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We present a novel analysis technique that can measure the densities and oligomerization states of fluorescent macromolecules within individual images obtained using conventional single detection channel laser scanning microscopy. The method is based on fitting intensity histograms from images with super-poissonian distributions to obtain density maps of fluorescent molecules and their molecular brightness in cellular samples. The approach represents a transposition of the temporal photon counting histogram (PCH) to the spatial domain and can thus be applied to analysis of chemically fixed tissue as well as live cells. The technique does not rely on spatial correlations, which frees it from biases due to subcellular compartmentalization that can be problematic for spatial image correlation based approaches. Analysis of computer simulated images and immunochemically stained $GABA_B$ receptors in spinal cord samples shows that the approach provides accurate estimates of monomer/dimer distributions over a broad range of densities within limits set by spatial sampling (areas of 6 μm^2) and the yield of the fluorophore. We use this method to show, for the first time, that the G-protein coupled receptor for substance P (NK-1r) forms almost exclusively homodimers on the plasma membrane in native spinal neurons, in contrast to within the cytoplasmic compartment where it is composed primarily of monomers. The density of NK-1r homodimers on the surface of the membrane was estimated to be 38±7 μm^{-2}. Triggering receptor internalization with capsaicin caused a measurable decrease in homodimer density at the membrane to 21±8 μm^{-2}. Independent immunocytochemical analysis using electron microscopy confirmed the differential distribution of NK-1r monomers and homodimers in distinct subcellular compartments. This new method opens the door for quantification of protein oligomer distribtions with tissue samples prepared by standard immunocytochemistry.

Imaging and Optical Microscopy - III

823-Pos Design Sensitive Protease Sensors for Living Cell Imaging

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Proteases are essential for regulating a wide range of physiological and pathological processes and for regulating quality control of life cycles. Currently there is a strong need to develop protease sensors that are capable of quantitatively measuring protease activity in real time and monitoring activation and inhibition of enzymatic activity in various subcellular compartments. In this paper, we report a novel strategy to create protease sensors by grafting an enzymatic cleavage site into a chromophore-sensitive location of the enhanced green fluorescent protein (EGFP). Our designed protease sensors exhibit a large ratiometric optical signal change, and a wide dynamic range in both absorbance and fluorescence, as a response to the action of proteases. These engineered protein variants exhibit high enzymatic selectivity and kinetic responses that are comparable or better than current small peptide probes. Additionally, our developed protease sensors can be utilized for real real-time monitoring of cellular activation of zymogens and the effects of inhibitors in living cells. This designed strategy opens a new avenue for developing other specific protease sensors to investigate enzymatic activity in real time, diagnose diseases related to proteases *in vitro* and *in vivo,* and screen protease inhibitors with therapeutic effects.

Keywords

Enhanced green fluorescent protein, protease sensor, ratiometric change, protease activity and protease inhibitor

824-Pos Fluorescence Microscopy Investigations of Ligand Propagation and Accessibility to the Basal Membrane of Adherent Cells

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Board B669

Fluorescence microscopy methods including total internal reflectance (TIRF), and confocal laser scanning microscopy (CLSM) allow researchers to obtain images of fluorescently labeled components of cell membranes. As such these methods are often used to examine interactions occurring between membrane receptors and ligands such as antibodies and growth factors. For quantitative biophysical applications based on these imaging methods, one often assumes that the maker of interest has the ability to access all areas of the membrane equally. Our findings suggest that there is limited accessibility of ligands under the basal membrane of adherent cells plated on bare glass. We present a detailed examination of the extent to which ligands are able to propagate under adherent cells which have been grown on a variety of biologically compatible substrates. Furthermore, we examine the steric proproperties that limit basal membrane accessibility using a number of typical fluorescent labels, including antibodies, fluorspheres, small organic dyes and quantum dots. Finally, we examine the kinetics of the process by fluorescence photobleaching studies using both fluorescent ligands, and GFP-labeled cells, on the different substrates. Taken together we determine which of the substrates examined provides the ideal balance between cell growth/proliferation and ligand propagation for quantitative fluorescence microscopy studies on the basal membrane.

