three dimensional (3D) resolution. In this study, both pure and fluorescently labeled (3% by weight) collagen gels are assembled over a range of concentrations (0.1, 0.5, 1.0, 2.0, 5.0, and 5.5 mg/mL) and at various temperatures (37, 32, 27, and 22°C). The networks are investigated using confocal reflectance microscopy (CRM) and confocal fluorescence microscopy (CFM). Comparison between CRM and CFM reveals that they are not equally sensitive to details of network structure, with CRM (CFM) displaying higher sensitivity to fibers perpendicular (parallel) to the optical axis. Furthermore, analysis of background signal in CFM images suggests the existence of small fibrillar structures that are not resolved by CRM. Despite these differences, image analyses performed on 2D slices of CFM and CRM images to quantify mesh size, number of fibers, and fiber length reveal identical trends as a function of gel concentration and gelation temperature. Fiber width approximated from both CRM and CFM is in good accord with fiber width determination using electron microscopy. Overall network structures (as quantified via mesh size, fiber number, fiber length and fiber width) are related to bulk mechanical properties measured by rheology. Finally, we demonstrate the ability to form collagen gels of varied mesh size at identical collagen concentration, all of which are compatible with cell health and 3D cell studies.

1510-Pos Board B354

Monitoring the Granulomal Micro-environment in a Monkey Model of Tuberculosis Using a Novel Fluorescence Bronchoscope

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Tuberculosis is a disease that infects one in three humans today. The long, expensive drug course required to cure the active form, combined with its increasing resistance to conventional antibiotic treatment, necessitates the development of a new class of TB drugs. To evaluate the efficacy of these drugs, as well as to gain increased basic knowledge of the disease's progression, we are building a novel 0.8 mm diameter bronchoscope as part of a multi-institutional initiative to develop a primate-based tuberculosis model system. In order to monitor the *in vivo* microenvironment of the tuberculosis granuloma, monkeys will be inoculated with transgenic pH reporting tuberculosis bacilli and GFP expression measured to quantify the local pH and other micro-environmental parameters. CT scans will be used to reveal the induced nodules/lesions and guide the bronchoscope to the granulomas. The lung tissue itself contains many 488 nm excitable endogenous fluorophores (e.g. elastin, collagen) and autofluorescence limits the level of reporter quantification. To overcome this problem we are employing a photoactivatable protein (Dronpa) as the reporter expressed by the bacteria. Using a novel pulsed UV/Blue non-laser light source, the protein's fluorescence can be modulated to distinguish reporter signal from the constant autofluorescence background and therefore produce highly quantitative measurements of changes in the granuloma microenvironment during the progression of the disease and during drug treatment.
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1511-Pos Board B355

Towards Cardiac Safety Screens by Single Cell Imaging Procedures

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To address cardiac safety screens *in vitro*, neonatal cells may not represent a good model due to non-adult gene expression patterns. Therefore we explored a novel concept of high content screens utilising isolated adult cardiomyocytes. This approach based on a long term culture procedure developed and established for these cells enabling culturing of cardiomyocytes for one week without major dedifferentiation. Such an approach is suitable as a safety screen since experiments can be performed in acute and chronic stimulation conditions. In our hands fluorescence microscopy appears to be an ideal tool to perform such screens. An essential prerequisite for long term fluorescence recoding, ideally even using an identical cell population, was the application of genetically encoded biosensors. They represent an almost ideal sensor-system since transfection is easy, characterised by high (almost 100%) transfection rates, fast expression (<24h) and high biocompatibility. In addition appropriate transfection systems are available for both *in vitro* (Adenovirus) and *in vivo* (Lentivirus) situations. Here, we demonstrate the result of a long term expression of various calcium sensors while imaging individual cells for several hours. We compared results obtained in cardiac myocytes expressing various such sensors with respect to their biophysical properties and putative changes of calcium handling and biocompatibility.
The combination of various imaging techniques, such as fast video microscopy, total internal reflection fluorescence microscopy, fluorescence lifetime imaging and fluorescence redistribution after photobleach will allow flexible and complex screening protocols. Furthermore, online image processing algorithms will allow intelligent alterations of screening processes that depend on the cellular response. We demonstrate how all these component add up to use single cell models of adult cardiac myocytes for high-content safety screenings.
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1512-Pos Board B356

Obtaining Quantitative Information on the Cell-induced Deformation of Collagen with Digital Holographic Microscopy

