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 photoactivable GTPase Rac1 imaged by Lattice Light-Sheet Microscopy.

[1] A.L. Parker, W.S. Teo, E. Pandzic, J.J. Vicente, J.A. McCarroll, L. Wordeman, M. Kavallaris, "β-tubulin carboxy-terminal tails have isotype-specific roles on microtubule dynamics in human cells", *Life Science Alliance*, **1**(2), (2018).
[2] C. Triplet van Oostende, D. Guillet, T. Triplet, E. Pandzic, P. Wiseman and A. Geitmann. "Vesicle dynamics during plant cell cytokinesis reveals distinct functional phases.", *Plant Physiology*, **174**(1), pp.00343, (2017).
[3] G. Ashdown, G. Burn, D.J. Williamson, E. Pandzic, R. Peters, M. Holden, H. Ewers, L. Shao, P.W. Wiseman, and D. Owen."Live-cell super-resolution reveals F-actin and plasma membrane dynamics at the T cell synapse.", *Biophysical Journal*, **112**(7),1703-13, (2017).
[4] M.B.M. Maddens, E. Pandzic, J.A. Slotman, D. Guillet, B. Joosten, Svenja Mennens, L.M. Paardekooper, A. B. Houtsmuller, K. van den Dries, P.W. Wiseman and A. Cambi. "Actomyosin-dependent dynamic spatial pattern of cytoskeletal components drive mesoscale podosome organization." *Nature Communications*, **7**(13127), (2016).
[5] K. van den Dries, L. Nahidiazar, J.A. Slotman, M. Meddens, E. Pandzic, B. Joosten, M. Ansems, J. Schouwstra, A. Meijer, M. Wijers, J. Fransen, A. B. Houtsmuller, P.W. Wiseman, K. Jalink and A. Cambi. "Modular actin nano-architecture enables podosome protrusion and mechanosensing", *Nature Communications*, **10**(5171), (2019).
[6] M. Park, A. Richardson, E. Pandzic, E.P. Lobo, J.G. Lyons, N. Di Girolamo, "Peripheral (not central) corneal epithelia contribute to the closure of an annular debridement injury", *PNAS*, **116**(52), (2019).