Scanning Laser Image Correlation (SLIC) is a technique to measure the flow of small particles and to observe local flow patterns over an area. SLIC can be applied to situations including blood cells flowing through blood vessels or tracer particles flowing through microfluidic channels. The main advantage of SLIC over other flow measurement techniques is that SLIC can be scaled to measure flow in areas ranging from microns to centimeters wide. To accomplish this, an image is first acquired through laser scanning and analyzed with number and brightness analysis (N&B) to identify regions of flow. This is used as a guide to manually (or potentially automatically) select a pattern within the image, such as a line along the center of a channel, that is then scanned repeatedly with the laser beam. Since the entire image is not scanned in each measurement SLIC measurements can be obtained quickly and efficiently. The results of these scanned patterns are analyzed with the recently developed pair correlation technique to extract the rate of flow and to identify characteristic flow patterns such as turbulence, particles that adhere to the channel walls, and variable velocity along the length of the channel.

In the work presented here we demonstrate the effectiveness of SLIC by measuring blood flow in a zebra fish model. With SLIC, we are able to obtain blood flow measurements equivalent to those obtained with other techniques. We are also able to map the rate of flow and to observe variability in flow rate over time. This indicates that SLIC has potential to measure blood flow in other animals as well and may hold potential as the basis of a medical device.

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3004-Pos

Fluorescent Lifetime Imaging of Lignin in the Plant Cell Wall

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Lignin, a highly complex but integral part of plant cell wall , is indigestible and therefore a concern in biomass deconstruction for cost effective biofuel production. A way to address this issue is to manipulate the plant to generate a cell wall that is amenable to breakdown. However, little is know about the actual assembly of lignin during plant cell wall biosynthesis. Fluorescent Lifetime Imaging Microscopy (FLIM) utilizes the lifetime of the auto-fluorophore signal generated, rather than its intensity , to create an image. An interesting characteristic of lignin is that it is highly auto-fluorescent in the UV excitation region due to its phenolic ring composition. Thus, it may be possible to infer structural and organizational information of lignin polymerization using this imaging technique. In this study FLIM is used to resolve differences in lignification in the plant cell wall during development.

3005-Pos

Using High Resolution Photoemission Electron Microscopy to Quantify the Absorption Properties of Human Eumealnin and Pheomelanin

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A novel approach to photoemission electron microscopy is used to enable the first direct measurement of the absorption coefficient from intact melanosomes. Two cases are examined in detail: bovine melanosomes from choroid and iris tissue, and human iris melanosomes isolated from different colored irides. The difference in absorption between newborn and adult bovine uveal melanosomes is in good agreement with that predicted from the relative amounts of the monomeric precursors present in the constituent melanin as determined by chemical degradation analyses. The results demonstrate that for melanosomes containing eumelanins, there is a direct relation between the absorption coefficient and the relative 5,6-dihydroxyindole: 5,6-dihydroxyindole-2-carboxylic acid (DHI:DHICA) content, with an increased UV absorption coefficient associated with increasing DHICA content. The human iridal melanosomes from different colored irides contain both eumelanin and pheomelanin; the ratio of which varies with iris color. Taking pigment composition for these melanosomes into account, the absorption coefficient of natural eumelanin is determined to be a factor of six greater than that of natural pheomelanin. This contrasts synthetic models for eumelanin and pheomelanin, which exhibit comparable absorption coefficients at this wavelength. This difference between natural and synthetic systems underscores the care that must be exercised in using such polymeric systems as models for photophysical and photochemical properties of melanosomes. The determined absorption coefficients for the iridal melanosomes further suggest that the correlation between epidemioloigical data and the eumelanin:pheomelanin ratio reflects an increased exposure of tissues to UV light rather than a increased reactivity of pheomelanin.

3006-Pos

Correlative TEM/FISH Imaging of Microbial Communities

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Biofilms are the predominant lifestyle for most microbes. Mixed microbial communities are adapted to their specific environment allowing them to live under sometimes extreme conditions and to perform complicated metabolic pathways. They often show high species interdependencies suggesting that such communities are highly organized. However, little is known about detailed interaction between the microbes. This is due to difficulties in both studying them at sufficient resolution to visualize the individual community members in their biofilm context and at the same time identifying each community member.

To overcome this problem we have developed a technique that combines TEM sample preparation and imaging with 16S rRNA Fluorescence In-Situ Hybridization (FISH). This technique can be applied both to thin resin sections and to cryo TEM, allowing the identification of the microbes for both TEM techniques.

