3D+T Spatio-Temporal Image Correlation Spectroscopy for Flow Mapping of Molecules and Organelles in Live Cells

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Molecular and organellar flows are of fundamental importance for the delivery of nutrients and essential components used in cellular functions such as motility and division. Spatio-temporal Image Correlation Spectroscopy (STICS) is the tool of choice that was successfully applied to study the fluxing of beta tubulin in human cells [1], vesicle dynamics during plant cell cytokinesis [2], f-actin dynamics at T-cell synapse [3] podosomes dynamics in dendritic cells [4,5] and stem cell migration in corneal wound healing experiments [6]. Nevertheless, this technique is usually performed on 2D image time series, where every image of the small local subset, or region of interest (ROI) is temporally correlated. Each spatio-temporal correlation function (CF) is characterized to extract the local flow in 2D and ultimately produce a 2D flow vector map. While the current approach works well for 2D+T datasets, it was never fully adapted to characterize flow mapping in 3D+T data. We demonstrate here the 3D STICS for vector mapping of flows in 3D data sets and verify the robustness of the approach by simulations with varying flow speeds, data signal-to-noise and sampling window sizes. As a proof of concept, we analyze experimental data sets of actin-GFP fluxing in live cells acquired as 3D+T stacks on Zeiss 880 Airyscan laser confocal microscope as well dynamics of photoactivatable GTPase Rac1 imaged by Lattice Light-Sheet Microscopy.