 4 categories: phosphate buffer solution, cell periphery, body and center (Figure 1(B)). Next, we calculated average BFSs specific to each region for cells treated with 0 mg/mL (control), 0.05 mg/mL, 0.5 mg/mL and 5 mg/mL of Timothy grass pollen protein extracts. Figure 1(C) shows that the cells treated with the highest concentration of pollen extract have increased localisation of F-actin in the central cell region. This finding, supported by the direct measurement of F-actin fluorescent intensity, points to the remodeling of actin network in cells as well as compromised junctional integrity as the result of Timothy grass pollen exposure [3]. Overall, this can shed light on the cellular mechanisms involved in the cell sensing of its local environment and disease pathways [3].

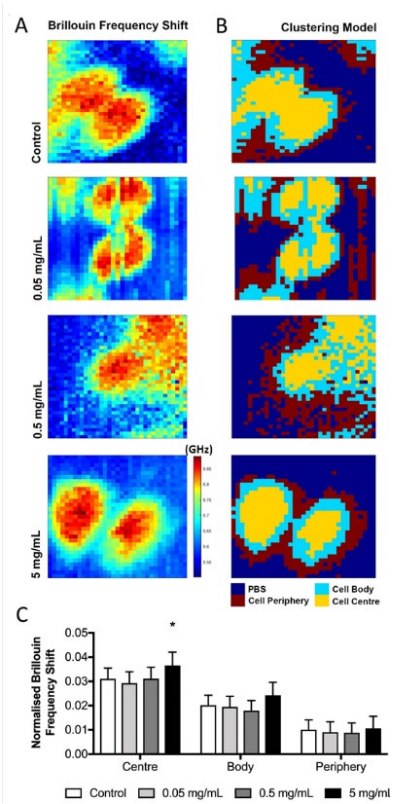

Fig. 1: Measurements of the Brillouin frequency shift (A) cells treated with 0 mg/mL (control), 0.05 mg/mL, 0.5 mg/mL and 5 mg/mL concentration of Timothy grass pollen protein extract. (B) Results of the clustering analysis applied to data in (A). (C) Averages across all clusters calculated from (B).

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[3] Bradbury *et al.* Timothy Grass Pollen Induces Spatial Reorganisation of F-Actin and Loss of Junctional Integrity in Respiratory Cells. *Inflammation* **45,** 1209–1223 (2022).