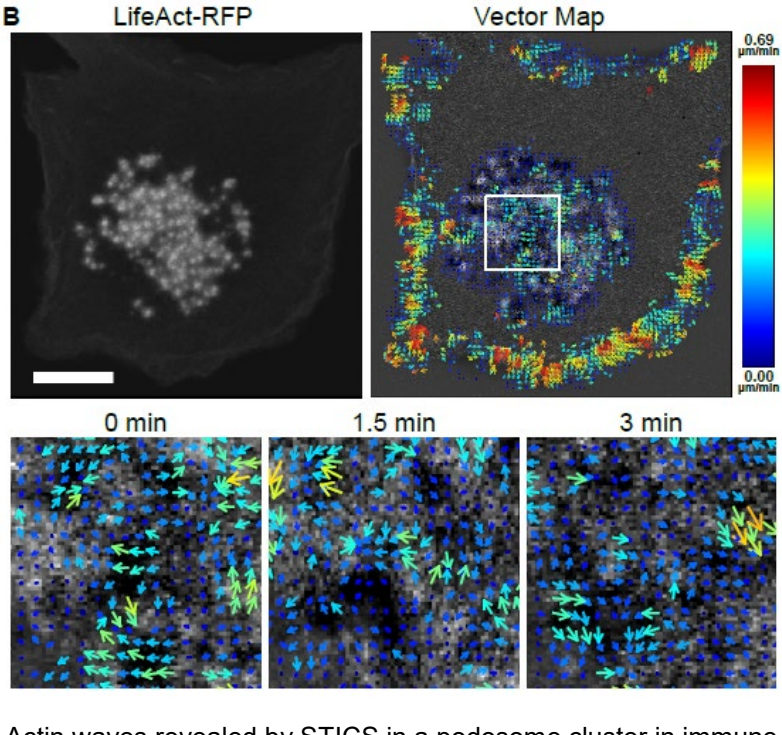
Abstract

The transport properties of biomolecules in cells can reveal a great deal about the functional interactions regulating cells at the molecular level. Various biophysical methods have been developed to measure these properties in cells, although most have relied on fluorescence microscopy imaging as the window for measurement of labeled macromolecules in living cells. Image correlation methods are an extension of fluorescence fluctuation spectroscopy that can measure protein-protein interactions and macromolecular transport properties from input fluorescence microscopy images of living cells. These approaches are based on space and time correlation analysis of fluctuations in fluorescence intensity within images recorded as a time series using a fluorescence or super-resolution microscope. I will introduce spatio-temporal image correlation spectroscopy (STICS) and its 2 color cross-correlation variant (STICCS) and show how the analysis can reveal hidden coupling between retrograde cellular actin flows and the plasma membrane lipids for activated Jurkat cells. I will then describe the application of the STICS and pair vector correlation for measuring cellular waves of adhesion related macromolecules talin and vinculin as well as cytoskeletal actin between assembling and disassembling podosomes in dendritic immune cells. Podosomes are cylindrical membrane complexes with an integrin adhesive ring and an actin rich core that are associated with cellular migration and invasion in specific cell types. EM and super-resolution microscopy of cells shows radial actin filaments that connect neighboring podosomes. The image correlation analysis combined with pharmacological perturbation experiments show that podosome turnover is coordinated within local clusters in cells with a correlation length scale extending to next nearest neighbor podosomes. Moreover,



Actin waves revealed by STICS in a podosome cluster in immune dendritic cells

recent work pairing the analysis with live cell super-resolution microscopy reveals that the dynamic coordination between podosomes differs for cells on soft versus stiff substrates which provides clues to the mechanistic function of podosomes.