revealed that neutrophil rolling on P-selectin at a shear stress of 6-8 dyn/cm^2 is facilitated by four-fold larger footprints than expected and three to four long membrane tethers at the rear. When mouse bone marrow neutrophils were stained with an intercalating membrane dye (DiI), allowed to roll on P- selectin in a microfluidic device at a shear stress of 6-10 dyn/cm^2 and footprints recorded using dual-color qDF (DqDF), we found that following tether bond failure, tethers did not retract. Instead, the detached tethers landed as 'slings' in front of the rolling neutrophils. Slings were confirmed in an acute model of inflammation *in vivo* using epifluorescence-intravital microscopy of mouse cremaster. In each sling, PSGL-1 was distributed in patches 1.6 μm apart while the integrin LFA-1 was expressed over the entire length of slings. As the cells rolled on slings, they were wrapped around the rolling neutrophils and underwent a step-wise peeling at the rear of the rolling neutrophils enabled by the tandem failure of PSGL-1 patches under hydrodynamic force. The failure of each load-bearing PSGL-1 patch on the sling resulted in loading of the next patch downstream of it and the cycle continued until the sling was completely peeled off from the substrate. 'Peeling of slings' is distinct from 'pulling of tethers' reported previously and the two mechanisms probably work synergistically to facilitate neutrophil rolling at high shear stress during inflammation. This study was supported by the American Heart Association-Scientist Development Grant 11SDG7340005 (P.S.) and NIH EB02185 (K.L.).

978-Pos Board B764

Label-Free Hyperspectral Imaging of Intracellular Hemoglobin in Human Erythrocytes

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A broadband hyperspectral imaging (HSI) technique, originally developed for remote sensing and imaging in geophysical applications, recently has been employed to enable noninvasive label-free optical imaging of biological specimens. Here we report our results on hyperspectral microscopy of non-labeled erythrocytes to study the distribution of hemoglobin. We have chosen to focus on cellular hemoglobin because it is an endogenous chromorphore that is present in high concentrations, which makes it a good candidate for establishing proof of principle. In our imaging technique, hyperspectral data cubes (x, y, λ) are collected by either a wide-field or confocal microscope equipped with a broadband light source, such as a white light laser or spectral light engine in which narrow-band selection and wavelength scanning is achieved by fast optoelectronic devices. The obtained data cubes then are analyzed by algorithms based on a spectral angle mapper, yielding a set of unique absorption spectral signatures (i.e. endmembers) corresponding to subforms of hemoglobin, such as oxyhemoglobin and methemoglobin. Wavelength-dependent scattering signatures of cell membranes are also resolved. Unique endmembers of specific hemoglobin subforms are identified and used to build a map of intracellular hemoglobin distribution by estimating the abundance of each specific endmembers from the hyperspectral data cube. Our ongoing efforts include technology development for the determination of the local thickness of a single erythrocyte, to enable single cell volumetric measurement. The technique may be further developed for label-free molecular and chemical imaging of a broad range of endogenous biomarkers in cells and tissues, and ultimately for in vivo molecular imaging as well.

979-Pos Board B765

Quantifying Bacterial Growth and Gene Expression using New Long-Term Single-Cell Observation System

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Although the phenotypic differences between individual organisms can often be ascribed to underlying genetic and environmental variation, even genetically identical organisms in homogeneous environments are phenotypically heterogeneous due to the stochastic effects in biological reactions, which is considered to play an important role in the biological processes such as differentiation, development, adaptation and evolution. In general, the single-cell distribution of the state of a cell (e.g., protein concentration) can be obtained by repeating a single-cell measurement over an ensemble of cells. However, we must be careful that of the result reflects not only the statistical property of individual cells, but also the difference of cell proliferation rate that depends on the temporary state. Conversely, a careful treatment of the statistical properties of cell lineage and population might allow a reliable detection of the relation between proliferation and the other phenotypic states. To address the question along this line, we asked how cell growth fluctuations and gene expression fluctuations are interrelated. For this purpose, we developed a new microfluidic device that allows rigorous environmental control and tracking of a large number of individual growing cells for hundreds of generations and designed a new custom software to analyze timelaps images using imageJ and C. Analysis of long-term data of individual histories obtained by our new system demonstrated the discrepancies of the growth rate as well as of the gene expression level distributions between the single-cell lineages and the population. We confirmed that a simple mathematical relation, which was in fact discovered more than 60 years ago, can explain the difference of growth rate. This study shows the significance of large-scale and careful examination of individual lineage trees, which provides the non-trivial statistical relations between individual cells and population.