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Principles of holography are applied to study the dynamics of cells and their extracellular matrix with digital hologram microscopy (DHM). The goal of this study is to measure the deformation of collagen matrix induced by cell migration. DHM has appeared as a unique tool to study the displacement at the nano-scale by resolving differences in refractive index. DHM is capable of conducting quantitative size and depth measurements in 3-D. A digital holographic microscope, in transmission, is designed and built to record two-dimensional holograms on a CMOS camera. The digitally recorded holograms are computationally reconstructed using the angular spectrum method (ASM) providing a better signal to noise ratio in comparison with the traditional Fresnel approximation method. The ASM method also outputs the phase image that is used to perform quantitative phase-contrast analysis. The phase images represent the optical pathlength disturbance caused by the sample. These images are unwrapped by applying the Flynn's algorithm to account for the 2π ambiguity. The importance of conducting quantitative phase analysis rises up when one needs to reveal the optical thickness profile of a transparent specimen with sub-wavelength accuracy. Quantitative phase information concerning cell morphology and volume along with those of the extracellular matrix could be obtained with digital holographic microscopy images. One of the advantages of DHM is that, this method is completely non-invasive and there is no need to dissect the sample or to stain it. Also the numerical reconstruction of different object planes from a single hologram enables one to focus on various planes of the image without any mechanical or optical components. This work is funded by NIH grant number 447904-23909.

1513-Pos Board B357

Vascular Smooth Muscle Cell Response to Transglutaminase 2 Cross-linked Collagen Fibril Thin Films

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Tissue transglutaminase 2 (TG2) is a ubiquitous protein thought to play an important role in both the normal and abnormal progression of the wound healing response through extracellular matrix (ECM) cross-linking. However, how TG2 cross-linking of ECM affects cell behavior is still ill-defined. Here we use a model ECM system to show that vascular smooth muscle cell (vSMC) spreading, proliferation, actin polymerization, and myosin activation increase with increasing exposure of type 1 collagen fibrils to TG2 activity. A10 vSMC ligate fibrillar type 1 collagen through beta(1) integrins, and beta(1) integrin ligation appeared to be identical before and after TG2 cross-linking of collagen. This result suggests that the observed changes in cell response were not induced by changes in surface chemistry or receptor recognition. Atomic force microscopy (AFM) studies show that untreated fibrils are more susceptible to lateral movement on the surface than cross-linked fibrils, which suggest that the observed cell response is solely due to TG2-induced changes in the mechanical properties of collagen fibrils. The results provide valuable insight into a mechanism by which TG2-modified ECM proteins can influence cell behavior.

1514-Pos Board B358

Segmentation-Less 3D Quantitative Image Analysis of Tissue Architecture with Application to the Localization of Organelles in MDCK Cysts

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To identify the physical and biological factors that influence tissue morphogenesis, 3D imaging tools are essential. However, obtaining quantitative information about the spatial organization of observed objects from the resulting images is not trivial. Often researchers are forced to choose ''representative'' image regions due to the complexity of analyzing the tissue as a whole. This may lead to false conclusions, and complicates comparisons across different biological systems. In this work we have used quantitative projection methods that reduce the dimensionality of the problem while encoding its essential

features. In particular, we developed an unsupervised segmentation-less projection method in which the whole intensity volume is expanded in four dimensional spherical harmonics (4DSH). To demonstrate our technique, we used projections to compare the spatial distributions of actin, Golgi apparatus and nucleus within cells of an MDCK epithelium regardless of tissue size and support. We found a clear dependence of the internal architecture of individual cells on tissue size and type of support. We conclude that when information on the general spatial distribution of cell and tissue components is needed, and when the tissue geometry permits it, projection methods in general, and the 4DSH representation in particular, eliminate the need for choosing representative image regions or performing cumbersome image segmentation.

1515-Pos Board B359
Light Scattering Detects Changes In Subcellular Structure And Organization With Connections To Cell Function
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By incorporating new models into our light scattering analysis techniques that better account for the ellipsoidal shape of cellular organelles, we can determine not only the average size, but also the average shape of an ensemble of cell nuclei in culture. This advance permitted new insight into the nucleus structure, and by providing an accurate depiction of its contribution to the light scattering signal, has also enabled an enhanced ability to analyze density correlations and therefore subcellular organization in biological cells. We will present verification of our methods and results of two new studies facilitated by these recent developments. In the first study, we used light scattering to detect statistically significant structural changes in breast cancer cells within one hour after treatment with apoptosis-inducing drugs. Two conclusions emerge: First, the ability of this technique to discern early onset of apoptosis makes it a promising tool for monitoring cancer treatments; and second, monitoring the organization of subcellular organelles could be a powerful method for studying the mechanisms of apoptosis, and perhaps other functional changes in cells. In the second study, we evaluated the deformation of stem cell nuclei as a response to engineered nanotopographical cues and the mechanical properties of their substrate. As verified by image analysis and comparison to control samples, the changes in nuclear shape due to materials' properties and nanotopography are highly significant. Additionally, these shape changes relate to modifications in stem cell adhesion and mobility, and provide a connection between environmental cues, nuclear deformation, and cellular behavior. Both studies solidified light scattering as a promising tool to assess structure in biological samples, and indicate the potential to link these structural changes to corresponding alterations in cell function.