825-Pos An Automated System For Screening Pharmaceutical Agents As Potential Multiphoton-excited Cancer Contrast Reagents

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Board B670

Multiphoton microscopy has proved to be a powerful tool for imaging live tissue providing sub-cellular resolution at depths 2 to 3 times possible using confocal microscopy, as well as for imaging UV excited fluorophores using much less damaging NIR light. This success has driven research into clinical applications for the technique, such as cancer detection via a two-photon endoscope. However, there is a real difference between "live tissue" and "live human tissue" imaging, as the majority of the current dyes and contrast agents used in laboratory experiments visualizing tumors in animal models have a toxicity that precludes their use in a clinical setting. Multiphoton imaging using intrinsic signals can provide a unique view of tissue morphology without the use of added fluorophores, however with the exception of collagen SHG, these signals are extremely weak due to low two-photon action cross-sections and require high excitation power to generate detectable signal. One possible solution is to employ drugs already pre-authorized by the FDA, many of which are fluorescent. To this end we have built a fully automated two-photon action cross-section measuring instrument to easily screen these drugs for use as two-photon excited clinical markers.

826-Pos Back-scattered Detection Provides Atomic-scale Localization Precision, Stability, and Registration in 3D

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Board B671

State-of-the-art microscopy techniques (e.g., atomic force microscopy, scanning-tunneling microscopy, and optical tweezers) are sensitive to atomic-scale (100 pm) displacements. Yet, sample drift limits the ultimate potential of many of these techniques. We demonstrate a general solution for sample control in 3D using back-scattered detection (BSD) in both air and water. BSD off a silicon disk fabricated on a cover slip enabled 19 pm lateral localization precision ($\Delta f = 0.1$–50 Hz) with low crosstalk between axes ($\leq$3%). We achieved atomic-scale stabilization (88, 79, and 98 pm, in x, y, and z, respectively; $\Delta f = 0.1$–50 Hz) and registration ($\approx$50 pm (rms), $N = 14$, $\Delta t = 90$ s) of a sample in 3D that allows for stabilized scanning with uniform steps using low laser power (1 mW). Thus, BSD provides a precise method to locally measure and thereby actively control sample position for diverse applications, especially those with limited optical access such as scanning probe microscopy, and magnetic tweezers.

827-Pos Real Time Quantitation Of An Endogenous mRNA In Single Living Cells

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mRNA is a key player in gene regulation. However, the characterization of endogenous mRNA is difficult using conventional approaches. In our previous work we visualized endogenous *c-fos* mRNA in a living COS-7 cell using antisense oligonucleotide probes bearing 2′-*O*-methyl RNA backbone under a fluorescence microscope. Endogenous *c-fos* mRNA was distributed diffusely throughout the cytoplasm of COS-7 cells. In this study, fluorescence correlation spectroscopy (FCS) was chosen to quantify the target mRNA visualized by fluorescent antisense probes. When antisense probes hybridize with the target mRNA, they form large complexes with slower diffusion constants than unbound probes. Antisense probes labeled with Cy3 were microinjected into the cytoplasm of COS-7 cells and the fluorescence intensity was analyzed by FCS. As we expected, probes hybridized with mRNA showed slower diffusion times than those of unbound probes. Two components having different diffusion times were observed, suggesting that we could detect both antisense probes hybridized with endogenous *c-fos* mRNAs and unbound probes. The fraction ratios of bound and unbound forms were different among cells, reflecting the different concentration of endogenously expressed *c-fos* mRNA. By using the number ratio of these two forms obtained in each cell and K_d value of the hybridization, the concentration of endogenous *c-fos* mRNA in each cell was determined. The concentration of endogenous *c-fos* mRNA in COS-7 cells ranged from 99.2 nM to 752 nM and its average was 274 nM $\pm$ 123 nM (n = 178 cells). This novel method to quantify endogenous mRNAs in single living cells will help us to elucidate the characteristics of mRNA such asdistribution, kinetics and functions.

828-Pos Time-resolved Anisotropy Imaging combined with FCS in a Confocal Microscope

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Board B673

We present a technique which provides time-dependent fluorescence anisotropy images next to fluorescence correlation spectroscopy measurements (FCS) in a single confocal mirror-scanning microscope. The recording process is based on a multi-dimensional TCSPC (time-correlated single photon counting) technique. The optical part deploys a conventional microscope, picosecond diode laser excitation, fast galvo-mirror scanning and confocal detection. A polarizing beamsplitter splits the fluorescence signal into the detection channels. In order to minimize signal crosstalk and to increase the throughput both detectors are connected to independent TCSPC channels. The laser beam is scanned over the sample for anisotropy imaging and can be parked at a specific location for FCS enabling a direct combination of the results of both techniques in a single specimen. A comparison of rotational correlation times and lateral diffusion of fluorophores inside lipid vesicles is performed and discussed in the framework of a common model.

