

### 3D TRACKING OF ENDOCYTIC EVENTS USING LATTICE LIGHT SHEET MICROSCOPY

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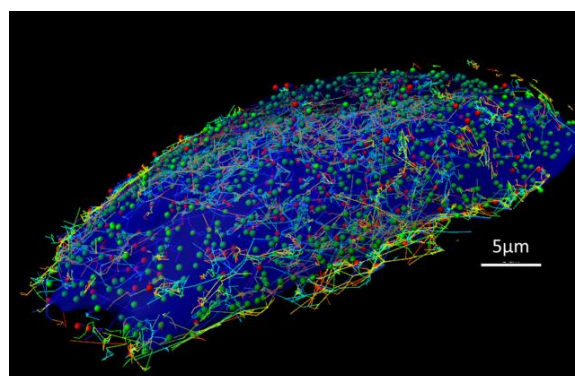
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**KEYWORDS:** light sheet microscopy, 3D tracking

Live cell imaging has been helpful in discovering various phenomenon and mechanisms that a static image cannot. Nevertheless, live cell imaging has been restricted by the microscopy techniques available to mainly two dimensions or limited by the speed. The study of the whole cell dynamics of endocytic 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 Microscopy (LLSM) allows overcoming these difficulties. This microscope uses an ultra-thin structured laser light sheet to image successive planes of the specimen [1]. The benefits in relation to previous techniques are low phototoxicity and photobleaching during image acquisition. In addition, high resolution images (~250 nm in xy and ~700 nm in z) can be acquired at high speed (~200 planes per second) [1]. This allows 3D images to be captured over longer time, and enables the study of signaling, transport, and stochastic self-assembly in complex environments with single molecule sensitivity. However, facing the amount of information provided by LLSM, cutting-edge image processing algorithms need to be investigated, at a time regime compatible with live cell imaging.

We present a quantitative analysis of endocytosis dynamics of AP2 adaptor complex, Galectin-3 (Figure 1) and Transferrin using two colour single particle tracking analysis of 3D+time data. These examples demonstrate the advantage of lattice light sheet microscopy for imaging endocytic events in single cells.



**Figure 1.** 3D tracking of Galectin-3 (red) vs AP2-eGFP (green) in SUM159 cell.

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