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Visualizing Early HCoV-229E Viral Infection Events Using Correlative 2D Light and 3D Electron Microscopy Techniques

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Infection with the human coronavirus (HCoV) 229E, a member of the alpha coronaviridae family, can result in cases of moderate upper- and lower-respiratory tract illnesses [1-3]. The enveloped HCoV-229E virus particle of approximately 100 nm in diameter contains a lipid membrane that encloses the viral capsid and contains transmembrane protein receptors, including the spike protein that aids viral attachment and entry into target cells [1-5]. In this project, fluorescence and electron microscopy were used to visualize HCoV-229E virus binding activity. Using the selectivity and specificity of fluorescence microscopy, virus aggregates and viral binding sites were located and identified for further electron microscopy investigation to visualize the ultrastructural detail of HCoV-229E viral binding interactions. Key HCoV-229E viral membrane proteins have been investigated using 3D electron tomography (ET), especially cryogenic ET to understand their morphology and involvement in virus binding to target cells [6-7]. However, this electron microscopy modality is limited by the required small field of view during image acquisition and constrains analysis of viral and cell interactions. Here, we utilized focused ion beam scanning electron microscopy (FIB-SEM) nanotomography to visualize and characterize HCoV-229E virus binding to lung fibroblast cells to capture the viral-cell interactions at multiple scales while preserving nanoscale resolution to identify key features on the HCoV-229E virus. The combination of 2D fluorescence microscopy and 3D FIB-SEM nanotomography provides nanoscale contextual ultrastructural information of HCov-229E viral binding activity at relevant cell-virus length scales.

In brief, MRC-5 human lung fibroblast cells were cultured on 1 cm x 1 cm aclar squares (Ted Pella) while HCoV-229E viruses were incubated with the lipophilic dye, DiO (Invitrogen), to fluorescently label the lipid membrane of the virus (Figure 1). MRC-5 cells were incubated with fluorescently labelled HCoV-229E virus for 1 hour at low temperatures (4^{O}C) to capture early viral infection events including virus entry and attachment to cells. Widefield fluorescence microscopy was used to locate regions of interest using the AxioImager.M2 microscope (Zeiss) for further investigation with electron microscopy techniques. Infected cell samples were then stained using an osmium-thiocarbohydrazide-osmium (OTO) protocol, dehydrated with a graded ethanol series, and finally embedded in Embed 812 epoxy resin (Electron Microscopy Science) [8]. Bright-field light microscopy mosaics were taken across the sample using the Axio Zoom.V16 microscope (Zeiss) to locate regions of interest noted from the fluorescence microscopy investigation. Once key sites of interest with virus aggregation and binding to cells were relocated, 3D FIB-SEM nanotomography was performed using Atlas 3D nanotomography interface (Fibics) on the Crossbeam 540 (Zeiss). The 2D and 3D images were processed and aligned in the Atlas5 software (Fibics) to identify virus infection activity.

The FIB-SEM nanotomography run was set to have 3 nm voxels to allow for the identification of nanoscale features including the transmembrane spike protein. In this study, HCoV-229E virus attachment to the MRC-5 cell membrane was captured at the early viral infection stage (Figure 2a and b). The spike protein of HCoV-229E virus was also visualized on attached and unattached virions captured in the 3D FIB-SEM dataset (Figure 2b and c). In these images, we can visualize the viral spike binding to the cell membrane while preserving capturing the overall cellular context in the environment.

In conclusion, the combination of light and electron microscopy modalities facilitated the localization of HCoV-229E viral activity and the visualization of viral membrane proteins on viruses attached and unattached to the MRC-5 cells. This study to the author's knowledge presents the first high-resolution 3D FIB-SEM nanotomography of HCoV-229E virus where the viral proteins are visible on the outside of the virus lipid membrane. The advantages of combination of light and electron microscopy techniques allow for site-specific analysis of viral-cell interactions and show the viability of FIB-SEM for visualizing virus attachment with similar resolving power as TEM. This demonstrates the advantages of a correlative workflow for specimen identification while achieving high-resolution nanoscale images of viral infection events with FIB-SEM nanotomography.

