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