829-Pos Applications Of Quantum Dots For Single Molecule Imaging In Cells And Substrate-supported Planar Membranes

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Board B674

Quantum dots have several advantages compared to other probes for single molecule imaging. These include enhanced brightness and photostability as well as in some cases smaller size. Perhaps the major advantage of quantum dots for single molecule imaging is the possibility of simultaneous imaging of multiple species at fast repetition rates over long periods of time. With this in mind, we have begun assembling a microscopy system eventually capable of imaging multiple colors of single quantum dots at high repetition rates over long periods of time in cells and substrate-supported planar membranes. With our current system, which consists of an Olympus IX81 microscope equipped with a 100 W Hg arc lamp for excitation and an electron-multiplied CCD (Andor DV887-ECS) for detection, we can in some cases image single quantum dots with 100 μs signal integration or at rates up to about 250 Hz. These results are however very dependent on the particular emission color characteristics of the quantum dots, as we find that certain quantum dot colors are dimmer and/or primarily in a non-fluorescent state. We will present data on the intensity and on/off characteristics of a variety of quantum dots. We will also give examples of single molecule imaging with quantum dots for tracking membrane proteins and biotin lipids in cells and biotin lipids in planar substrate-supported membranes.

830-Pos Protein Diffusion Vs. Biopolymer Volume Fraction In Live *Escherichia coli*

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We have previously measured green fluorescent protein (GFP) diffusion in the cytoplasm of *Escherichia coli* grown in minimal media by fluorescence recovery after photobleaching (FRAP). Systematically increasing the mean biopolymer volume fraction $<\phi>$ by either increasing the growth osmolality (allowing cells to adapt) or sudden osmotic upshift (plasmolysis) enables us to investigate the severity of possible crowding, binding, and confinement effects *in vivo*. While increasing $<\phi>$ by varying the growth osmolality causes a small decrease in $<D>$ from 14.8 ± 3.4 to 6.1 ± 2.4 $\mu m^2\text{-}s^{-1}$, plasmolysis causes a factor of 70 decrease in $<D>$ to 0.20 ± 0.16 $\mu m^2\text{-}s^{-1}$ over the same range of $<\phi>$. Crowding models, such as scaled particle theory (SPT), alone are unable to account for the difference between the two methods. We are investigating the hypothesis is that there are two-domains of diffusion in the cell: slower local GFP diffusion within the nucleoid due to larger crowding/confinement effects and faster local GFP diffusion in the ribosome-rich cell periphery due to ineffective crowding by the larger ribosomes. The effective D would then be a composite of motion within these two domains. According to this model, adapted cells will maintain the segregation of the two domains, while in plasmolyzed cells the periphery volume decreases and proteins diffuse more through the nucleoid, decreasing the effective D dramatically. Time resolved fluorescence anisotropy and a careful measurement of the spatial extent of the nucleoid and ribosomes will test this hypothesis.

831-Pos Role of Various *in Vivo* MR Methodologies in the Evaluation of Prostate Cancer

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Prostate cancer is the most common and leading male cancer. Various diagnostic tests like estimation of prostate specific antigen (PSA) level, digital rectal examination (DRE), and transrectal US biopsy are routinely used. In this presentation, we present the results of a comprehensive study carried out for evaluating the role of MRI, MR spectroscopic imaging (MRSI) and diffusion weighted imaging (DWI) in prostate cancer diagnosis.

MR imaging with endorectal coil provides improved sensitivity and high-resolution T2-weighted images can be used to identify tumor of the prostate. The normal peripheral zone (PZ) of prostate is bright in the T2-weighted images while tumor is darker on the same images. MRSI provides metabolic information and ratio of [Citrate/(Cho+Cr)] metabolite ratio is used to predict malignancy. A cut-off value of the metabolite ratio was obtained using ROC analysis from 67 patients and was tested in another group of 70 patients for estimation of sensitivity and specificity.

In addition, DWI was carried out on prostate cancer patients to investigate its role in the differentiation of malignant and normal prostate tissue. An ROC curve analysis was carried out to obtain a cut-off value of ADC to predict malignancy. A correlation between metabolite ratio and ADC value of the peripheral zone of patients was also established.

