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We present a novel multi-confocal Fluorescence Correlation Spectroscopy (mFCS) technique that allows simultaneous FCS measurements in different locations within a cell. Standard FCS experiments are usually limited to one observation volume, so that information can only be obtained from one position at a time. In contrast, mFCS makes it possible not only to monitor fast temporal and spatial changes in the dynamics of cellular proteins, but also to increase the amount of data collected per measurement and thus reducing the time necessary to produce statistically significant results. Our mFCS technique takes advantage of a Spatial Light Modulator (SLM) to create several distinct observation volumes at a time. Parallel detection is performed using an Electron-multiplied CCD camera, where pixels act as pinholes for confocal detection. We were able to show that the spatial resolution and the sensibility of our mFCS system is close to that of an classical FCS setup. Employing a special camera readout mode, a temporal resolution of 14 µs is reached, which is adapted to the dynamics of most cellular proteins. The mFCS technique is applied to study the cellular response to thermal stress, by monitoring Heat Shock transcription Factor 1 (HSF1), which is a key regulator of heat shock response. Conducting experiments on living cells, we observed clear changes in the dynamics of HSF1 when heat shocking: its diffusion slows down, together with an increase in the bound fraction and in the residence time.

1003-Pos Board B789

Real-Time Tracking of Lanthanide Ion Doped Upconverting Nanoparticles in Living Cells

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Lanthanide ion-doped upconverting nanoparticles (UCNPs), which emit in the visible range upon absorption of NIR photons, have attracted great attention in the area of biological imaging owing to their unique properties. First, two-photon upconversion of NIR excitation to the emission of a visible photon is so efficient that a tiny CW laser with the output of tens of milliwatts is sufficient as the excitation source. Second, by employing NIR excitation, one can suppress cellular autofluorescence, hardly induce photodamage to cells, and achieve relatively deep penetration into tissues. Finally, UCNPs exhibit neither photoblinking nor photobleaching, and their cytotoxicity is very low. As a result, UCNPs became one of the most promising nanoparticle systems for biological imaging and there are continuing efforts to improve their properties (e.g., increasing luminescent intensity and reducing the particle size) by designing new synthetic strategies. In this study, we demonstrated the benefits of using UCNPs as the probe for real-time imaging and particle tracking in living HeLa cells. Combined with the low cytotoxicity and photostability of UCNPs, NIR excitation enabled uninterrupted long-term imaging of living cells. For the first time, we obtained real-time images of endocytosed UCNPs at the single vesicle level for 6 h continuously at the rate of 20 frames sec^{-1}. The dynamics of particle transport was composed of multiple phases within a single trajectory including the active transport by motor proteins such as dyneins and kinesins.

1004-Pos Board B790

Photoswitchable Biocompatible Polymer Dots Doped with Diarylethene

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Molecular photoswitches can be employed for the study of protein trafficking in living cells and applications in optical memories. Especially, to switch fluorescence, fluorescence quenching mechanism via energy or electron transfer is one of the most fundamental pathways to realize the system of photoswitching. In order to achieve fluorescence photoswitching, photochromic compounds such as diarylethene have been used to toggle fluorescence on and off. For example, photochromic diaryethene induces absorption changes upon light irradiations via cyclization reaction, which would trigger the fluorescence toggling. On the other hand, polymer dots (P-dots) is one of the promising fluorescent probes for the biological applications. We assumed that doping diarylethene into P-dots would realize fabrication of photoswhitchable P-dots via energy transfer mechanism between fluorescent polymer and diarylethene. In this study, we synthesized photoswitchable P-dots doped with diarylethene to toggle the fluorescence back and forth via energy transfer mechanism. We also tried to apply synthesized photoswitchable P-dots toward biological imaging. First, we examined the photoswitching properties with absorption and fluorescence measurements. Fluorescence of P-dots was dramatically quenched upon photoirradiation with UV light and recovered after visible light irradiation. Those photoswitching processes were reversible and could go through at least 5 cycles. We are now applying photoswitchable P-dots synthesized as mentioned above to biological imaging. Details will be discussed at the meeting.

1005-Pos Board B791

Back-Scattered Detection Provides Viable Signals in Many Conditions

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Precision position sensing is required for many microscopy techniques. One promising method, back-scattered detection (BSD), provides position sensing at the level of several picometers, and is compatible with platforms that have restricted optical access (e.g. magnetic tweezers, atomic force microscopy, and microfluidics). However, widespread adoption of BSD may be limited by recent theoretical modeling that predicts diminished signals under certain conditions. In BSD the position of a micron-sized bead is measured by back-scattering a focused laser off the bead and imaging the resulting interference pattern onto a detector. Theoretical modeling of the detector response assumes the bead acts as a Mie-Debye scatterer and creates a first order interference pattern in the back-focal-plane of the collection lens. According to this Mie-Debye scattering model the BSD signal reverses sign many times for bead radii between 100 nm and 2000 nm and that for some radii (e.g. 1000 nm) the BSD response would be vanishingly small, limiting the applicability of BSD. We directly measured the BSD response while varying the experimental conditions, including bead radius, medium refractive index, and numerical aperture of the objective. Contrary to the proposed theory, we find that the signal increases with bead radius. Furthermore, the signal sign does not fluctuate, as predicted, over the tested parameters of radius, numerical aperture, and medium refractive index. We conclude that BSD provides a viable signal in a plurality of conditions.

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Use of Fluorescent Sphingolipid Precursors for Biophysical Studies of Sphingolipids

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Sphingolipids are one of the major components of cell membranes and also play critical roles in cell signaling. Many studies of disrupted cells have expanded our understanding of sphingolipid metabolism and function. Nonetheless, investigations of dynamic sphingolipid events, such as trafficking, diffusion, and organization in cell membranes, require observation of fluorescent sphingolipid analogs within living cells. However, sphingomyelin or ceramide analogs that contain a fluorophore-labeled N-acyl fatty acid cannot be used to track sphingosine or sphingosine-1-phosphate in cells. Additionally, the catabolism of these fluorescent sphingolipids may also result in fluorophore incorporation into glycerolipid species. Though various fluorescent sphingosine analogs have been developed that permit the study of sphingosine and its metabolites, poor photostability of the fluorophores limits long term data collection. Here, we report the use of fluorescent sphingolipid precursors in which a borondipyrromethene (BODIPY) fluorophore is incorporated into the sphingosine backbone. The enhanced photostability of the BODIPY fluorophore improves the ability to observe dynamic sphingolipid events. The fluorescent sphingosine analogs are incorporated into cells by addition to the cell culture media. To verify metabolic incorporation of the fluorescent sphingosine into cellular sphingolipids, lipid extracts from labeled cells were analyzed by thin layer chromatography and mass spectrometry. We demonstrate that the fluorescent analogs of sphingosine can be used to study dynamic events of sphingolipids, such as transport and trafficking.

1007-Pos Board B793

Exploiting Fluorescence Lifetime Plasticity in Flim: Target Molecule Localisation in Cells and Tissues

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The mechanisms of drug-receptor interactions and the controlled delivery of drugs via biodegradable and biocompatible nanoparticulate carriers are active research fields in nanomedicine. Many clinically used drugs target G-protein coupled receptors (GPCRs) due to the fact that signaling via GPCRs is crucial