980-Pos Board B766

3D Imaging by Multiphoton Selective Plane Illumination

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Light-sheet based techniques, such as single plane illumination microscopy (SPIM) [1] and digital scanned laser microscopy (DSLM) [2], have been found particularly useful in developmental biology applications since they provide the capability to perform fast imaging of living samples reducing photobleaching effects. In particular, single plane illumination provides high signal to noise ratio and optical sectioning capability due to the planar excitation, representing a useful tool for biological investigations of thick biological samples.
On the other side, two photon excitation microscopy (2PE) has been demonstrated to be a suitable tool for improving penetration depth since it allows for reduction of the scattering effects and light-sample interactions. To improve image quality and penetration depth of light sheet illumination microscopy, two photon excitation can be coupled with planar illumination [3] thus reducing the scattering effects due to light-sample interactions.
We characterized the two photon excitation process within the light sheet and we applied this technique to image large biological samples [4]. 3D imaging of mammary cell spheroids in depth has been performed using the resulting hybrid architecture.
Keywords: Two photon excitation, Single plane illumination microscopy.
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(4) Cella Zanacchi et al. *Proc. of SPIE*, vol.7903 ''Multiphoton Microscopy in the Biomedical Sciences XI'' (2011)

981-Pos Board B767

Characterization of Scattering Effects in Phantom Samples using Single and Two-Photon Excitation Light Sheet Microscopy

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Scattering in thick biological samples plays a relevant role in imaging degradation and reduction of signal to noise ratio and contrast. Even if there are existing techniques that allow imaging of very thick samples, they can suffer from aberrations and distortions related to scattering properties of the sample. Recently, the capability of coupling light sheet techniques with Two Photon Excitation(TPE) (1,2,3) provided a powerful tool for deep imaging of biological samples. This has been proved to be a promising trend in thick-imaging microscopy, because it combines the advantages of light sheet techniques with the increased penetration depth intrinsic of TPE. For this reason, in this work a detailed characterization of the effects induced by scattering on the excitation profile of a light sheet based microscope is provided. In particular, different phantom samples, with different scattering coefficients mimicking the optical properties of various biological tissues, are used. Experiments were performed to investigate the shape distortions of the excitation intensity distribution provided by the light sheet, both in single and in two-photon excitation. This aspect represents a crucial point, since the effective light sheet intensity distribution is strictly related to the optical sectioning capability and image quality of the system. Results show that the light sheet intensity distribution is less affected by light-sample interactions and the shape is preserved in the case of TPE in comparison with single photon excitation regime. Furthermore, contrast measurements has been performed using fluorescent beads embedded in different

scattering samples in both excitation modes. These experiments showed again the better performances of TPE compared to single photon excitation.

(1) Cella Zanacchi et al. Proc. of SPIE, vol.7903 (2011)
(2) Planchon et al. Nature Methods 8, 417-423, (2011)
(3) Truong et al. Nature Methods 8,757-760, (2011)

982-Pos Board B768

Angular Morhpomechanics in the Establishment of Multicellular Architecture

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We address a fundamental question: what are the physical laws that govern the assembly of acinar structures? We report a novel type of human cell motility where single cells undergo multiple rotations. This motion is maintained as these cells cohesively divide to assemble into polarized multicellular spherical structures (acini) when placed in a 3D basement membrane surrogate gel. We link the functional relevance of coherent angular motion (CAMo) to spherical architecture, and determine the importance of molecules involved in cell-cell adhesion and tissue polarity to the outcome. CAMo is observed in both primary human cells and established breast cell lines where the final realized geometry is spherical. Breast cancer cells do not display CAMo but are randomly motile. Upon 'phenotypic reversion' of malignant cells, both CAMo motility and correct architecture are restored

983-Pos Board B769

Separation of NADPH and NADH Fluorescence Emission in Live Cells using Flim

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Observing changes in the intensity of the endogenous fluorescence of the enzymatic cofactors NADH and NADPH has been used to monitor the metabolic state of cells and tissues for upwards of half a century. The fluorescence arising from NADH and NADPH is spectrally identical, leading to the observed combined fluorescence being labelled NAD(P)H.

In this work, we investigate the application of fluorescence lifetime imaging microscopy (FLIM) to separate the fluorescence arising from NADPH from that of NADH in live cells.

The fluorescence lifetime of NADH in solution (~0.4ns) increases more than 5-fold when NADH is enzyme bound, with a precise lifetime governed by the particular enzyme to which the cofactor is bound.

We have used FLIM to measure the fluorescence lifetime of NAD(P)H in HEK293 cells overexpressing NAD-kinase, in which NADPH levels are elevated ~15-fold compared to NAD-kinase knock down cells. The fluorescence lifetime of free NAD(P)H remains unchanged at ~0.4ns, but the fluorescence lifetime arising from enzyme-bound NADPH was significantly increased to $3.9(\pm 0.4)$ns compared to its value in the knock down cells, $3.0(\pm 0.2)$ns.