We applied the technique first to already well characterized Acid Mine Drainage biofilms, and we currently transfer the same technique to the termite hindgut microbial community. Our goal here is to clarify the mechanism how the up to 200 reported species in the hindgut (Warnecke et al. (2007), Nature 450) manage to efficiently degrade lignocellulose. TEM shows the attraction of the bacteria towards the wood particles and interesting extracellular features such as vesicles, which seem to be involved in the digestion process. While cells with different morphologies are usually densely packed, even single bacteria prove the ability to digest the material by themselves as they manage to create tunnel-like structures through the wood. Since the termite hindgut community is regarded one of the most effective lignocellulose degrading systems, it is of great interest for the research on second generation biofuels.

Fluorescence Spectroscopy

3007-Pos

Exploiting the Environmental Sensitivity of Fluorescent Proteins Allows Unambiguous Discrimination of Genetically Identical Labels

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The application of transcriptional fluorescent fusion proteins has revolutionized the field of single molecule biophysics. Recent efforts to expand beyond GFP by way of mutagenesis have generated nearly comprehensive libraries of fluorescent proteins exemplified by a wide variety of spectral excitation/emission profiles, photostabilities, and quantum efficiencies. A different, but similarly applicable approach to expand the usefulness of these fluorescent constructs can be achieved by combining spectral imaging with multivariate analysis to quantitatively separate each of the emitting species present in a sample. A recent demonstration of this imaging methodology, under the extreme condition of two genetically identical fusion proteins (YFP) conjugated to two different membrane receptors (TLR4 and the BK channel), reveals that the slight perturbation of the local environment of the fluorescent reporter is sufficient for spectral separation, and quantitatively interpretable images. In this talk we will highlight several recent discoveries enabled by multivariate analysis of environmentally specific perturbations of fluorescence in both prokaryotic and eukaryotic systems, and demonstrate the implications of these findings on the commonly used analytical tools, fluorescence correlation spectroscopy (FCS) and fluorescence resonant energy transfer (FRET). Finally, the potential for mapping local chemical environments based on multivariate analysis of spectral images will be discussed.

3008-Pos

Accurate Color Tuning of Firefly Chromophore by Modulation of Local Polarization Electrostatic Fields

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In molecular biology, fluorescent proteins have become a unique marking tool for gene expression, environmental pollutants, and monitoring the dynamics of AIDS virus and single-molecule motors [1-3]. Particularly, the mutagenesis

method is employed to produce mutants of fluorescent chromophore for color shifts [4, 5]. However, due to the uncertainty and complexity of the mutation effect on the wavelength of emitted light, real color modulation for target marking remains unavailable. In this work, we report a top-down method for the accurate and continuous color tuning of firefly chromophore (oxyluciferin) by controlling the surrounding polarization electrostatic fields. Systematic investigations of the absorption spectra of oxyluciferin molecules are carried out in the framework of time-dependent density functional theory. Results show that the polarization electrostatic field applied on the long molecular axis significantly changes the optical properties. However, if the field is applied on the out-of-plane axis, its effect is almost negligible. Under long axis electric fields, the wavelength of the two main peaks shifts continuously, covering a wavelength range of about 100 nm. Such a wide range of wavelength shift provides us a realizable modulation technique for very accurate color tuning of fluorescent proteins. The need of any special marking application can be met by careful design of the local polarization electrostatic fields. On the other hand, the peak intensity is also associated with the electrostatic fields, which shows that the efficiency of light emission can be well enhanced as well.

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3009-Pos

Sequence-Dependent Enhancement of Cy3 Fluorescence on DNA

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Cy3 is a cyanine fluorescent dye extensively used as a fluorescent probes in molecular biology, biochemical and biophysical applications. We investigated the fluorescent properties of Cy3 covalently attached to the 5' terminus of DNA oligonucleotides, and demonstrated that its fluorescence efficiency and lifetime depend strongly on DNA sequence. Fluorescence quantum yields and mean fluorescence lifetimes ranged from 0.18 to 0.39 and from 533 ps to 1.2 ns respectively. DNA sequence determines the extent and nature of the interactions between the dye and the DNA bases, which are responsible for the unusual enhancement in fluorescence observed for a large number of oligonucleotides. Results are discussed in terms of a photoisomerization mechanism that deactivates the excited state and thus competes with fluorescence. The efficiency of isomerization decreases when Cy3-DNA interactions prevent rotation around the double bonds, resulting in an increase in the lifetime of the singlet excited state. We have shown that the ability of Cy3 to interact with DNA depends on the flexibility of the oligonucleotide and the presence of purines in the chain.