The results of these *in vivo* MR techniques indicate the diagnostic potential and management of prostate cancer especially in clinically challenging cases of patients with PSA level in the range of 4 – 20 ng/mL and/or abnormal DRE.

832-Pos Imaging Subcellular Structure by Using 4Pi Confocal Fluorescence Microscopy

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Compared with standard confocal microscopy, the 4Pi technique yields a substantial improvement of resolution in the axial direction (~100 nm), which is extremely helpful in the study of organelles and other subcellular structures. In 4Pi microscopy, the spherical wavefronts of two opposing objective lenses are superimposed for excitation (type A), emission (type B) or both (type C). The sharp interference maximum is accompanied by two side lobes, which can be strongly reduced by using two-photon excitation (2PE). Therefore, bright 2PE fluorescent labels are required for routine application of 4Pi microscopy.

Genetically encoded fluorescent proteins (FPs) of the GFP family are particularly useful in cellular imaging. EosFP, a fluorescent protein which can be switched from a green- to a red-emitting state by 400-nm illumination (1), is an excellent FP for 4Pi microscopy with 2PE (2). Moreover, its ability to switch its emission color allows for regional optical marking in 3 dimensions. We present examples of 4Pi multi-color imaging of subcellular structures of cultured human cells using both FPs and synthetic dyes.

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833-Pos A Novel Interaction Assay Using The Rotational Correlation Time As A Ruler For The Molecular Volume

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Biological processes are frequently regulated by multiprotein complexes where the spatial proximity of the components facilitates resp. enables their function. High resolution fluorescence spectroscopic methods nowadays allow for the spatio-temporal analysis of localisation and reactivity of proteins in multiprotein complexes.

Interactions are usually studied by fluorescence resonance energy transfer (FRET). This requires labelling of the putative partners in the complex. However, the FRET method can not be applied for the identification of yet unknown partners. In molecular biology the green fluorescent protein (GFP) is usually used to localise coexpressed proteins inside living cells. A main goal of this project is to additionally characterise possible interactions using only a single fluorophore. This approach harbours the potential to screen for unknown interaction partners in a cellular context. Therefore we want to use the rotational diffusion as a ruler for molecular volume. The rotational diffusion time provides information on whether binding occurs (rotational diffusion time increases) and the size of the interaction partner (to what extent increases the time). In order to test this we fused GFP to proteins of different molecular weights to determine their rotational correlation time. With multiparameter fluorescence detection (MFD) we were able to record fluorescence correlation spectra from picoseconds to seconds. The determined rotational correlation times are compared to results from corresponding anisotropy decay measurements and to hydrodynamic properties deduced from known molecular structures of the crystallised proteins.

We confirmed the correlation of molecular volumes with rotational diffusion times over more than three orders of magnitude (volumes: 20 to 80000 nm^3, diffusion times: 16 ns to 20 µs). The applicability of this interaction assay for imaging of biological interactions in cells will be discussed.

834-Pos LED Illumination For Video-Enhanced DIC Imaging Of Single Microtubules

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In many applications high-resolution video-enhanced differential interference contrast microscopy is used to visualize and track the ends of single microtubules. We show that single ultrabright light emitting diodes from Luxeon can be used to replace conventional light sources for these kinds of applications without loss of function. We measured the signal-to-noise ratio of microtubules imaged with three different light emitting diode colors (blue, red, green). The blue light emitting diode performed best, and the signal-to-noise ratios were high enough to automatically track the ends of dynamic microtubules. Light emitting diodes as light sources for video-enhanced differential interference contrast microscopy are high performing, low-cost and easy to align alternatives to existing illumination solutions.

835-Pos A New Microscope Optics For Laser Darkfield Illumination Applied To High Precision Measurement Of Specimen Displacement

