Utilization of FIB-SEM Nanotomography to Visualize Early HCoV-229E Virus Mediated Endocytosis Nanoscale Interactions

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Focused ion beam scanning electron microscopy (FIB-SEM) nanotomography has widely been used in several biological applications to gain structural and spatial information about nanoscale biological events. FIB-SEM utilizes an iterative ion beam milling and electron beam imaging process during nanotomography acquisition to image 3D volumes of specimens. This destructive imaging technique can achieve high resolution ~3 nm voxel sizes to capture impressive ultrastructural details. In this study, the resolution of FIB-SEM nanotomography was pushed to achieve ~2 nm resolution to visualize early human coronavirus 229E (HCoV-229E) virus particles binding to lung fibroblast cells, particularly to visualize the spike proteins that facilitate this activity. HCoV 229E virus particles were fluorescently labelled with DiO, incubated with lung fibroblast cells and then imaged with the AxioImager.M2 microscope (Zeiss) to locate virus-cell binding events. Once samples were prepared for electron microscopy, virus-cell binding regions of interest were relocated in the FIB-SEM microscope, where site-specific 3D FIB-SEM tomography acquisition using the Atlas 3D nanotomography program (Fibics) was performed. FIB-SEM nanotomography data shows HCoV-229E spike protein attachment (~10 nm) to the lung fibroblast cell membrane, while still achieving a ~ 20 µm field of view that captures the context of the cell environment. This investigation displays early virus-mediated endocytosis stages of HCoV-229E virus activity and demonstrates displays the advantageous application of FIB-SEM nanotomography to visual whole cell imaging while still capturing nanoscale virus interactions at nanoscale resolution.