3010-Pos

Determination of Fluorophore Orientation and Energy Transfer from MD Simulations

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Fluorescence Resonance Energy Transfer (FRET) spectroscopy is a technique that is widely used to obtain co-localization and structural information of proteins in their native environment. The technique is based on the mechanism of energy transfer by dipole-dipole induced, non-radiative interaction between a fluorescent donor and a suitable acceptor. While the rate of energy transfer depends on the distance between the donor and acceptor, the use of FRET as a spectroscopic ruler is complicated by it also being dependent on the relative orientations of the fluorescent probes. In general these orientations are difficult to determine experimentally making the technique uncertain for measuring absolute distances.

Simulations may offer an alternative means of understanding the behavior of the fluorophores at the molecular level, thus enabling distances between specific sites in the sample to be determined more accurately by calculating the orientation factor for a given system. To examine this possibility, we attempt to simulate FRET in a simple model that allows atomistic simulations in the 10s of ns. The system contains individual donor and acceptor molecules in an aqueous solution.

Preliminary results from standard MD simulations show that the simulation accurately predicts the probability density of the orientation factor κ^2 and reproduces experimental values of the anisotropy decay for donor and acceptor molecules. By simulating FRET in a simple system we hope to gain insight into the process of the energy transfer and the factors affecting the behavior and orientation of the fluorophores in order to better understand and analyze data from FRET experiments. The results of this study might also be useful as indications of when simulation may help to understand and analyze data from more complicated FRET experiments.

3011-Pos

Fretting About FRET: Breakdown of the Ideal Dipole Approximation

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Fluorescence-detected resonance energy transfer (FRET) experiments have been a useful tool in structural biology for four decades and have enjoyed resurgence in the last several years due to improved fluorescent labeling techniques and the rapid growth of single-molecule methods. As modern experiments examine a variety of complex systems, the validity of the assumptions that underlie analysis of FRET data is unclear. In this talk I will examine one of these, the ideal dipole approximation (IDA). Calculations showing the breakdown of the IDA in several commonly-used FRET probes (e.g. Fluorescein, AlexaFluor 488 and 594, Cy3, Cy5) will be presented and connections will be drawn to the impact on FRET experiments. In particular, breakdown of the IDA exacerbates problems due to limited sampling of dye orientations (i.e. the kappa squared problem). Guidelines will be suggested for planning a FRET experiment to avoid potential issues with the IDA and other assumptions employed in analysis of FRET data.

3012-Pos

The First All-Nucleobase Analog FRET Pair

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The fluorescent nucleobase analogs of the tricyclic cytosine (tC) family are promising nucleic acid probes capable of being inserted into double-stranded DNA as a replacement for one of the natural bases without perturbing the overall double helical structure. The high fluorescence quantum yields in both single- and double stranded DNA, combined with a rigid and well-defined position inside the DNA double helix, make these molecules particularly well suited as fluorescence resonance energy transfer (FRET) probes in nucleic acid studies. Recently we reported the first all-nucleobase analog FRET-pair, consisting of tC^O as the donor and the newly developed tC_{nitro} as acceptor which will be the focus of this presentation.[1-3] The FRET-pair successfully monitors distances covering up to more than one turn of the DNA duplex and, more importantly, the rigid stacking of the two base analogs, and consequently excellent control of the their exact positions, results in a very high control of the orientation factor in the FRET efficiency. A set of DNA strands containing the FRET-pair at wisely chosen locations will, thus, make it possible to accurately distinguish distance- from orientation-changes using FRET. We believe the development of this new tool opens up a wide range of possibilities in the structural investigation of nucleic acids, *e.g.* in characterizing DNA-protein complexes and in monitoring the inherent dynamics and the structural changes of nucleic acids in response to all kinds of stimuli.

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3013-Pos

A Fluorescent Indicator Monitors in Vivo Acetyl-Transferase Activity

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Lysine acetylation was identified in histones as a posttranslational modification that plays an important role in chromatin regulation. Following that discovery many other nuclear and cytoplasmatic proteins have been identified as targets of this modification. Acetylated proteins are often involved in the regulation of DNA transcription, cell growth, differentiation and epigenetic information. Moreover, aberrant levels of acetylation were reported in various human diseases such as neuropathologies and cancer (Watson, J.A. et al. 2009). To date, however, no methods for real-time monitoring of acetyltransferase activity are available for application in living cells.

We shall present the first cell-permeable fluorescent indicator of acetyltransferase activity in live cell cultures. The sensor consists of the basic domain of the HIV-1 trans-activator protein (Tat) labeled with a pair of fluorescent dyes