receptor ($GABA_AR$). Super-resolution techniques [2] based on Individual Molecule Localization (IML) [3] provide the capability to quantitatively study these subcellular structures, giving access to its protein distribution at the molecular level.
In this work the highly heterogeneous spatial distribution of Gephyrin cluster along the 3D neuronal network has been investigated through dual color STORM (*Stochastic Optical Reconstruction Microscopy*) to characterize the post-synaptic area, for example reveling the interaction between Gephyrin and $GABA_ARs$.
To monitor the response of Gephyrin cluster to Long-Term Potentiation of inhibition (iLTP) their functional link with the synaptic terminals has been highlighted. The insight provided at the molecular level makes IML techniques the suitable tool for a quantitative study of Gephyrin distribution at synaptic level. Quantitative approach based on clustering analysis [4] provided access to a more comprehensive set of parameters able to define the response to iLTP, such as the area and the density of the scaffold protein. At the fluorescent tag level, to fulfill the requests of the quantitative analysis, an irreversibly photoactivatable fluorescent protein has been used (mEos), knowing its photophysical properties [5].
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2415-Pos Board B552
Single Molecule Analysis of Endogenous mRNA in Stress Granules
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In eukaryotic cells, mRNA is transcribed from DNA in the nucleus, and conveys genetic information to the ribosome in the cytoplasm. There are various regulation steps in the life of mRNA, including transcription, translation and degradation. When cells are exposed to environmental stresses, cytoplasmic mRNA and several proteins affecting mRNA functions assemble and form densely packed granular structures, which are called stress granule (SG). Previous studies showed that SG play an important role in mRNA remodeling for translational repression, but the underlying mechanism remains unclear. This is partly because there has been no method to visualize and analyze the spatiotemporal regulation of endogenous mRNA with sufficient performance in highly dense environments like SGs. Here, we investigated the behavior of endogenous mRNA in SG at the single-molecule level by using super-resolution localization microscopy and single-particle tracking. Super-resolution imaging revealed that mRNA was distributed heterogeneously in SG, whereas it was elusive in conventional fluorescence imaging. The results obtained by single-particle tracking represent that mRNA motility was suppressed inside SG compared to outside SG. These results suggest that SG has highly organized composition that might be responsible for the physiological functions in cellular stress responses. Therefore, the combination of super-resolution imaging and single particle tracking is a powerful tool to investigate the detailed organization of densely packed granular architectures in cells.

2416-Pos Board B553
Super-Oscillatory Imaging of Nanoparticle Interactions with Neurons
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We are developing a super-resolution imaging technology, called the Nanoscope, to allow label-free imaging of sub-cellular processes in living neural cells at nanometre resolution. This technique is an enhancement to current confocal microscope technologies and, unlike most super-resolution technologies, does not rely on fluorescent labelling. The phenomenon of super-oscillation means that by precisely controlling the interference of many band-limited waves, it is possible to generate a local region containing very high spatial frequencies. In the context of optics, this allows us to break the diffraction limit by making arbitrarily small spots far from any lens, within a dark field-of-view, surrounded by sidebands - trading efficiency for resolution. We select scattered light from the central spot using conventional optics, eliminating the sidebands and achieving resolution determined by central spot size. Our approach replaces a conventional microscope objective with a 'super-oscillatory lens' which sculpts the input light to form our strongly-confined spot. We have two methods of achieving this: the first uses a carefully designed set of concentric metal rings milled on a glass substrate to focus the light directly. The second method uses a spatial light modulator to structure the beam incident on a standard objective, shaping the standard focal spot into a sub-wavelength super-oscillatory spot.
We will use the super-oscillatory microscope to further the understanding of cellular function, during health and neurodegenerative diseases. Our initial experiments utilise unlabelled gold nanorods (500x75nm) in low-density primary neuronal cultures. Localisation and tracking of individual gold nanoparticles will allow us to determine the effect of nanoparticles on cell function, mechanisms of uptake and potential clearing. This technique can also be used to investigate the general mechanisms of uptake and subcellular trafficking, providing information on the cell's essential abilities to dynamically compartmentalise materials within the neuronal architecture.

