Investigating the functional role of the TFG protein in situ using cryo-ET
Preliminary Result

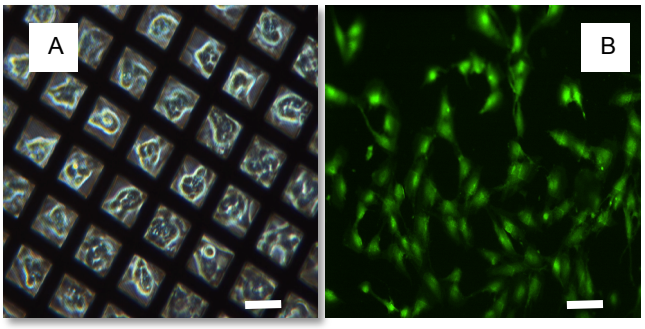


Figure 1. Depiction the consistent growth of the RPE cell line, expressing the GFP-tagged Sec16A protein on an UltraAuFoil TEM grid. A) Phase contrast Optical microscopy of the cells. B) Fluorescent microscopy image, cells are fixed and stained at room temperature using the glutaraldehyde and the osmium tetroxide. The fluorescent signal is more intensified at the nucleus, highlighting the locations of Sec16 and TFG proteins. Scale bars are 10μm and 15μm, respectively.

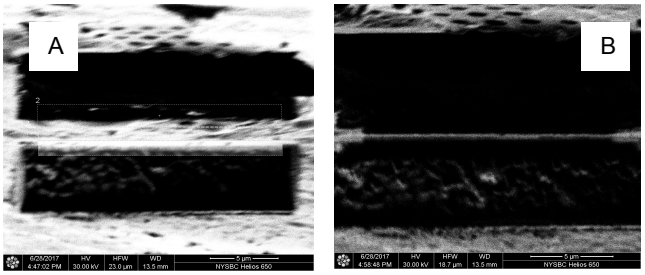
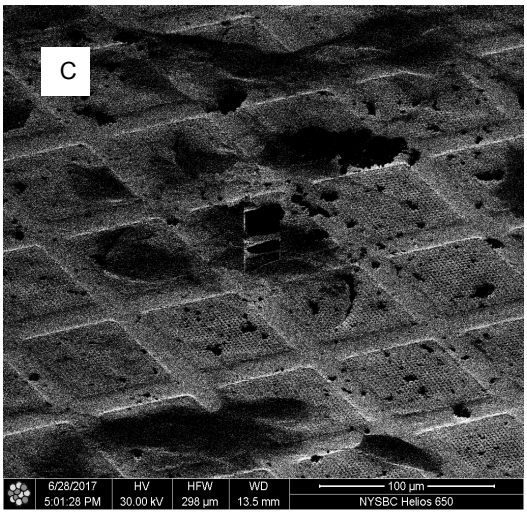


Figure 2. Cryo-FIB/SEM procedure performed by Alex Noble at NYSBC. A) Intermediate state of sectioning (2 μm thick) and B) final lamella (~250 nm thick) C) Zoomed out view showing the relative position of the FIB-milled cells compared to the intact cells on an EM grid.



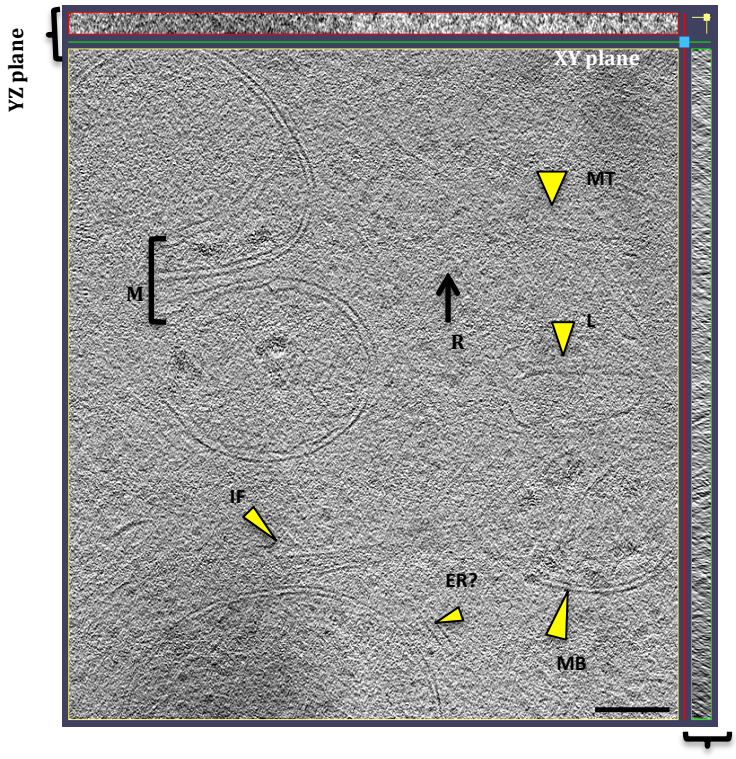


Figure 3. Single slice of a tomogram, aligned and reconstructed by Appion-Protomo. The image is recorded using IMOD software. Certain organelles at the center slice of RPE cell line are feasible. The scale bar corresponds to 85 nm. Putative densities are attributed to the subcellular organelles based on the similarity of visualized objects in the literature. The letters stand for; ER: Putative ER IF: Intermediate Filaments L: Lysosome, M: Mitochondria, MB: Multi Vesicular Bodies, MT: Microtubules, R: Ribosomes.