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Board B680

To get detailed information of the dynamic features of biomolecules, developing new techniques for the higher accuracy and precision measurement of specimen motions is one of the major tasks. In particular, measuring techniques with a sub-nanometer precision and with a micro-second time-resolution, which are 10–100 times better than previous ones, have been awaited, since it would enable us to analyze the atomic-scale and real-time configuration changes of organelles or biomolecules. With conventional light microscopy, precision to determine the displacement of specimen depends on signal-to-noise ratio when we measure the light intensity of magnified images. Therefore, to improve measuring precision, getting brighter images and reducing background light noise are both inevitably required. In the present study, to meet these requirements, we developed a new optics for laser darkfield illumination. We used a laser-beam and a pair of axicons (conical lenses). One of the axicons was used to convert a Gaussian laser beam into a beam of a conical shape and the other was to make a parallel ring beam, which was introduced to a conventional condenser lens without light source energy loss. For the precise evaluation of light intensity distribution produced by this optics, a microbead used as a positional probe was scanned around the focusing area of laser beam under an objective and the intensity of scattering light was measured by a quadrant-photodiode. With the procedure, we found an optimized condition for the displacement measurement with high stability and reproducibility. The observed noise level of our apparatus was 0.1nm/$\sqrt{}$Hz, *i.e.*, a 0.1 nm measuring precision and a 0.1ms time-resolution was achieved. The method was applied to 2-D motion analysis of the high-frequency nanometer-scale vibration of sliding microtubules in reactivated sea-urchin sperm flagellar axonemes.

836-Pos New LED-based TIRF Microscopy To Study Molecular Permeability, Cell Membrane And Associated Cytoplasmic Dynamics

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We have developed a novel light emitting diode (LED) based device for quantitative Total Internal Reflection Fluorescence Microscopy (TIRFM). TIRF illumination is achieved using high power LEDs coupled into high index cover glass. High resolution TIRF signals are captured by an EMCCD camera. This TIRFM can be an add-on to any inverted optical microscope for stand-alone operation and integrated with scanning probe microscopes for multimodal imaging and thus enabling direct structure-function biophysical studies. Significantly, this TIRFM uses 10× to 100× magnification objectives. Absence of any laser source limits potential sample damage from conventional laser TIRFM. As an example, we imaged cells expressing connexin43-YFP (Figs A1,2). Plasma membrane hemichannels with little or no interference from the cytoplasmic hemichannels could be identified. As a further biophysical relevance of this technique, we measured dye permeability of individual reconstituted hemichannels supported over 70 nm diameter silicon nanopore in buffers with different [Ca++] (Fig B1). Closed connexons (in 1.8 mM Ca++) show no dye transfer (B2). Open connexons (in 0 mM Ca++) show time-dependent dye permeability (B3), consistent with calcium-dependent hemichannel gating.

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837-Pos A Novel Protocol For Scanning Ion Conductance Microscopy Of Biological Samples With Elaborated Surface Profile

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Biological samples exhibiting complex surface profile with large features extending vertically into the outer or inner space (e.g. a network of dendrites on a flat dish or a bacterium interacting with cell microvilli) have always represented a daunting task for scanning ion conductance microscopy. Due to limited lateral sensitivity of the scanning pipette, features protruding substantially higher than the pipette tip diameter may not be detected properly and collide with the pipette during the horizontal movement, resulting in irreversible blocking of the pipette tip and/or damage of the sample. Previous attempts to solve this problem resulted in substantially decreased scan rate making it difficult to apply for live imaging. We have developed a novel protocol that overcomes the lack of lateral sensitivity of the scanning probe and allows acquiring topographical images of highly elaborated sample without compromising the scan rate. This is achieved by the adjustable and automated on-the-fly compression of topography acquisition that helps to redistribute horizontal resolution more effectively so that information-poor areas such as flat dish consume less scanner resources than highly structured areas. Varying parameters of scan compression it is also possible to shift resolution focus between areas with finer and coarser details. Test measurements on a non-biological sample of known surface profile proved vertical resolution indistinguishable from the conventional mode. Experiments on cultured neurons and epithelial cells showed that the new protocol produces clear topography image in cases where conventional scan mode produces either heavily distorted topographical images or damages sample due to pipette-sample collisions. This technical advance should allow scanning ion conductance microscopy imaging of tissues and brain slices.

