nanodroplets suspended in a low index-of-refraction fluorocarbon medium. The index of refraction mismatch between the nanodroplets and fluorocarbon is such that individual hydrosomes can be optically trapped. Using optical tweezers, the hydrosomes are held within a confocal observation volume, and we interrogate the encapsulated molecule by means of fluorescence excitation. Hydrosome encapsulation has an important advantage over liposome encapsulation techniques in that hydrosomes fuse on contact, thereby mixing the encapsulated components. Optical tweezers are used to manipulate the hydrosomes and to induce a fusion event. Custom fabricated microfluidic channels are used to sort the hydrosomes containing different molecule species. We demonstrate the use of hydrosomes as microreactors by fusing two hydrosomes, each containing a complementary single strand of DNA, and observing the subsequent hybridization via FRET (Fluorescence Resonance Energy Transfer).

3042-Pos

Using PNA and LNA as Handles for Tethering Single DNA Molecules

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Single-molecule manipulation (SMM) instruments, such as the optical trap or magnetic tweezer, require a means to immobilize the studied biomolecule on a solid substrate. In the case of DNA, this is accomplished by adding moieties, e.g. biotin, using enzymatic labeling; however, this strategy introduces extra steps in the processing of the sample, and makes direct application of SMM to DNA/protein interactions and chromatin structure difficult. Here, we develop a novel tethering strategy based on the properties of the nucleic acid analogs (NAAs) peptide nucleic acid (PNA) and locked nucleic acid (LNA). PNA and LNA are known to stably bind to double-stranded DNA in a sequence-specific manner, either through triplex formation or strand-invasion. Using a magnetic tweezer, we explore the ability of biotinylated NAAs to immobilize a DNA molecule in a sequence-specific fashion, and to remain bound under applied force. Our results indicate that both LNA and PNA can tether a DNA molecule and withstand mechanical force, but that PNA suffers from non-specific binding, particularly to DNA extremities. We discuss rules for the optimal design of NAA probes for single-molecule experiments.

3043-Pos

Precision Force Spectroscopy of Bacteriorhodopsin

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Single-molecule force spectroscopy studies have produced rich insights into the unfolding of individual proteins and nucleic acid structures. In a typical force spectroscopy experiment, an AFM tip is coupled to a surface-adsorbed protein by pressing the tip into it. Force-extension curves are then generated by retracting the tip at a constant velocity using a piezoelectric (PZT) stage. Force is measured by cantilever deflection. Extension, or more precisely tip-sample separation, is deduced from the PZT stage position used to control the vertical tip position. Thus, this deduced extension is sensitive to the vertical mechanical drift of the AFM assembly (~10 nm/min). We have previously developed an ultrastable AFM in which the tip and the sample positions are independently measured by, and stabilized with respect to, a pair of laser foci in three dimensions. These lasers establish a local reference frame that is insensitive to long-term mechanical drift of the AFM assembly. This new measurement of position is complementary to the cantilever deflection sensing, which measures force. We have now extended the ultrastable AFM capabilities into liquid and can routinely mechanically unfold proteins at slow pulling velocities (2 nm/s), which allows averaging to increase precision. We can also stop pulling altogether and hold the molecule at constant force while independently measuring tip-sample separation ($\sigma = 0.2$ nm, $\Delta f =$ 1-50 Hz). Alternatively, we can stabilize tip-sample separation and measure force ($\sigma = 5$ pN, $\Delta f =$ 1-50 Hz) over 100s of seconds . Using these techniques, we are studying the unfolding and re-folding of bacteriorhodopsin (BR), a model transmembrane protein.

