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**Review of Scientific Instruments**
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Not out yet.

**Proceedings of the National Academy of Sciences of the USA**
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Nothing of interest

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**Heterogeneity of AMPA receptor trafficking and molecular interactions revealed by superresolution analysis of live cell imaging**

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**Abstract**

Simultaneous tracking of many thousands of individual particles in live cells is possible now with the advent of high-density superresolution imaging methods. We present an approach to extract local biophysical properties of cell-particle interaction from such newly acquired large collection of data. Because classical methods do not keep the spatial localization of individual trajectories, it is not possible to access localized biophysical parameters. In contrast, by combining the high-density superresolution imaging data with the present analysis, we determine the local properties of protein dynamics. We specifically focus on AMPA receptor (AMPAR) trafficking and estimate the strength of their molecular interaction at the subdiffraction level in hippocampal dendrites. These interactions correspond to attracting potential wells of large size, showing that the high density of AMPARs is generated by physical interactions with an ensemble of cooperative membrane surface binding sites, rather than molecular crowding or aggregation, which is the case for the membrane viral glycoprotein VSVG. We further show that AMPARs can either be pushed in or out of dendritic spines. Finally, we characterize the recurrent step of influenza trajectories. To conclude, the present analysis allows the identification of the molecular organization responsible for the heterogeneities of random trajectories in cells.

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**Counting single photoactivatable fluorescent molecules by photoactivated localization microscopy (PALM)**

Sang-Hyuk Leeab, Jae Yen Shin, Antony Leea, and Carlos Bustamanteabcde
Abstract

We present a single molecule method for counting proteins within a diffraction-limited area when using photoactivated localization microscopy. The intrinsic blinking of photoactivatable fluorescent proteins mEos2 and Dendra2 leads to an overcounting error, which constitutes a major obstacle for their use as molecular counting tags. Here, we introduce a kinetic model to describe blinking and show that Dendra2 photobleaches three times faster and blinks seven times less than mEos2, making Dendra2 a better photoactivated localization microscopy tag than mEos2 for molecular counting. The simultaneous activation of multiple molecules is another source of error, but it leads to molecular undercounting instead. We propose a photoactivation scheme that maximally separates the activation of different molecules, thus helping to overcome undercounting. We also present a method that quantifies the total counting error and minimizes it by balancing over- and undercounting. This unique method establishes that Dendra2 is better for counting purposes than mEos2, allowing us to count in vitro up to 200 molecules in a diffraction-limited spot with a bias smaller than 2% and an uncertainty less than 6% within 10 min. Finally, we demonstrate that this counting method can be applied to protein quantification in vivo by counting the bacterial flagellar motor protein FliM fused to Dendra2.

Automatica

Vol. 48, no. 11

Exploiting packet size in uncertain nonlinear networked control systems

L. Greco, A. Chaillet, and A. Bicchi

This paper addresses the problem of stabilizing uncertain nonlinear plants over a shared limited-bandwidth packet-switching network. While conventional control loops are designed to work with circuit-switching networks, where dedicated communication channels provide almost constant bit rate and delay, many networks, such as Ethernet, organize data transmission in packets, carrying larger amounts of information at less predictable rates. We adopt a model-based approach to remotely compute a predictive control signal on a suitable time horizon. By exploiting the inherent packets payload, this technique effectively reduces the bandwidth required to guarantee stability. Communications are assumed to be ruled by a rather general protocol model, which encompasses many protocols used in practice. An explicit bound on the combined effects of the maximum time between consecutive accesses to each node (MATI) and the transmission and processing delays (MAD), for both measurements and control packets, is provided as a function of the basin of attraction and the model accuracy. Our control strategy is shown to be robust with respect to sector-bounded uncertainties in the plant model. Sampling of the control signal is also explicitly taken into account. A case study is presented which enlightens the great improvements induced by the packet-based control strategy over methods that send a single control value in each packet.

Biophysical Journal

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3D Single Molecule Tracking with Multifocal Plane Microscopy Reveals Rapid Intercellular Transferrin Transport at Epithelial Cell Barriers

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Abstract

The study of intracellular transport pathways at epithelial cell barriers that line diverse tissue sites is fundamental to understanding tissue homeostasis. A major impediment to investigating such processes at the subcellular level has been the lack of imaging approaches that support fast three-dimensional (3D) tracking of cellular dynamics in thick cellular specimens. Here, we report significant advances in multifocal plane microscopy and demonstrate 3D single molecule tracking of rapid protein dynamics in a 10 micron thick live epithelial cell monolayer. We have investigated the transferrin receptor (TfR) pathway, which is not only essential for iron delivery but is also of importance for targeted drug delivery across cellular barriers at specific body sites, such as the brain that is impermeable to blood-borne substances. Using multifocal plane microscopy, we have discovered a cellular process of intercellular transfer involving rapid exchange of Tf molecules between two adjacent cells in the monolayer. Furthermore, 3D tracking of Tf molecules at the lateral plasma membrane has led to the identification of different modes of endocytosis and exocytosis, which exhibit distinct temporal and intracellular spatial trajectories. These results reveal the complexity of the 3D trafficking pathways in epithelial cell barriers. The methods and approaches reported here can enable the study of fast 3D cellular dynamics in other cell systems and models, and underscore the importance of developing advanced imaging technologies to study such processes.

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Video-Rate Confocal Microscopy for Single-Molecule Imaging in Live Cells and Superresolution Fluorescence Imaging

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Abstract

There is no confocal microscope optimized for single-molecule imaging in live cells and superresolution fluorescence imaging. By combining the swiftness of the line-scanning method and the high sensitivity of wide-field detection, we have developed a, to our knowledge, novel confocal fluorescence microscope with a good optical-sectioning capability (1.0 µm), fast frame rates (<33 fps), and superior fluorescence detection efficiency. Full compatibility of the microscope with conventional cell-imaging techniques allowed us to do single-molecule imaging with a great ease at arbitrary depths of live cells. With the new microscope, we monitored diffusion motion of fluorescently labeled cAMP receptors of Dictyostelium discoideum at both the basal and apical surfaces and obtained superresolution fluorescence images of microtubules of COS-7 cells at depths in the range 0–85 µm from the surface of a coverglass.
Compressive sensing based reconstruction in bioluminescence tomography improves image resolution and robustness to noise

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Abstract

Bioluminescence Tomography attempts to quantify 3-dimensional luminophore distributions from surface measurements of the light distribution. The reconstruction problem is typically severely under-determined due to the number and location of measurements, but in certain cases the molecules or cells of interest form localised clusters, resulting in a distribution of luminophores that is spatially sparse. A Conjugate Gradient-based reconstruction algorithm using Compressive Sensing was designed to take advantage of this sparsity, using a multistage sparsity reduction approach to remove the need to choose sparsity weighting a priori. Numerical simulations were used to examine the effect of noise on reconstruction accuracy. Tomographic bioluminescence measurements of a Caliper XPM-2 Phantom Mouse were acquired and reconstructions from simulation and this experimental data show that Compressive Sensing-based reconstruction is superior to standard reconstruction techniques, particularly in the presence of noise.