laser source. For SHG, these VSDs were found to exhibit moderately large voltage sensitivities in addition to fast kinetic responses. Our results suggest that voltage sensitive dyes can be developed which have both large SHG signal changes and the requisite speed for use as a practical tool for measuring electrical activity in neuronal systems.
(Supported by NIH grant EB001963).

1504-Pos Board B348
Application of Higher Harmonic Generation Microscopy in Assisted reproductive technologies
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It is known that the quality of the mammal oocytes and embryos greatly influence the outcomes of assisted reproductive technologies. Noninvasive imaging tools with high 3D resolution are thus needed to provide as much information about the embryos as possible. In previous studies, the images of *in vitro* cultured mouse oocytes and embryos were acquired by the harmonic generation microscopy (HGM). Various sub-cellular structures of the whole mouse oocytes and embryos were identified. In our presentation, we will report our study on application of harmonic generation microscopy in assisted reproductive technologies. We use Cr:forsterite laser as the excitation source to obtain the HGM images of mouse oocytes and embryos. First of all, several safety tests are performed to ensure the proper exposure doses of the embryos to the laser. Second, we try to figure out what characteristics in the HGM images of the oocytes and embryos are possibly related to their quality. As to our experimental setup, the HGM signals are collected in both the forward and the backward direction by high N.A. objectives. With limited available working distances, the thickness of the chamber containing the embryos must be within 2 mm. A homemade sterile glass-cover-and-bottom culture dish is designed to meet the need. The dish, containing the embryos, is then placed in a CO_2 stage micro-incubator for HGM observation. Those embryos are later transferred to female mice for pregnancy. The viability of the mouse embryos will be investigated to see if the harmonic generation microscopy can play a role in embryo-selection.

1505-Pos Board B349
Second Harmonic Generation Imaging Microscopy of Ovarian Cancer
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We report the new technique for quantifiable differentiation between normal, high risk, and malignant human ovarian biopsies based on combination of 3D Second Harmonic Generation (SHG) imaging microcopy and Monte Carlo simulation, where we comparatively utilize SHG depth-dependent profiles and bulk optical parameters. We determined that malignant ovaries exhibit larger scattering coefficient, μ_s, and scattering anisotropy, g, than normal tissues. The increased scattering is likely due to higher collagen concentration and fibril density resulting from increased cellular activity. Similarly, the increased anisotropy is consistent with the visual observation of remodeled and more highly ordered fibrils and SHG polarization anisotropy. The underlying structural dissimilarities also lead to significant differences in the measured forward-backward ratio of SHG intensity, which is the metric sensitive to sub-resolution structural effects (local packing of SHG-producing domains). We find that SHG from normal tissues is statistically more forward-directed compared to malignant ovaries, while the high risk ovaries show intermediate behaviors. Data from 100μm below the surface epithelium in the malignant biopsies trend towards the behavior of high risk and normal tissues, suggesting this method is a means of quantifying disease progression into stroma.
Monte Carlo simulations of the photon propagation confirm our experimental data. Here we include the directionality of initially emitted SHG, which is more forward-directed in normal ovaries. Based on our recently developed model using quasi-phase matching conditions relating SHG emission directionality to tissue structure, this finding is consistent with the collagen fibrillar assembly determined through electron microscopy.

1506-Pos Board B350
Analysis Of Multiphoton Imaging Of Thick Biological Scattering Samples
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Non linear optical scanning microscopy has became a useful tool for tissue imaging. Biological tissues are highly scattering media and this lead to an exponentially attenuation of the excitation intensity as the light moves into the sample. While performing imaging of biological scattering tissues in non linear excitation regime, the localization of the maximum 2PE intensity was found to shift closer to the surface [1] and the 2PE imaging depth limit appears strongly limited by near surface fluorescence [2]. In this work we computed the illumination and the photobleaching distribution [3] for different scattering coefficients in order to characterize the effects induced by scattering. An experimental test has been carried out by imaging, with medium numerical aperture objective (N.A.=0.8), thick scattering fluorescent immobile sample (polyelectrolyte gel). Results confirm that in this conditions no photobleaching effects due to scattering occur close to the surface.
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1507-Pos Board B351
SHIM And 2PEM: Getting More Information For Tissue Imaging
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Goal of this communication is to report about a recent study on several different biological samples: bone, cartilage, tendon, tumoral tissues. We show that is possible to couple SHIM (Second-harmonic imaging microscopy) and 2PEM (2Photon excitation microscopy) in a powerful way including polarization properties. SHIM on a laser-scanning system is a unique tool for high-resolution, high-contrast, three-dimensional studies of live cell and tissue architecture. The physical origin of SHG within these tissues is addressed and is attributed to the laser interaction with dipolar protein structures that is enhanced by the intrinsic chirality of the protein helices [2]. Although is a coherent process the multiple scattering through the tissue give us the capability to acquire signal in both backward and forward direction [3]. The orientation of collagen fibers within tissues such as tendons or ligaments is of primary importance. In this study, we propose a simple method based on second harmonic generation (SHG) microscopy to map, pixel by pixel, the orientation of the symmetry axis of the second-order nonlinear susceptibility tensor of collagen fibers of a tendon. The method uses only few images acquired at specific polarizations of the input laser beam. In addition to orientation information, the method would provide polarization independent images and an estimation of the ratio of the nonlinear susceptibility components. This procedure is implemented in both backward and forward scattering pathway. The approach could allow mapping fiber orientation fields, independently of individual fiber contrast in the SHG image. The relationship between images acquired in forward and backward scattering configuration provides more information about sample organization.
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1508-Pos Board B352
Vibrational Imaging Based On Stimulated Raman Scattering Microscopy
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We demonstrate a new implementation of coherent vibrational optical microscopy where image contrast is based on stimulated Raman scattering (SRS). SRS detection inherently maps the imaginary part of the third-order nonlinear susceptibility of a molecular vibration. The chemical contrast in SRS microscopy is thus inherently free of nonresonant nonlinear background signal and of spectral interferences between overlapping Raman bands. Experiments are presented that confirm these fundamental advantages of SRS microscopy when compared to coherent anti-Stokes Raman scattering (CARS) microscopy and demonstrate its potential for the noninvasive vibrational imaging of biological systems.

1509-Pos Board B353
Investigating The Network Structure Of Type I Collagen As A Function Of Temperature And Concentration Via Confocal Microscopy
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Three dimensional *in vitro* approximations to extracellular matrix (ECM) are increasingly being used in biophysical experiments investigating cell behavior. One advantage to using collagen I gels as ECM approximations in such experiments is the ability to image the collagen fibers within the gel. This allows simultaneous imaging of cells and their local environment simultaneously with

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.
(Supported by the Gates Foundation and NIH/NIBIB P41 RR04224 to WRZ.)

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.
This work is supported by the Federal Ministry for Education and Research (BMBF) in the framework ''Biophotonics III''.

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