Two types of signaling noises underlie spatiotemporal PTEN heterogeneity

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Cells employ various intracellular signaling molecules to determine their front/back polarity. PtdIns(3,4,5)P3 phosphatase PTEN is known to be excluded from the front and is necessary for correct pseudopod formation. However, the role of noise in the formation of PTEN polarity has not been elucidated. Here we studied spatiotemporal PTEN heterogeneity in individual cells by imaging PTEN molecules conjugated with green or red fluorescent probes. Spatial and temporal correlation functions of the fluorescence intensities revealed the kinetics of PTEN and its binding sites. The results suggest that two types of noises, one from PTEN itself and the other from its binding sites, contribute to PTEN heterogeneity.

Fluorescent Single Molecule Orientation Imaging in Living Cells

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We are proposing a light microscopy to detect changes in intra-molecular structure or inter-molecular organization based on orientation imaging of fluorescent single molecules. We have developed robust instrumentation for polarized fluorescence imaging exhibiting the speed and sensitivity required to monitor 3D angular changes of individual fluorophores that are rigidly connected to proteins of interest. While developing the optical arrangement and required acquisition and processing algorithms, we use the system to monitor the organization of cytoskeletal molecules in a filamentous fungus, Ashbya gossypii, and in budding yeast. In this presentation we describe our single-molecule approaches which include instrumentation, image acquisition and processing algorithms.