Title
Dynamic Imaging and Fluctuation Spectroscopy on Single Microvilli in Opossum Kidney Cells by the Modulation Tracking Method

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Nuclear pore complexes (NPCs) mediate bidirectional transport of proteins, RNAs, and ribonucleoproteins across the double-membrane nuclear envelope. We recently introduced a method based on pair correlation functions (pCF) which measure the time the same molecule takes to migrate from one location to another within the cell (1). The spatial and temporal correlation among two arbitrary points in the cell can provide a map of molecular transport, and also highlight the presence of barriers to diffusion with very high time resolution (in the microsecond scale) and spatial resolution (limited by diffraction).

Here we report the use of this method to directly monitor a model protein substrate undergoing transport through NPCs in living cells, a biological problem in which SPT has given results that cannot be confirmed by traditional FCS measurements because of the lack of spatial resolution. Our substrate is composed by a GFP linked to a functional nuclear localization sequence (NLS) and transfected into living CHO-K1 cells: the recombinant NLS-GFP protein can bind to molecular carriers mediating cytoplasm-to-nucleus active import as well as shuttle across the NPC by passive diffusion (its molecular weight is below the cut-off size limit of the NPC).

We show that obstacles to molecular flow can be detected and that the pCF algorithm can recognize the heterogeneity of NLS-GFP intracompartment diffusion as well as the presence of barriers to its transport between compartments (i.e. the NPCs of the nuclear envelope).


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