838-Pos One-photon Raster Image Correlation Spectroscopy (RICS) on Model Systems and Living Oligodendrocytes

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Board B683

The microfluorimetric Raster Image Correlation Spectroscopy (RICS) approach has recently been introduced to measure diffusion coefficients over a wide dynamic range. Principles of RICS apply to confocal laser scanning microscopes (CLSM) in general but most often results for two-photon excitation systems with photon counting detection have been reported so far. This work describes implementation and performance of RICS on a one-photon CLSM with analog detection. We report on the influence of sample monodispersity and spatial and temporal homogeneity, refractive index mismatch, point spread function (psf) shape and stability, optimum scan speed choice and (un)correlated detection noise on the recovery of diffusion coefficients and particle concentrations in model systems and live cells. Model systems included fluorescent beads and FITC-Dextran of various size and molecular weight in dilute aqueous buffer and more viscous sucrose solutions at 23 and 37 °C. Measurements were carried out on a one-photon Zeiss LSM 510 META running at 488 nm excitation with 10 μW at the sample position and 1 Airy pinhole size using 10×/0.3 air and 40×/1.3 oil objectives. Hydrodynamic radius, temperature-dependent viscosity and refractive indices were also determined. Images were corrected for bleaching. Simulations and image analysis were carried out both with the original RICS (UCI) and an in-house developed Matlab code. A global analysis approach for related experiments was performed with the latter one. Results were compared with the Stokes-Einstein (SE) model for free unhindered diffusion of hard spheres. (Dis)agreement of RICS analysis and SE applicability is discussed. Extraction of radial and axial psf was carried out with Huygens Essential (SVI), ImageJ (NIH) and Origin (OriginLab) and Matlab software packages.

This work was supported by a tUL imaging grant and an FWO grant.

839-Pos Surface Morphology Study of cDNA Microarray Probe Spots

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Board B684

cDNA microarray contains densely packed spots with known DNA sequences. The deposited DNA sample is referred to as the probe. After the fluorescently-labeled target DNA is hybridized to its complementary probe strand, the microarray was scanned to measure the intensity of the fluorescent signals on each spot. The intensity of each spot represents the expression level of a specific gene. However, the DNA distribution within each spot will influence the analysis of the fluorescent signals. This research aims at applying the spectral images technology and other analytical methods to study the distribution of DNA within the probe spot and analyze the cause of its irregular distribution.

The spectral-scanning system was applied in this study to scan the spectrum of probes labeled with Cy3 dye on the cDNA microarray. The characteristics of spectrum were used to distinguish the fluorescence, which may overlap with that of Cy3, emitted from the other materials on the cDNA microarray. SSC, which was added in the DNA spotting solution, do not produce fluorescent signal, therefore cannot be detected by the fluorescence and spectral imaging systems. The non- fluorescence morphologic image acquired by EMCCD camera represents the distribution of SSC crystal, after the spotting solution was dry out. We compared the morphologic and fluorescent images of each spot. The pixel correlation between the distribution of DNA and SSC crystal is higher than 0.9. This result indicats that DNA was diffused outward from SSC crystal in the process of drying out of the spotting solution. In the drying out process after rehydration, DNA molecules tend to gather inward, thus the spots become more rounded, enlarged and the DNA is more evenly distributed. The degree of homogenization of DNA distribution on the glass surface affects the reliability of cDNA microarray results.

840-Pos A Confocal Microscopy Enabled Langmuir Trough for Visualizing Competitive Adsorption at the Air-Liquid Interface

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Board B685

Lung surfactant (LS) is a complex mixture of lipids and proteins that occupies the air-liquid interface of the alveoli. LS is responsible for modulating the surface tension at the interface which both greatly reduces the work of breathing and prevents collapse of the alveolar air sacs. Inhibition of LS, and the ensuing loss of surface tension modulation, is observed during acute respiratory distress syndrome (ARDS) which afflicts 150,000 patients annually with a 40% mortality rate. LS inactivation similar to that of ARDS cases occurs *in vitro* when surface-active serum proteins prevent the interfacial adsorption of LS bilayer aggregates. However, the addition of select hydrophilic polymers reverses the inhibition of replacement LS and such polymers may help increase the effectiveness of LS therapy. A newly-designed Langmuir trough coupled with confocal microscopy was used to characterize the competitive adsorption between serum proteins and LS. Confocal microscopy proved advantageous because it allowed characterization both laterally and axially, which was an improvement over traditional interfacial fluorescence microscopy. Additionally, multiple dyes were imaged simultaneously,

and their relative positions determined both laterally and axially, yielding insight into the competitive adsorption of LS and serum protein. Finally, the continuous steel-ribbon barrier of the new Langmuir trough maintained very low surface tensions, permitting *in situ* imaging of LS monolayer collapse.

