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 trans-

portation. 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

sophisticated process and expensive instruments. Hence, We propose the method to use optical anisotropy and scattered light intensity as indices for accurate and robust discrimination between single AuNPs and dimers using single-particle polarization microscopy in aqueous solution. This method is called a direct light scattering(DLS) method. This is homogeneous, rapid low-cost, sensitive method. We tested several way to deduce distance between two AuNP in the step of fabricating AuNP dimers and evaluated these AuNPs to utilize DLS method in colloidal solution. In this presentation, we report the result and conclusion about the influence of inter-particle distance in our experiment.

1640-Pos Board B591
Optically Tracing Electrical Synapses with Proton Channel-Based Voltage Sensing Protein

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The field of optogenetics has extensively improved over the last couple years allowing optical detection of single action potentials [CITE PAPERS]. In order to improve voltage sensors, a search strategy using the highly conserved S2 trans-membrane motif sequence identified 8 novel voltage sensing proteins. These proteins were fused to the fluorescent super ecliptic pHluorin A227D and transfected to HEK293 cells. The voltage sensing domain of a proton channel from liver fluke resulted in a sensor, Pado, which gave large optical signal and also responded to the induced pH change in the cell. This proton channel exhibits inhibition by extracellular Zn^{2+} and shifted response curves in differing pH environments. Higher buffering of the internal solution resulted in diminished pH dependent fluorescence change. With the ability to controllably manipulate pH of a cell, gap junctions can be traced through a network of HEK cells. Pado also provides a tool to screen fps for pH and voltage related activities. Ultimately Pado would make it possible to map electrical synapses and voltage activity simultaneously.

1641-Pos Board B592
Using Exploratory Data Analytics to Identify Deficiencies in mCherry Red Fluorescent Protein and Suggest Improvements

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Red fluorescent proteins are a valuable class of genetically encoded indicators for in vivo imaging. Even so, development of brighter variants has stagnated despite extensive efforts. To address this need, we have generated a library of over two hundred thousand simulated structures of mCherry fluorescent protein variants. The atomic positions of non-hydrogen atoms was then correlated with the planarity of the mCherry chromophore, a key determinant of molecular brightness. These studies revealed geometric instability of the mCherry chromophore. Further, we identified ten positional changes in mCherry side chains that strongly correlated with changes in the mCherry chromophore conformation. Site directed mutagenesis of key amino acids was then used to improve the mCherry quantum yield by approximately ten percent. These results suggest that a data analytics approach can be applied to structural engineering of fluorescent proteins for the first time.

1642-Pos Board B593
Design of a Theoretical Model to Identify Specific SSDNA Aptamers for Biosensing Applications

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Aptamers are single stranded DNA or RNA oligonucleotides which bind to proteins, small molecules, cells or organs with high affinity and specificity. Aptamers are designed to work in such a way that they only bind to their target proteins/cells, leaving other neighboring and non-targeting proteins/cells intact. This targeting efficiency makes them a promising solution to the problem associated with affinity and specificity of other bioactive agents, reducing harmful side-effects and costs. Target specific Aptamers can be produced by Systematic Enrichment of Ligands by Exponential Amplification, a method usually known as "SELEX Protocol". However, this SELEX protocol needs a large pool of ssDNA/RNA library containing ~10^{14} unique ssDNA/RNA sequences. Selection of only one aptamer for a target takes 15-18 rounds of selection and each round can take 2-3 days, which makes the process very time consuming and costly. In our model, we are trying to identify specific aptamer sequence for a specific target prior to experimental SELEX by a purely theoretical approach. This is done by rigorous theoretical studies on aptamer behavior in different biological environments. A molecular mean field theory is used which takes into account the size, shape, electrical properties and physical configurations of the aptamers along with the size, shape and physical properties of other chemical species associated with a particular biological environment. Coupling between different interactions, acid-base equilibrium and inhomogeneous dis-

tribution of different species along with the minimization of strong electrostatic repulsions gives us the most stable structure of aptamers specific to the target. So, this model can be a very efficient tool for designing aptamers to use in wide range of biosensing applications.

1643-Pos Board B594
Experimental Determination of Transition Dipole Moment Directions in Representative Fluorescent Proteins

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Fluorescent proteins are the workhorses of biological molecular imaging. Important imaging modalities (such as polarization microscopy or FRET imaging) exploit anisotropic optical properties of fluorescent proteins. The anisotropy (directionality) of optical properties of fluorescent proteins is described by a vector, the transition dipole moment (TDM). Despite the importance of molecular TDM orientation for quantitative structural interpretation of many imaging experiments, experimental data on TDM direction in fluorescent proteins is very limited. Here we present the results of our optical measurements on crystals of representative fluorescent proteins, as well as mathematical interpretation of these results, yielding information on the orientation of TDMs within the investigated fluorescent protein molecules.

1644-Pos Board B595
Glycine Protects Hepatocytes through a Chloride Independent Mechanism

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Cytoprotection by the amino acid glycine was first described more than two decades ago. Accumulated evidence shows that glycine protects against hypoxia, ischemia-reperfusion and several toxins in hepatocytes, renal tubular cells and other cell types. The exact mechanism of the protection is still under investigation and multiple models have been proposed. One working model is that the protection exerted by glycine is mediated by inhibition of ligand-gated chloride channels. In our study, we used a genetically encoded chloride biosensor to test this working model. Isolated rat hepatocytes infected with adenovirus for expression of the chloride biosensor were treated with the mitochondrial uncoupler, carbonyl cyanide m-chlorophenyl hydrazone (CCCP), in the presence and absence of 3 mM glycine. With and without glycine, intracellular chloride concentration increased upon addition of CCCP. Without glycine, CCCP led to swelling, membrane bursting and death of hepatocytes in Krebs-Ringer-Hepes buffer. By contrast in the presence of glycine, cell death was markedly delayed. Moreover instead of swelling, hepatocytes underwent cell shrinkage after CCCP. These findings suggest that glycine alters cell volume regulation after CCCP and prevents cellular swelling leading to plasma membrane rupture and consequent loss of cell viability. DK073336, DK037034 and 14.Z50.31.0028.

1645-Pos Board B596
Small Angle Neutron Scattering Studies of Glucose Oxidase Immobilized on Single Layer Graphene: Relevant to Protein Microfluidic Chip

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Chronic diseases like diabetes has become more pervasive. Blood glucose monitoring is a valuable tool in the health management and consequently point-of-care devices like glucometers has now become indispensable. Since, obtaining sufficiently accurate and reliable measurements are very essential, a promising solution is to incorporate enzymes into the biosensor [1]. Enzymes like glucose oxidase (GOx) are ideal for consistent measurements because of their specificity and the ability to electrochemically transduce from the enzymatic reaction. Consequently, the presence of glucose in blood plasma can be detected by the change in the potential of the electrode adjacent to GOx during catalysis of glucose [2]. Further, the oxidation overpotential of the electrode