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