Atomic Force Microscopy

841-Pos Combined Atomic Force/ Fluorescence Microscopy Technique to Select Aptamers in a Single Cycle from a Small Pool of Random Oligonucleotides

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Board B686

We are developing a method, which utilizes a combined atomic force microscope (AFM)/fluorescence microscope and small copy number PCR, to affinity-select individual aptamer species in a single cycle from a small pool of random-sequence oligonucleotides (oligos). In this method, a library of small beads, each of which is functionalized with fluorescent oligos of different sequences, is created. This library of oligo-functionalized beads is flowed over immobilized target molecules on a glass cover slip. High-affinity, target-specific aptamers bind tightly to the target for prolonged periods and resist subsequent washes, resulting in a strong fluorescence signal on the substrate surface. This signal is observed from underneath the sample via fluorescence microscopy. The AFM tip, situated above the sample, is then directed to the coordinates of the fluorescence signal and is used to capture a three-dimensional, high-resolution image of the surface-bound bead and to extract the bead (plus attached oligo). The extracted oligo is PCR-amplified, sequenced and may then be subjected to further biochemical analysis.

Here, we describe the underlying principles of this method, the required microscopy instrumentation and the results of proof-of-principle experiments. In these experiments, we selected aptamers in eight trials from a binary pool containing a 1:1 mixture of thrombin aptamer oligo and a nonsense oligo. In each of the eight trials, the positive control aptamer was successfully detected, imaged, extracted and characterized by PCR amplification and sequencing. In no case was the nonsense oligo selected, indicating good selectivity at this early stage of technology development.

842-Pos AFM Study Of DNA Complexes With The V(D)J Recombination Proteins RAG1/RAG2

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RAG1 and RAG2 proteins are critically involved in the DNA rearrangement process responsible for the assembly of functional antigen receptor genes from component gene segments. These proteins bind to specific sites called recombination signal sequences (12-RSS or 23-RSS) and mediate recombination of antibody and T-cell receptor genes in developing lymphocytes via formation of synaptic complexes with a 12-RSS and a 23-RSS (the 12/23 rule). This 12/23 rule ensures that the correct regions are joined when recombination takes place. To elucidate the mechanism leading to synaptic complex formation and elucidate the architecture of RAG-RSS complexes, we have used atomic force microscopy (AFM) to directly image RAG complexes bound to RSS substrates. Various fragment designs have been used to compare protein-DNA complexes formed with the RAG proteins and intact 12-RSS and 23-RSS motifs, signal end (SE) motifs which resemble post-cleavage fragments, and nonspecific DNA. We have characterized complexes of the RAG proteins bound to a single DNA fragment (presynaptic complex), as well as complexes of the RAG proteins bound to two DNA fragments (synaptic complexes). The site specificity of RAG binding was examined by measuring the position of the protein complexes on each fragment, and the stoichiometry of the RAG proteins in these complexes was estimated by measuring the volume of the complexes. Our results show that the volume of the RAG proteins in synaptic DNA complexes is larger than in presynaptic complexes, suggesting an association model for the synaptic complex assembly. Also, when the RAG proteins are bound at the end of the stand, fragments are considerably shorter, raising the possibility that the DNA wraps around, coils within, or undergoes strand separation in such complexes. These findings highlight the structural differences between RAG pre-cleavage and post-cleavage signal end complexes.

843-Pos Probing Protein Conformations at the Oil-water Interface Using Single-Molecule Force Spectroscopy

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Beta-lactoglobulin (BLG), a globular protein that is abundant in the milk of several mammals, adsorbing to the interface of oil-in-water emulsions and forming a protective coating that stabilizes the oil droplets against flocculation and/or coalescence. The present work aims at a deeper understanding of the conformational changes in BLG adsorbed onto the emulsion interfaces due to variations in pH. Mechanical unfolding of BLG using AFM-single-molecule force spectroscopy (AFM-SMFS) was performed on single oil droplets that were mechanically trapped in a polycarbonate filter. The changes in the contour length upon each unfolding event were determined by fitting the WLC model of polymer elasticity to each of the BLG peaks. Our results show clearly that at pH 2.5 BLG exists as a dimer in which each monomer is similar to two Immunoglobulin domains with contour lengths of 32 nm. At neutral pH (6.8) BLG on the oil droplets adopts a conformation that is different from that in its native state consisting of domains with a contour length of 11 nm. Furthermore, at pH 9 the interactions between the AFM tip and the BLG layer on the oil droplet surface are dominated by a huge repulsion due to the highly negatively charged BLG layer. This study demonstrates a novel application of AFM-SMFS to investi-