FLIM studies of mammalian cochlear explant cultures also revealed a layer of supporting cells which showed a similar long lifetime of $3.6(\pm 0.1)$ns, suggesting that these cells exhibit elevated NADPH. This lifetime was significantly reduced by biochemical interventions to decrease NADPH (pentose phosphate pathway inhibition), supporting this conclusion.

These results represent a first step towards a functional imaging protocol for separating NADH and NADPH levels in live tissue.

984-Pos Board B770

Comparing Photodamage Induced by Confocal Microscopy and Light Sheet Fluorescence Imaging of Zebrafish Skeletal Development

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Understanding the formation of multicellular structures during embryogenesis requires high quality three dimensional microscopy over long periods of time, a task for which confocal fluorescence microscopy has become the standard technique. Extended viewing of developing structures in vivo, however, exposes the organism to prolonged illumination that runs the risk of being detrimental to the processes being studied. Furthermore, the effects of phototoxicity may not be apparent over the course of an experiment, as they may become significant well before any photobleaching of the sample is observed. Selective plane illumination microscopy (SPIM), or ''light sheet'' microscopy, has been developed as an imaging platform that can address the issues of phototoxicity, acquisition rate, and photon efficiency present in confocal methods of imaging. While it is known that SPIM requires orders of magnitude less light intensity than confocal techniques for similar imaging conditions, it has not been previously demonstrated that this difference is relevant to maintaining normal biological function. Using the development of the opercle, a cranio-facial structure in larval zebrafish (Danio rerio), we have tested the effects of long term imaging using a commercial spinning disk confocal microscope as compared to our own self-built SPIM instrument. Examining a range of exposure times and scan durations of several hours, we find that confocal imaging can generate significantly abnormal growth of bone forming cells (sp7:eGFP transgenic), especially if high signal to noise is desired. In contrast, we find normal opercle development with SPIM throughout the entire examined range of conditions.

985-Pos Board B771

Fluorescent Live Cell Imaging Under Pressure

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Perturbing equilibria of molecular interactions within a cellular environment provides a versatile method for dissecting the cellular functions of proteins. To date this has been achieved by modulating the temperature of the sample under investigation. When the sample contains a temperature sensitive mutant protein it allows this method to be used to investigate specific protein functions. However increasing temperature often has associated disadvantages of having non-reversible and destructive effects upon the cell/molecule under investigation.

Hydrostatic pressure offers a number of advantages over temperature as a variable for probing protein-protein interactions. High pressure does not usually disrupt native protein structure at pressures below ~ 2GPa. The effects are fully reversible; pressure can be applied and released from a sample extremely rapidly in the sub-millisecond; and the sample reaches uniform equilibrium pressure quickly, which is in contrast to conventional temperature techniques, which can take several minutes. The ability to rapidly increase and decrease pressure also allows the opportunity to determine whether the proteins/cells under investigation are irreversibly effected/damaged during the experiment.

We have recently developed a pressure chamber which allows rapid modulation of the hydrostatic pressure acting upon a biological sample and is used in combination with a conventional magnification fluorescence live-cell imaging system with up to 60x objective magnification. This system can be used to establish the effect pressure has upon the organisation and function of a plethora of cellular processes and protein-protein interactions.

Having established conditions which have no observable effect upon yeast cell viability, we are now using this live cell pressure perturbation imaging system to alter the dynamic behaviour of the actin and microtubule cytoskeleton, and thereby probe their regulation and function.

986-Pos Board B772

Visualizing Hyroynamic Transgene Delivery and Expression in Live Mammalian Kidneys

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While gene transfer has been difficult to accomplish in the kidney, reports have indicated that tissue cavitation may facilitate such genetic modifications. Accordingly, we investigated this theory, and designed and characterized a method that utilizes renal vein-guided, retrograde pressurized injections to illicit prompt transgene expression in mammalian kidneys. In devising this approach we hypothesized that the hydrodynamic forces generated from pressured injections were sufficient to facilitate the widespread delivery of exogenous matter throughout the kidney. As a consequence, this infusion process would assist the passage of transgenes across epithelial and endothelial tissue layers, and ultimately support their cellular uptake. We tested this hypothesis by introducing naked plasmid DNA and baculovirus vectors, which express fluorescent proteins, into 60 rodent kidneys. Gene expression was then examined in live animals using intravital multiphoton fluorescence microscopy, and in vitro with confocal laser scanning microscopy and electron microscopy. We recorded transgene expression in various glomerular, tubular and vascular segments throughout a period of 3 weeks. Moreover, we were able to generate transient, yet stable targeted and non-specific genetic modifications. Overall, these results outline a potentially attractive approach for renal gene transfer in live animals.