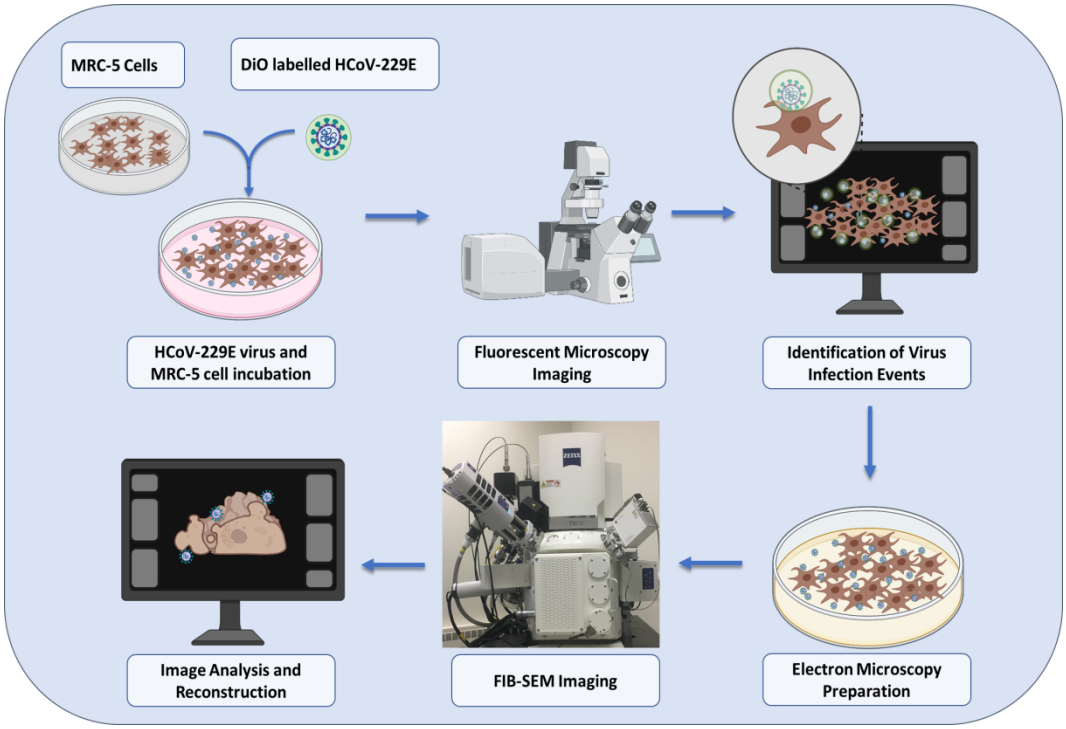

Figure 1. Schematic overview of the correlative 2D light and 3D electron microscopy workflow using 2D widefield fluorescent microscopy and 3D FIB-SEM nanotomography imaging techniques (Created with Biorender.com).

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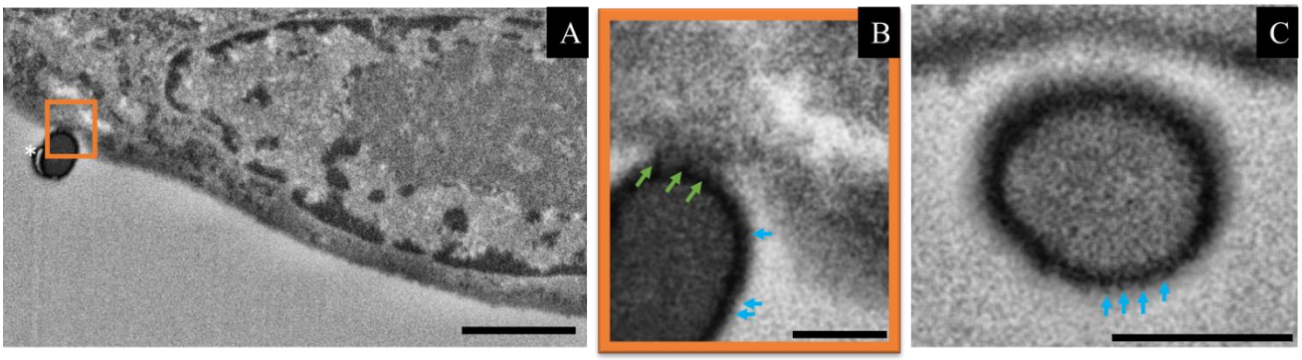

Figure 2. Visualization of early events of infection of MRC-5 cells with HCoV-229e using FIB-SEM. A) Attachment of a HCoV-229E viral particle to the MRC-5 cell membrane. A second viral particle can also be seen coming into plane from behind the attached viral particle to the cell surface (white asterisk). B) Magnification of A) (orange inset) captures the moment viral spike protein S binding to the cell membrane (green arrows) before the virion enters the cell. Unbound viral spike protein S (blue arrows) can also be found on the membrane of the virion attached to the cell. C) Unbound HCoV-229E virion where spike glycoprotein S (blue arrows) can be visualized on the virion surface. Scale bars A) 1 μm, B) 350 nm, and C) 300 nm.

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