accuracy in distance measurements between two different color dye-molecules attached at known positions along a surface tethered bio-molecule. The statistical uncertainty in the mean for an ensemble of N~10 identical single molecule samples is limited only by the total number of collected photons to ~0.3nm, or ~0.002 of the width of the optical PSF. We further show how our method can be used to improve the resolution of many sub-wavelength, far-field imaging methods such as those based on co-localization of stochastically excited fluorescent molecules.

Conclusion: We demonstrate sub-nanometer resolution in measurements of molecular-scale distances using far-field fluorescence imaging optics, at room temperature and in physiological buffer conditions. The improved resolution will allow deciphering in real-time, at the single molecule level the structure and dynamics of large, multi-subunit biological complexes.

1984-Plat

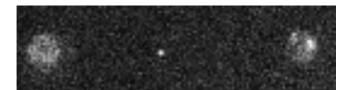
Visualizing Single-proteins On A Single DNA Molecule With Superresolution

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DNA-proteins interactions can be studied in great detail when precise and dynamic control of the mechanical state of a single DNA molecule can be achieved together with direct visualization of proteins interacting with the DNA. Our custom-built experimental setup combines optical trapping and wide-field epifluorescence microscopy. In this setup we can visualize single DNA-bound proteins (see figure) with an accuracy of tens of nanometers while at the same time we can control the tension applied on the DNA at a sub-PicoNewton level.

Here we report the limit of localization accuracy in combined DNA-trapping/ fluorescence experiments by fitting a point-spread function to the fluorescence image of single DNA-bound proteins.

We investigate the impact of DNA dynamics on the maximum attainable accuracy for the localization of these DNA-bound fluorescent proteins (in this case a Ca2+ inactivated restriction enzyme EcoRV). In particular, we study the effect of tension on the DNA and identify the force regime in which single-proteins can be localized with super-resolution.



1985-Plat

Visualizing the Receptor Assembly Into Clathrin-coated Pits with Superresolution Two-color Palm and sptPALM

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The dynamic assembly of receptors into endocytic structures such as clathrincoated pits underlies cellular response to many external signals. However, the biophysical mechanisms for the regulated uptake of select receptors are unresolved. While commonly used bulk measurements access only ensemble-averaged behaviors, single molecule measurements have largely been limited to looking at only a few molecules in a single cell, lacking true ensemble information. Thus, until recently, the study of receptor capture by clathrin-coated pits has been limited by a scarcity of experimental methods capable of accessing information on ensembles of individual molecules within an individual cell. We addressed this by combining two recent technological advances to image receptors and clathrin-coated pits in living cells: two-color imaging with the photoswitchable PA-mCherry fluorescent label, and single particle tracking photoactivated localization microscopy (sptPALM).

As compared to other monomeric red photoactivatable proteins, PAmCherry1 has higher pH stability, faster maturation and photoactivation kinetics, better photostability, and similar number of photons in single-molecule imaging. Furthermore, lack of background green fluorescence makes PAmCherry1 an advanced probe for two-color diffraction-limited microscopy and super-resolution techniques such as PALM. Two-color PALM imaging of PAmCherry1 tagged to the transferrin receptor (TfR) and PAGFP fused with the clathrin light-chain (CLC) were performed. Pair correlation analysis suggests clusters of less than 200 nm in size with both distinct and overlapping distributions of the TfR and CLC chimeras at sub-diffraction 25 nm resolutions. In addition, sptPALM was used to create spatially resolved maps of the trajectories of single receptor motions in conjunction with SPT of diffraction-limited clathrin-coated pits.

1986-Plat

A Single-Molecule Study of Gene Regulation in Real Time Zach Hensel, Christine L. Hatem, Jie Xiao.

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Gene expression is inherently stochastic, yet must be tightly regulated to properly carry out essential cellular processes. However, many of the transcription factors responsible for this precise regulation are expressed at extremely low copy numbers in cells. To understand how noise in gene expression is controlled to overcome this challenge, it is important to directly monitor gene regulation in real time at the single-molecule level.

In this work, we use the genetic switch of lambda phage as a model system to demonstrate continuous monitoring of autoregulation of lambda repressor CI in living *E. coli* cells. CI exhibits both positive and negative feedback on its own expression at low and high intracellular concentrations, respectively. We generated a single-molecule, gene-expression reporter that enables counting of the exact number of CI molecules expressed in real time without compromising CI's regulatory activity. Over several cell generations, we observed highly clustered expression of CI separated by relatively long periods of low expression, which we attribute to the alternating positive and negative autoregulation of CI. Our results suggest that the noise in CI expression is controlled by coupled positive and negative feedback, a mechanism also implicated in regulation of the eukaryotic cell cycle and circadian rhythm. This method should be applicable to similar studies probing gene regulation in other systems.

1987-Plat

Pitfalls In Single Particle Tracking In Living Cells

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An increasing body of evidence for subdiffusion of biopolymers under typical in vivo conditions has been reported recently. The physical foundation of this subdiffusion remains unidentified although it is commonly ascribed to molecular crowding. Single particle tracking provides crucial information on the mechanisms behind the subdiffusion. In several such experiments the measured mean squared displacement shows a characteristic scatter (e.g., [1,2]).

Using the widely accepted continuous time random walk framework we demonstrate that pronounced scatter in time averaged quantities such as the mean squared displacement is no artefact but arises naturally from the nonexistence of a characteristic time scale separating microscopic and macroscopic events [3,4]. An expression for the broad distribution of diffusion coefficients in such measurements is derived and confirmed by simulations. The most crucial finding from our theory is that the subdiffusive nature of the particles will be masked in the time averages: What looks like normal diffusion in an experiment may in reality be subdiffusion in an ageing system. Interpretations of the reported data in [1,2] will be discussed. We provide general guidelines to properly intepret single molecule tracking data.

We also argue that ageing properties in biopolymer diffusion in living cells may be advantageous for the accuracy of genetic regulation at minimal concentrations of transcription factors. The physical picture emerging from our theory provides additional support for a more local picture of gene regulation and confirms the importance of colocalisation in the genome.

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1988-Plat

New Single Molecular Detection System from Three-Dimensional Tracking of Single Nanocrystals using Scanning Electron Microscope Yuji C. Sasaki^{1,2}, Akihiro Ohsaki³, Akira Ishikawa³.

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Recent technological progress in dynamical observations of individual functional single protein molecules in living cell has been achieved with several single molecular techniques and systems. In order to improve monitoring precisions and stability of the signal intensity from single molecular units under physiological conditions, we have proposed that single molecular techniques using shorten wavelength, for example, X-rays, electrons, neutron, and other accelerated ion probes. In this work, we demonstrate three-dimensional tracking of single nanocrystals using Scanning Electron Microscope. We called Diffracted Electron Tracking (DET).

Diffracted X-Ray tracking (DXT) [1] has been developed for obtaining the information about the dynamics of single molecules. This method can observe the