3044-Pos

Fast Millisecond Imaging of Single Fluorescent Protein Molecules Using a Simple "Slimfield" Optical Trick

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Fluorescence microscopy offers a non-invasive probe for biological processes *in vivo*, but there are limitations in sensitivity for commonly used fluorescent proteins typically utilized as genetically encoded fusion constructs as molecular reporters. Here we present a simple optical trick bundled with some relatively straightforward custom-written analysis software which allows quantification of single fluorescent protein molecules over a rapid ~millisecond time scale. This optical trick has been used previously to image bright organic dyes *in vitro* and a similar approach can be used to change confocal volume sizes for use in fluorescence correlation spectroscopy, but never to image single photophysically poor fluorescent proteins in living cells. We have called the illumination mode which results ''slimfield''. Slimfield is cheap and simple and can be implemented on existing commercial microscope systems with relatively little modification. It permits excitation intensities ~100 times greater than those of widefield imaging, facilitating single-molecule detection at high speed. We demonstrate it using many different purified fluorescent proteins in common laboratory use. Controlled *in vitro* experiments indicate single protein molecules over a field of view 30 microns^2 area, large enough to encapsulate single bacterial and yeast cells. Using our custom software we can automate detection and quantification of single molecules using true 2D imaging at ~500 frames per second with a localization precision for these photophysically poor dyes of typically a few tens of nm. We show that you can image the dim enhanced cyan fluoresecent protein (ECFP) and CyPet at a single-molecule level. Simple modifications then allowed us to perform simultaneous dual-color slimfield imaging for use in co-localization and FRET. We then report some preliminary *in vivo* data using bacteria and show ~millisecond time scale functional imaging at a single-molecule level with negligible photodamage.

3045-Pos

Zero Mode Waveguides: a Powerful Tool for Single Molecule Optical Studies

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Single-molecule fluorescence studies of enzymes that incorporate fluorescently labeled substrate nucleotides typically operate at substrate concentrations well below their K_m values. While this is inevitable in conventional fluorescence microscopy, the biological relevance of the insights gained into enzyme mechanism may be compromised. Zero-mode waveguides (ZMWs) provide an excellent solution to this problem by greatly reducing the observation volume. We report the nanofabrication of ZMWs, the surface treatment for controlled immobilization of biomolecules and the reduction of background noise. We also present the development of an assay to monitor in real time the incorporation of fluorescently-labeled nucleotides, which paves the way for the studies of nucleic acid polymerizing enzymes, e.g. DNA/RNA polymerase, reverse transcriptase, telomerase, etc.

3046-Pos

Comparison of Three-Dimensional Imaging Configurations for High Resolution Microscopy Measurements

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In recent years, a class of fluorescence microscopy imaging techniques has emerged which enables the imaging of single fluorophores at high resolution by reducing the problem of resolution to one of localization. The photoactivated localization microscopy (PALM) technique, for example, constructs finely resolved images by way of accurately localizing closely spaced fluorophores that are detected separately in time by successively photoactivating small and stochastically different subsets of fluorophores.

Due to the optical microscope's poor depth discrimination capability, the resolution of three-dimensional (3D) versions of techniques like PALM is limited by the z-localization accuracy of a single fluorophore, which can be especially poor when a fluorophore is near-focus. An imaging technique that overcomes the near-focus problem is multifocal plane microscopy (MUM) (Prabhat, P. et. al., IEEE Trans. Nanobiosci., 2004), which allows the simultaneous imaging of a fluorophore from distinct focal planes. Images from multiple focal planes enable MUM to accurately localize a near-focus fluorophore (Ram, S. et. al., Proc. SPIE, 64430D1, 2007) and to support high accuracy 3D localization over a wide depth range.

Here we compare 3D fluorescence imaging configurations which employ different combinations of conventional excitation, PALM excitation, conventional emission, and MUM emission. Using a Cramer-Rao lower bound-based 3D resolution measure (Chao, J. et. al., Opt. Commun., 2009), comparisons are made in terms of the accuracy with which the distance separating two closely spaced fluorophores can be estimated. Such distance information can be important as it can help to characterize the interaction between two biomolecules. Our results show that configurations incorporating PALM excitation provide superior distance estimation accuracies for fluorophore pairs characterized by small distances of separation and orientations near parallel to the optical axis. Meanwhile, configurations incorporating MUM emission provide the best accuracies for near-focus fluorophore pairs.

3047-Pos

Investigating Structural Heterogeneity in Dimers of Amyloid-β Peptide

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