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The study of the whole cell dynamics of endocytic/exocytic-recycling events has proven difficult until recently because of lack of sensitivity, limited speed, photobleaching and phototoxicity associated with conventional imaging modalities. The Lattice Light Sheet Microscope (LLSM) [1] allows overcoming these difficulties, yet reaching high spatial resolution. This allows 3D images to be captured over long time at a high acquisition frequency, and enables the study of signalling, transport, and stochastic self-assembly in complex environments.

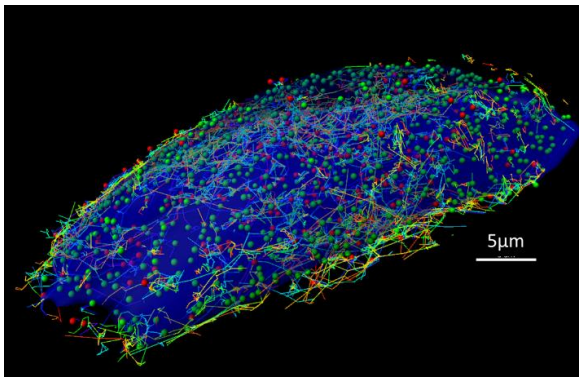

Figure 1. 3D tracking of Gal3-Atto647n (red) vs AP2-eGFP (green) adaptor protein in SUM159 cell.

In addition, this imaging technique and 3D-tracking will allow to look at molecular machinery throughout the full sequence of events that lead to exocytic fusion event or endocytic carrier formation, from initial membrane recruitment and budding and intracellular trafficking throughout the entire endocytic membrane system. Using 3D segmentation maps will permit to quantify different intracellular distribution pathways from the plasma membrane to a particular cellular destination and *vice et versa*.

We present preliminary results of the coordination of vesicle recycling from the endosomal recycling compartment up to the plasma membrane using LLSM imaging and 3D tracking. In addition, we introduce a quantitative analysis of endocytosis dynamics of AP2 adaptor complex, Galectin-3 (Figure 1) and Transferrin using single particle tracking analysis of 3D+time data. These examples demonstrate the advantage of lattice light sheet microscopy for imaging endocytic/exocytic events in single cells.

[1] Chen, B.C. et al. Science. 346 (6208): 1257998–1257998. (2014)