the trapping-laser wavelength in the medium. The optimal filling ratio for the lateral direction depended on the microsphere size, whereas for the axial direction it was nearly independent. Our findings are in agreement with Mie theory calculations and suggest that apart from the choice of the optimal microsphere size, slightly under-filling the objective is key for the optimal performance of an optical trap.

2947-Pos Board B717

Two-Photon Fluorescence Imaging of Single Fluorescent Proteins Inside Mammalian Cells

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Exciting progress has been made recently in biophysical techniques that allow optical imaging of single molecules in live cells. However, imaging of single fluorescent proteins in live cells is usually limited to bacteria that are small in size or proteins that are on the cell surface, where excitation through total internal reflection can be used to reduce fluorescence background and achieve single-molecule sensitivity. Eukaryotic cells are typically larger in size with higher autofluorescence background. To achieve single-molecule sensitivity deep inside a eukaryotic cell is thus challenging. We have used two-photon fluorescence excited by infrared lasers to reduce autofluorescence background. We show that single green fluorescent proteins can be imaged deep inside a mammalian cell using two-photon fluorescence. Discrete stepwise photobleaching of enhanced green fluorescent proteins was observed. The single-molecule fluorescence intensity analysis and on-time distribution indicate that two-photon fluorescence is detectable at the single-molecule level deep inside a eukaryotic cell. Moreover, it is not necessary to use a pulsed laser for two-photon fluorescence excitation. A continuous-wave laser that is routinely used for optical tweezers can be used to excite two-photon fluorescence and achieve single-molecule sensitivity. These advantages could significantly benefit future application of this single-molecule technique in biological studies. We present applications of our technique, and demonstrate two-photon fluorescence imaging of eukaryotic cells at single-molecule level.

2948-Pos Board B718

Inertial Effects of a Small Brownian Particle Cause a Colored Power Spectral Density of Thermal Noise

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The thermal agitation of fluids leads to the random, jiggling motion of suspended particles known as Brownian motion. The random thermal force acting on the particles is often approximated in Langevin models by a ''white-noise'' process. However, fluid entrainment results in a frequency dependence of this thermal force giving it a ''color''. While theoretically well understood, direct experimental evidence for this colored nature of the noise term is still lacking. Here, we tracked the motion of a particle confined in a very strong and ultrastable optical trap near a surface. By the confinement, we were able to directly measure the color of the thermal noise intensity. Far away from the surface, the noise intensity increased with the frequency approaching a square-root dependence with hints of a resonant enhancement. Close to the surface, the colored-noise amplitude strongly decreased and even reversed its sign. All our measurements are in quantitative agreement with the theoretical predictions, experimentally verifying a key aspect of Brownian motion. Since Brownian motion is important for microscopic, in particular, biological systems and high-resolution biophysical measurements, the colored nature of the noise and its distance dependence to nearby objects need to be accounted for and may even be utilized for advanced sensor applications.

2949-Pos Board B719

A Method for Time-Resolved Ratiometric Detection of Plasmon Coupling Between Gold Nanoparticles for Single-Molecule Binding Assays

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Fluorescence-based single-molecule techniques (and in particular, FRET) are the current gold standard for observing and measuring dynamic, molecular interactions that occur on millisecond timescales. However, these techniques suffer from the inherent trade-off between fluorescence intensity and photostability of the fluorescent probes. With unmatched photostability and high scattering brightness, gold nanoparticles (GNPs) are in many ways ideally suited to overcoming the limitations of fluorescence microscopy. Additionally, through the unique plasmonic coupling effects between GNPs, a FRET-like distance measurement with nanometer resolution is theoretically possible over distances that are roughly twice the GNP diameter, far exceeding the detectable range of FRET. Previous plasmonic coupling measurements have been largely limited by the use of white light excitation and detailed spectral characterization, both which limit the spatial and temporal resolution because of the low efficiency in collecting and measuring the scattered light. We have overcome these limitations through the ratiometric detection of scattered light from GNPs using only two excitation wavelengths. Using monochromatic laser excitation and total internal reflection-based dark-field microscopy, we collected the scattered light from GNPs at the two excitation wavelengths and used a dichroic mirror to spatially separate the channels on a CCD camera. As a proof-of-principle to establish this ratiometric approach, we measured the plasmonic coupling of surface-bound biotin-functionalized GNPs upon binding neutravidin-conjugated GNPs from solution. Here, we report the first demonstrated detection of plasmonic coupling between two particles with >25 Hz temporal resolution. At this time resolution, we observe individual GNP scattering intensities >100 times above background, and observe that the intensity ratio more than doubles upon binding a second GNP. Importantly, and in contrast with previous methods, our technique is fully extendable to faster timescales, limited largely by the choice of detector.

2950-Pos Board B720

Visualizing Single DNA Molecules in Nanofluidic Channels

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Nanofluidic channels have become an important tool for studies of single DNA molecules. Studies have been done on DNA polymer physics as well as DNA mapping and DNA/protein interactions. Common for these studies is that the DNA is homogeneously stained, typically with the bis-intercalating dye YOYO. While this is beneficial for some studies, there are several applications where the dyes interfere with the study. YOYO changes physical properties, such as charge, persistence length, contour length and winding of the DNA, as well as causes DNA damage by photonicking when exposed to light. Furthermore, for studies of interactions between DNA and ligands or proteins intercalating YOYO competes with the molecule of interest for the available binding sites. In order to avoid these obstacles we have designed a 50 kbp DNA construct with quantum dots (QDs) in each end. This construct allows us to study single DNA molecules in nanochannels without any intercalated dyes, by measuring the distance between the two fluorescent ends. We demonstrate the effect YOYO-labelling has on DNA polymer physics and show how this novel DNA construct can be used for DNA/ligand interaction studies on the single molecule level. Furthermore, nanostructures have extreme surface-to-volume ratios, leading to that the negatively charged surfaces are sticky to most hydrophobic and/or positively charged molecules and particles, including the streptavidin coated QDs used for end-labelling. We therefore developed a passivation-scheme, based on PEG, that is resistant to the QDs sticking. The absence of QDs sticking to passivated nanochannels serves as a general demonstration that can be extended to most proteins, allowing us to do DNA/protein interaction studies in the nanochannels using end-labeled DNA.

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Inter-Domain Dynamics of Phosphoglycerate Kinase Studied by Single-Molecule FRET

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Well pronounced domain movements in 3-Phosphoglycerate kinase (PGK) are assumed to be crucial for a phosphor transfer reaction which is catalyzed by this enzyme. Using a cysteine double mutant, with fluorescent dyes attached at the distal ends of each domain of PGK from yeast [1], we performed single molecule Förster Resonance Energy Transfer (smFRET) measurements together with substrates: ADP, ATP and 3-Phosphoglycerate (3PG). 2D-plots of the FRET-efficiency vs. the donor lifetime were analyzed with a two-state model showing the fast interconversion between two conformations [3]. The amplitude of the inter-dye movement is obtained together with the population of each state. The connection between dye and domain trajectories was exploited troughout the analysis of the slowest normal modes, calculated with a Normal Mode Analysis (NMA). Characteristic times of the domain dynamics were measured with fluorescence correlation spectroscopy (FCS). The results are discussed together with earlier findings from neutron spectroscopy [2] and ensemble FRET measurements [4].

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