Combined 3D-SIM and STORM to determine the localization of endocytic APP in intraluminal vesicles
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Structured Illumination Microscopy (SIM) can improve two times of the resolution than conventional microscopy, and provide 3D super-resolution imaging of whole cell through easy operating. Stochasitic Optical Reconstruction Microscopy (STORM) utilizing single molecular localization reaches even higher spatial resolution to 10-20nm in XY direction[1]. However, it is not easy implementing to image whole cell by STORM. Here, we used a gridded glass bottom dish to combine SIM and STORM in capturing endosomal proteins with high position accuracy that discovering protein localization in intraluminal vesicles (ILVs) of multivesicular endosomes.

APP is a type I transmembrane protein, that trafficking continually form plasma membrane to endosomes through endocytosis[2]. Endocytic APP can be selectively sorted into the ILVs subsquently for lysosomal degradation. Multicolor 3D SIM of whole cell showed that the overexpressed 3HA-APP-GFP and endocytic anti-HA labelled APP localized in enlarged endosomes (Rab5-Q79L-RFP labelling). STORM imaging of the same endosomal vesicle showed endocytic APP in the ILVs. Therefore, correlative 3D SIM and STORM provide a way for accurate 3D molecular mapping in cellular organelles.

REFERENCES: