The availability of a system such as the MS2-GFP fusion protein, which directly labels the mRNA, has allowed obtaining an estimation of the RNA polymerase elongation speed in living cells. There is however a large heterogeneity observed in RNA Polymerase II (PolII) elongation rates measured from fluorescence assays.
To shed further light on the source of this heterogeneity we introduce and discuss here a novel method based on the phasor analysis of steady state MS2-mRNA fluorescence trajectories. When applied to the study of PolII kinetics, we demonstrate that this approach allows resolving PolII elongation rates in a range from a few to hundreds of basepairs per seconds.
In order to couple this information to what happens to mRNA molecules once they leave the active transcription site, we combine 3D orbital particle tracking with Pair Correlation Analysis to investigate the diffusive routes taken by mRNA molecules within the nucleoplasm. With this approach we observe that the time an mRNA molecule takes to leave the transcription site is highly variable, ranging from a few to tens of seconds. Work supported in part by Grants NIH P41-GM103540 and NIH P50-GM076516

1630-Pos Board B581
Nanoscale Protein Diffusion by STED-Based Pair Correlation Analysis
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We describe for the first time the combination between cross-pair correlation function analysis (pair correlation analysis or pCF) and stimulated emission depletion (STED) to obtain diffusion maps at spatial resolution below the optical diffraction limit (super-resolution). Our approach was tested in systems characterized by high and low signal to noise ratio, i.e. Capsid Like Particles (CLPs) bearing several (>100) active fluorescent proteins and monomeric fluorescent proteins transiently expressed in living Chinese Hamster Ovary cells, respectively. The latter system represents the usual condition encountered in living cell studies on fluorescent protein chimeras. Spatial resolution of STED-pCF was found to be about 110 nm, with a more than twofold improvement over conventional confocal acquisition. We successfully applied our method to highlight how the proximity to nuclear envelope affects the mobility features of proteins actively imported into the nucleus in living cells. Remarkably, STED-pCF unveiled the existence of local barriers to diffusion as well as the presence of a slow component at distances up to 500-700 nm from either sides of nuclear envelope. The mobility of this component is similar to that previously described for transport complexes. Remarkably, all these features were invisible in conventional confocal mode.

1631-Pos Board B582
Analysis of Trabecular Bone Architecture using Two Photon Fluorescence Microscopy
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Biomechanical competence of trabecular bone is dependent on the makeup of its architecture. Alterations in the trabecular architecture can lead to fractures in metabolic bone diseases like osteoporosis. Therefore, it is paramount to understand the signaling mechanisms that dictate these changes in bone growth and fracture repair. Two photon fluorescence microscopy revolutionized the imaging of biological specimens utilizing its unique capabilities. The three-dimensional (3D) imaging based on nonlinear excitation of the fluorophores brings multiple advantages for imaging skeletal tissue. However, noise generated by the subsurface signal and auto-fluorescence of the local tissue make imaging of trabecular bone problematic. Imaging of calcified tissue presents a unique challenge to address the aberrations produced through the noise generated. Also a general practice of immunolabeling of the plasticized bone for antigen stability are to be optimized. We demonstrate here for the first time using two-photon fluorescence imaging of trabecular bone and its architecture identifying the structural differences and cell populations lining the trabecular cavity and also the cells embedded in it. Furthermore, we developed a shortened method of immunohistochemistry for plastic embedded bone tissue providing antigen stability for antibody labeling. Two photon fluorescence imaging greatly reduces photo damage and helps image of specimens of uneven planes to submicrometer resolution making this an ideal source for imaging in vivo signaling of trabecular bone. We demonstrate here labelling of multi colored fluorophores measuring Smad and ERK activity in trabecular bone growth in mice that are systemically injected with Bone Morphogenetic Protein 2 (BMP2). We optimized the conditions for in vivo imaging of bone tissue that is calcified and plasticized. We demonstrate here two photon fluorescence microscopy of the trabecular bone can be used for understanding the molecular mechanisms which control bone growth and development in vivo.

1632-Pos Board B583
Non-Linear Microscopy of Mitochondrial Damage and Abnormal Lipid Metabolism in Beta-Amyloid Expressing Yeast
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One of the earliest pathological hallmarks of Alzheimer's disease is the formation of soluble β-amyloid (Aβ) oligomers, also believed to be the primary neurotoxic agents long before the accumulation of amyloid plaques. However, the mechanisms by which the Aβ oligomers cause cell dysfunction and eventually cell death are poorly understood. The yeast *Saccharomyces cerevisiae* has here emerged as a valuable model for systemic studies of the intracellular cytotoxicity of Aβ species, revealing that Aβ transits through the different endocytic compartments and disrupts cell-, mitochondrial-, lysosomal- and ER membranes (for a review of the different aspects of amyloid-membrane interactions) finally activating the mitochondrial apoptotic pathway. In order to form a deeper understanding for the cause and consequences of mitochondrial damage, seemingly one of the central cytotoxic mechanisms, we have done a multi-parametric study on living GFP-Aβ42 expressing yeast using non-linear microscopy. The intracellular distribution of GFP-labelled Aβ42 was correlated with the corresponding distribution and morphology of mitotracker-labelled mitochondria by means of 2-photon fluorescence microscopy. Furthermore, the consequences of the dysfunctional mitochondria and the resulting oxidative stress were visualized by the monitoring of the general NADH levels based on their 2-photon-excited intrinsic fluorescence and the content and distribution/morphology of lipid stores by means of CARS microscopy (probing natural carbon-hydrogen vibrations). We could observe how Aβ-expressing yeast accumulates significant amounts of lipid stores and follow their coalescence to larger store units, which can be recognized as a general stress response, in this case most likely due to oxidative stress.

1633-Pos Board B584
Using Surface Plasmon Resonance to Study Species Transport across Lipid Membranes
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Studying species transport across lipid membranes by membrane transport proteins is important for various biological applications. Although patch-clamp technique is well developed for recording the ion transport across lipid membranes, the technique requires well trained personals for the challenging and delicate operation. In this study, we demonstrated using the surface plasmon resonance (SPR) based platform to detect the concentration change of the target species across the lipid membrane. We created sub-micron sized pore structure on the platform, in which the bottom surface is gold and the top surface is silica, and spanned lipid membranes over the pore. The process created a space inside the pore separated from the outside environment by the free-standing lipid membrane for further studying the species transport across the membrane. The platform geometry allowed us to combine plasmon-waveguide resonance (PWR) to the system to simultaneously monitor the refractive index change in the pore space, which is correlated to the target species concentration, and the refractive index change on the membrane above the top silica surface, which is correlated to the binding events occurring on the membrane surface. We expect to use this platform to monitor how various inhibitors or ligands could influence the transport dynamic of interested membrane transport proteins.

1634-Pos Board B585
Applications of High Resolution Surface Plasmon Resonance Imaging to Adherent Cells: Single Mammalian Cells to Bacterial Biofilms
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High resolution surface plasmon resonance imaging (SPRI) allows label-free imaging of subcellular features when performed using a high numerical aperture objective lens with a digital light projector to precisely position incident angle excitation. The SPRI signal is a result of the mass of material within

the evanescent field, and can be interpreted as a density or a refractive index of the cellular material. The quantitative nature of SPR images and the direct relationship to refractive index changes at the surface sensor allow for visualization new insights into mechanisms of cell biology at an interface. When applied to mammalian cells, such as rat aortic smooth muscle cells, cellular components near the sensor surface such as the cell membrane, focal adhesions, and cell nucleus are visualized in the SPRI image. Focal adhesion sizes measured by SPRI are similar with those highlighted with fluorescent antibody stained vinculin. In addition, a positive correlation between focal adhesion size and protein density is observed by SPR imaging. When SPRI is applied to pathogenic biofilms of Streptococcus mutans, distinct components of the bacterial biofilm at the surface including individual bacteria, bacterial microcolonies, and extracellular polymeric substance (EPS) are observed. SPRI shows that the refractive index of bacteria in a biofilm increases over time compared to that of bacteria not in a biofilm, which remains constant. SPRI also indicates that the EPS material generated in the biofilm at early time points is thicker near the bacterial microcolony periphery. This suggests that the EPS matrix is generated at the colony edge and that SPRI can be used to monitor the dynamics of EPS production in biofilms.

1635-Pos Board B586

Light Sheet Fluorescence Microscopy (LSFM) for Two-Photon Excitation Imaging of Thick Samples

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Over the last decades, fluorescence microscopy techniques have been developed in order to provide a deeper, faster and higher resolution imaging of three-dimensional biological samples. Within this framework, Light Sheet Fluorescence Microscopy (LSFM) became an increasingly useful and popular imaging technique able to answer several biological questions in the field of developmental biology [1]. Thanks to the spatial confinement of the excitation process within a thin sheet in the focal plane, it provides an intrinsic optical sectioning and a reduced phototoxicity. On the other side, Two-Photon Excitation (2PE), thanks to the use of IR wavelengths, has become an invaluable tool to improve imaging capabilities in terms of imaging depth and spatial resolution [2,3].
In this work we tested and compared the advantages provided by Two Photon Excitation in combination with two different light sheet based architectures: Selective Plane Illumination Microscopy (SPIM) and Inverted Selective Plane Illumination Microscopy (iSPIM) [4]. The two different optical approaches are characterized in terms of illumination intensity distributions and in terms of point spread function measurements, both in the linear and non linear regime. Additionally, particular attention has been addressed to the relationship between the sample holder and the specific sample geometry, showing the suitability of the inverted configuration when the sample geometry does not allow embedding in agarose gel (for example brain slices and retina). Furthermore, TPE-SPIM has been tested towards live imaging of nervous system in small animals, such as *Danio Rerio.*

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Biosensors I

1636-Pos Board B587

Sensing Elements Encapsulated within Hydrogel Matrix to Enhance the Signal-To-Noise Ratio

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In this study, we developed polydiacetylene(PDA) vesicle based colorimetric biosensor. PDA vesicle is a cluster of diacetylene(DA) lipid that has a unique property of color transition from blue-to-red upon eternal stimuli, such as temperature, pH, and mechanical stress. In our biosensor, PDA vesicle was conjugated with target-specific antibody by EDC/NHS coupled reaction to identify target protein. Antibody conjugated PDA vesicle was then encapsulated into Poly(ethylene glycol) diacrylate(PEG-DA) hydrogel at high density to enhance reaction sensitivity. Furthermore, PDA vesicle encapsulating hydrogel was formed in spherical shape by using droplet-microfluidic device to enable transportation. We named this colorimetric biosensor as immunohydrogel bead. Detection of PAT protein from genetically modified organisms (GMO) was attempted for practical use. The PAT protein allows herbicide resistance to GMO and was known as model protein of GMO. Using immunohydrogel bead, PAT protein was detected to 20 nM with naked eyes which means that even 1% of GMO can be detected. Thus, we expect that immunohydrogel bead can be used practically not only in GMO but also in other targets.

1637-Pos Board B588

Simulation Results for an Optically Active Semiconductor Nanopore

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Optical molecule detection is of increasing interest in biophysical applications especially for high speed molecule detection and DNA sequencing.
We present an optically active solid-state nanopore design for probing charged molecules. The proposed semiconductor nanaopore (SNP) is a hollow cylindrical semiconductor heterostructure consisting of a low band gap semiconductor in the center surrounded by a high band gap semiconductor. We use a single sub-band approximation to simulate the behavior of these SNPs and show that this structure exhibit a high quantum confinement effect that can be tuned by geometrical modification.
Our simulations indicate that a charge distribution within the SNP can change the energy state of the pore significantly. This effect is proposed to be usable for particle detection.

1638-Pos Board B589

Lipid Bilayer Coated Nanopipettes as Generic Nanopore Sensors with Enhanced Functionality

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Nanopore sensors show great promise for use as single-molecule diagnostic devices. Current limitations to nanopores include laborious fabrication, undesired interactions between species in solution and the nanopore walls, pore clogging and difficulties in controlling the speed of translocations.
Work in the literature shows the viability of using lipid coated solid-state pores and nanopipettes to overcome some of the aforementioned limitations to the development of nanopore biosensors.1,2 Such coatings offer increased control over the surface charge of the pore and the translocation speed, preconcentration of analytes on the pore surface prior to analysis, enhanced specificity and a reduction in pore clogging.
Our work revolves around developing lipid-bilayer modified pores as a generic platform to detect epigenetic modifications of DNA. Compared to pull-down assays, the combination with nanopore analysis provides enhanced information content, while being faster and cheaper than single-base resolution bisulfite sequencing. Our data further show that the coated nanopipettes remain stable for hours and suitable for DNA translocation experiments. DNA methylation is being investigated as a model system, with particular relevance as a biomarker for early cancer diagnosis.

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1639-Pos Board B590

Polarization-Based DNA Sandwich Assay with Au Nanoparticles using the Influence of Inter-Particle Distance

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Nucleic acid hybridization assay is widely used for application in biomedical research. In these field there is a considerable demand for the development of more sensitive , quantitative ,rapid and low-cost method for target detection. To meet these requirements, Nanoparticle-based controlled assembly method via DNA has become a useful tool for clinical diagnostics because of their strict selectivity. Gold nanoparticles(AuNPs) can be synthesized amenably and can be made highly stable. Moreover, they have unique optical response of surface plasmon resonance and this property can be easily adjusted by varying their size, shape, and the surrounding chemical environment. In this research, we focus inter-particle distance of AuNP dimer. Inter-particle separations are greatly sensitive for the resonance wavelength and interaction between AuNP. When it is small(<2nm), it can be observed that specific optical response in occurred such as significantly high intensity scattering, peak shift of absorbance spectra.
Recently, plasma mass spectrometry and surface enhanced raman spectroscopy(SERS) were reported as more sensitive method. However, they need