2417-Pos Board B554
Quantitative Super-Resolution Microscopy using Novel Meditope Reagents
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Pointillistic super-resolution microscopy techniques are excellent tools for quantitative interrogation of protein distribution and dynamics on a nanoscale level. To efficiently detect single molecules, proteins of interest need to be genetically tagged with optical highlighter proteins or affinity tagged with antibodies labeled with fluorescent (or caged) dyes. We leveraged advantages of both approaches by utilizing a unique peptide-binding site in the Fab framework of monoclonal antibody: the meditope [1]. In this manner we can achieve stoichiometric and site-specific labeling of endogenous proteins using meditope-enabled antibodies.
We coupled a high-affinity meditope to a series of optical highlighter proteins to perform advanced photo-activated localization microscopy (PALM) imaging of human epidermal growth factor receptor 2 (Her2) on the membrane of breast cancer cell lines SK-BR-3 and BT-474. We first characterized binding and we typically obtain 10 nm resolution. Next, we used pair-correlation analysis [2] to quantitatively investigate distribution of Her2. While imaging with Fab complexes (one fluorophore/Fab) results in random distribution of Her2, imaging with Ab complexes (two fluorophores/Ab), results in mostly dimeric distribution of Her2. We anticipate that in the future our approach will provide invaluable insights on molecular dynamics and nanoscale spatial organization of growth factor receptors.
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2418-Pos Board B555
Fourier Transform Infrared Spectroscopy and Imaging in Cancer Diagnosis and Characterization
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Fourier Transform Infrared (FTIR) spectroscopy and imaging techniques are formidable, novel, rapid and non-destructive tools for characterization of different biological systems from molecules to membranes, cells and tissues. These techniques with multivariate analysis tools can be used in the diagnosis/follow-up of diseases including cancer, and in monitoring drug or chemical induced alterations in tissues and cells. In the current study, Attenuated Total Reflectance FTIR spectroscopy together with chemometric (cluster and principal component) analysis were utilized as a diagnostic tool for urinary bladder cancer while FTIR imaging technique was used in the clarification of sodium butyrate (NaB) induced-differentiation in colon cancer cells since butyrate has an anti-proliferative effect in colon cancer. For bladder cancer studies, bladder wash samples of bladder cancer and control groups were used directly for spectra collection. ATR-FTIR studies revealed significant alterations in lipid, protein, and nucleic acid content of bladder wash samples of cancer groups as compared to the control ones. Based on these spectral variations, bladder cancer group can be successfully differentiated from control via chemometric analysis with a higher sensitivity and specificity than many other methods used currently in bladder cancer diagnosis. In colon cancer study, aggressive CaCo2 cell lines were treated with 3mM NaB and cultured for 48h for complete differentiation. Specific band ratio analysis such as lipid/protein, glycogen/phosphate and RNA/DNA were calculated from the chemical maps of control

and NaB-treated cells. Imaging results indicated the reconstruction of cancer induced changes in response to NaB treatment by modulating the saturated, unsaturated, triglyceride, protein and nucleic acid content in the treated cells. The findings of this study supported that FTIR spectroscopic and imaging techniques are valuable, label-free and sensitive bio-analytical tools for cancer diagnostics and investigating the global roles of drugs on cancer treatment.

2419-Pos Board B556

Quantitative Imaging of Proteome Degradation in Live Cells by Stimulated Raman Scattering

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Protein degradation is an indispensable regulatory process throughout cell division, growth and differentiation. Therefore it is highly desirable to develop a non-invasive live cell imaging technique to visualize protein degradation with subcellular resolution. Here we report a strategy that utilizes metabolic labeling of (13)C-phenylalanine and visualizes both the original and the nascent proteome by stimulated Raman scattering microscopy in a time dependent manner. We chose the ring breathing vibrational mode of (12C- or 13C-) phenylalanine as the spectroscopic marker of (old or new) proteome and quantified proteome degradation by (12)C ratio maps. We demonstrated the general applicability of our technique in revealing steady-state protein turnover in mammalian cell and yeast, and extended our efforts to map proteome degradation dynamics after perturbation such as oxidative stress, neurite outgrowth during PC12 cell differentiation and huntingtin protein aggregation.

2420-Pos Board B557

Vibrational Imaging of Glucose Uptake in Live Cells and Tissues by Stimulated Raman Scattering Microscopy

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Glucose is a ubiquitous energy source for virtually all living organisms. Its uptake activity closely reflects the cellular metabolic status in various physiological and pathological conditions. Extensive efforts such as positron emission tomography (PET), magnetic resonance imaging (MRI) and fluorescence microscopy have been made to image glucose uptake but all with technical limitations. Here, we report a novel vibrational microscopy platform to visualize glucose uptake in living cells and tissues with subcellular resolution and minimal perturbation by performing stimulated Raman scattering (SRS) on a new glucose analogue. Cancer cells with differing metabolic characteristics can be distinguished. Moreover, heterogeneous glucose uptake patterns are observed with clear cell-cell variations in tumor xenograft tissues as well as in neuronal culture and mouse brain tissues. Therefore, by offering the distinct advantage of optical resolution yet without the undesirable influence of bulky fluorophores, SRS imaging of glucose uptake will be a valuable tool to study energy demands of living systems, particularly in tumors and brain.