1516-Pos Board B360
Thermal Noise as a Probe for Cell Adhesion
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In the adhesion area of cells on solid substrates, there is a narrow cleft filled with electrolyte. The sheet resistance of the cleft is crucial for the interfacing of cells with semiconductors and metals. It can be estimated by applying intracellular or extracellular ac voltages and recording the response of current, of extracellular voltage or of transmembrane voltage. A more elegant approach relies on the Fluctuation-Dissipation Theorem which implies that an electrical resistance is related with voltage fluctuations. It was previously demonstrated that the voltage fluctuations in the area of adhesion can be recorded with transistors and that the resistance of the cell-chip junction can be estimated from the noise spectrum [1].
To attain a more reliable interpretation of the voltage noise, we measured spatial maps of the noise spectrum in the adhesion area. We used a Multi-Transistor-Array with a homogeneous surface of titanium dioxide [2]. The bandwidth of recording was 3 MHz at a spatial resolution of 7.8 µm. As a test system we used snail neurons that were cultured on chips coated with polylysine. We found a good agreement between the twodimensional maps of the noise spectra with a theory of thermal noise in a planar core-coat conductor. Sheet resistances on the order of 100 MOhm were obtained. Apart from the effect of the sheet resistance, the noise characteristics revealed changes of the membrane conductance and membrane capacitance. Thus thermal noise recording is a novel probe for the electrical properties of cell adhesion with subcellular resolution, with high bandwidth and without perturbation of the system.
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1517-Pos Board B361
Robust Pore Size Analysis of Filamentous Networks from 3D Confocal Microscopy
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We describe a robust method for determining morphological properties of filamentous biopolymer networks, such as collagen or other connective tissue matrices, from confocal microscopy image stacks. Morphological properties including pore size distributions and percolation thresholds are important for transport processes, e.g. particle diffusion or cell migration through the extracellular matrix. The method is applied to fluorescently labeled fiber networks prepared from rat tail tendon and calf skin collagen, at concentrations of 1.2, 1.6 and 2.4 mg/ml. The collagen fibers form an entangled and branched network. The medial axes, or skeletons, representing the collagen fibers are extracted from the image stack by threshold intensity segmentation and distance-ordered homotopic thinning. The size of the fluid pores as defined by the radii of largest spheres that fit into the cavities between the collagen fibers is derived from Euclidean distance daps and maximal covering radius transforms of the fluid phase. The size of the largest sphere that can traverse the fluid phase between the collagen fibers across the entire probe, called the percolation threshold, was computed for both horizontal and vertical directions. We demonstrate that by representing the fibers as the medial axis the derived morphological network properties are both robust against changes of the value of the segmentation threshold intensity and robust to problems associated with the point-spread function of the imaging system. We also provide empirical support for a recent claim that the percolation threshold of a fiber network equals the fiber diameter for which the Euler index of the networks becomes zero.

1518-Pos Board B362
Confocal Imaging Of Extracellular pH With Fluorescein Derivatives
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Extracellular pH (pH_e) is an important regulator of membrane-proteins, such as those involved in solute transport and matrix structure. In experimentally superfused cells or tissues, it is often assumed that pH_e is spatially uniform and invariant. Gradients of pH_e, however, may occur physiologically e.g. close to cell membranes or in tissue-regions with poor capillary perfusion. Fluorescein-derivatives are low-cost dyes for recording pH ratiometrically in dual-excitation mode (458nm/488nm). Fluorescein-DHPE is a phospholipid-conjugated dye for measurement of surface-membrane pH_e. Freshly isolated rat ventricular myocytes, membrane-loaded with the dye for 5min, produce a pH_e-sensitive signal that can be imaged confocally or measured using whole-cell epifluorescence. In low buffer superfusates (0.5mM Hepes), the dye reports transient acidification of surface pH_e during superfusion of 15mM NH_4Cl, owing to influx of NH_3 driving the local deprotonation of extracellular NH_4^+. On removal of NH_4Cl, surface pH_e alkalinises transiently. Activation of Na^+-H^+ exchange (by imposing an intracellular acid-load) acidifies surface pH_e. Fluorescein-sulfonic acid is a highly polar fluorescein-derivative with negligible membrane-permeability. It was used (30µM in superfusate) to image pH_e confocally in spherical (100-300µm radius) clusters (spheroids) of HCT116 cells. A pH_e gradient was observed, with low pH_e at the core (due to the long core-surface diffusion distance). Larger spheroids developed a more acidic core pH_e. Size-matched spheroids made from cells transfected with carbonic anhydrase 9, a membrane-tethered extracellular enzyme, produced steeper pH_e gradients. This is due to catalysis of cell-derived CO_2 hydration in the extracellular space. Fluorescein-derivatives may therefore yield novel insights into the regulation of pHe.
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Biophysical Modeling

1519-Pos Board B363
Modeling of Protein Adsorption on a Metal Surface: Brownian Dynamics Simulations
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The ability of proteins to bind selectively to different kinds of solid surfaces is widely used in advanced technologies in medicine, pharmacy, nanodevices and bioengineering. However, experimental data on the interfacial behavior of proteins is limited and our knowledge of the driving forces for protein-solid surface binding is still very poor. The present study is aimed at building