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High-Resolution Reconstruction of Human Lung Tissue Structure via Electron Cryo-Tomography

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Abstract: The 2017 Nobel Prize in Chemistry was awarded for advancements in cryogenic electron microscopy (cryo-EM) that were enabled by technologies such as direct electron detectors (DEDs) and the vitrification process, which allows for freezing specimens without forming crystalline ice. Traditionally, EM of biological specimens requires heavy modification, using methods like chemical fixation, heavy metal staining, and resin embedding. Cryo-EM approaches have mitigated many artifacts introduced by such methods. Vitrification prevents damage from crystalline ice, as well as preventing unwanted diffraction contrast in images. In recent years, the advancement of DEDs has eliminated the need to stain samples with heavy metals or image at high electron doses for adequate signal. Here, we aim to elucidate structures within the epithelial membrane of lung tissue, a system vital to understanding lung damage from viral infection. However, thick samples, such as tissue, still pose a significant challenge because they must be sliced into sections that are transparent to the electron beam, and physical sectioning with the knife of a microtome can introduce cutting artifacts. A solution to this is to prepare electron-transparent lamellae by a cryogenic focused ion beam (cryo-FIB) microscope. Cryo-FIB can be used to either thin a cell that has been frozen onto a substrate, or it can cut and lift out a region of interest in frozen tissue. After a lamella is produced via cryo-FIB, cryogenic electron tomography can be used to obtain high-resolution, 3D structural information from the lamella. The combination of cryo-FIB and cryo-TEM can provide high-resolution imaging of these native biological samples, as well as provide structural information at tomographic resolutions on samples not accessible by other methods. Here, we report progress on the cryo-FIB preparation and tomographic reconstruction of lung cells and tissues, correlated with structural data using traditional methods for larger length scale features.