2421-Pos Board B558

Beam-Scanning Broadband Cars Microscopy for Rapid Tissue Imaging

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A beam-scanning approach is demonstrated to improve signal-to-noise ratio and to reduce photo-induced sample damages in nonlinear hyperspectral imaging, such as broadband coherent anti-Stokes Raman scattering (BCARS) microscopy. Signals generated on a fast scanning axis are transferred to the slit of a monochromator, then onto the vertical axis of a CCD. This configuration acquires the whole BCARS spectra on the fast scanning axis at once, reducing the overall data transfer time. The fast scanning speed by a resonant beam scanner at 5 kHz greatly reduces sample damage from accumulative photo excitation, allowing for a higher excitation laser power to generate a stronger nonlinear signal. The optical characteristics related with this configuration and parameters are discussed and a few examples that demonstrate the advantage of the beam-scanning method for BCARS tissue imaging are presented.

Biosensors II

2422-Pos Board B559

Fluorophotometric Determination of Critical Micelle Concentration (CMC) of Ionic and Non-Ionic Surfactants with Carbon Dots Based on the Stokes Shift

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A new and facile method for the determination of critical micelle concentration (CMC) of ionic and non-ionic surfactants is proposed in this article. Carbon dots exhibited substantial fluorescence and therefore enhanced the sensitivity of this evaluation. Understanding the formation of surfactant micelles is vital for the applications of biomedicine such as drug fabrication and smart molecular vehicles in delivering therapeutic dosage to various molecular sites. The fluorescence property of carbon dots was utilized for the first time to estimate the critical micelle concentration of surfactants. The central concept of the approach is based on the Stokes shift determination of a system composed of constant amount of carbon dots with varying concentrations of ionic and non-ionic surfactants. The synthesized carbon dots were characterized by FTIR, TEM, XRD, Raman, UV, and fluorescence spectroscope. The carbon dots were excited at 280 nm so as to obtain maximum emission for the Stokes shift measurement. The CMC value of cetyltrimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS), Triton X-100, dodecyldimethyl(3-sulfopropyl)ammonium hydroxide (SB-12) evaluated by this approach was found to be 0.98, 7.3, 0.19, and 3.5 mM, respectively. The signals of spectra were assigned and explained in terms of both electron transitions between specific molecular orbital and the interaction with solvent.

2423-Pos Board B560

Novel Biosensors Based on Water-Soluble Fluorescent Silver Nanoclusters for Selectively Detection of Thiol-Amino Acid

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Noble metal (Au, Ag, Pt) nanoclusters, due to the attractive properties (such as brightness, photostability and biological compatibility), has recently become the subject of intense research and widely used in single-molecule studies, sensing, biolabeling, and biological fluorescence imaging. Numerous studies have shown that metal nanoclusters are excellent for detecting the poisonous metal ions (Hg)[1] and amino acid (cysteine, homocysteine)[2]. It would be significant to achieve detecting special amino acid in vivo or even intracellular. Herein water-soluble fluorescent silver nanoclusters with red emission band have been designed and successfully synthesized under UV photoreduction by using Poly (methacrylic acid) (PMAA) and other ligands as the scaffolds. The effects of reaction parameters, including UV irradiation time, pH, the initial ratio of COO^-/Ag^+, ageing time, and the type of scaffold, on the fluorescence of silver nanoclusters were studied. We have proposed a ligand-to-metal charge transfer (LMMCT) mechanism to explain the fluorescence from AgNCs.[3] Since the thiol group can coordinate with Ag^+, causing the quenching of fluorescence, we design a probe to detect the amino acid containing the thiol group, such as cysteine and glutathione. The detection limit can approach micro-mole concentration. The results should stimulate additional experimental and theoretical research on the molecular-level design of luminescent nano probes in biophotonics and other applications.

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2424-Pos Board B561

Novel Strategies for Micro-Contact Printing Based Protein-Protein Interaction Detection

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Originally developed for application in micro-electronics, micro-patterning has spread over most areas of science, including biology. Micro-patterning allows for the specific design of cell micro-environment and now covers a large number of cell biology applications, from stem cell culture and differentiation to printing of purified proteins or other biomolecules for in vitro assays. Recent efforts led to the development of much simpler, cheaper and more versatile methods. Nevertheless, there are still some technical difficulties and unresolved problems which need to be addressed.

We have recently introduced a method that is capable of detecting and characterizing protein-protein interactions (PPIs) in a live cell context by combining Total Internal Reflection Fluorescence microscopy and micro-patterned surfaces. Recent experiments showed that different biological questions require