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.
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1504-Pos Board B348
Application of Higher Harmonic Generation Microscopy in Assisted reproductive technologies
Hai-Yin Wu[1], Shee-Uan Chen[2,3], Cho-Shuen Hsieh[1], Szu-Yu Chen[1], Chi-Kuang Sun[1,4].
[1]Department of Electrical Engineering and Graduate Institute of Photonics and Optoelectronics, National Taiwan University, Taipei City 10617, Taiwan, [2]Department of Obstetrics and Gynecology, National Taiwan University College of Medicine, Taipei City 10051, Taiwan, [3]Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei City 10051, Taiwan, [4]Research Center for Applied Sciences, Academia Sinica, Taipei City 11529, Taiwan.
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
Oleg Nadiarnykh, Ronald LaComb, Molly Brewer, Paul J. Campagnola.
University of Connecticut Health Center, Farmington, CT, USA.
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
Francesca Cella, Zeno Lavagnino, Alberto Diaspro.
LAMBS MicroScoBio, Genoa, Italy.
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
Paolo Bianchini[1], Paola Ramoino[2], Cesare USAI[3], Alberto Diaspro[1,4].
[1]LAMBS, MicroSCoBiO University of Genoa, Genoa, Italy, [2]DIPTERIS, University of Genoa, Genoa, Italy, [3]IBF, National Research Council, Genoa, Italy, [4]NBT-IIT Italian Institute of Technology, Genoa, Italy.
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
P. Nanakumar[1], A. Kovalev[2], **A. Volkmer**[2].
[1]Birla Institute of Technology & Science Pilani, Goa Campus, Goa, India, [2]University of Stuttgart, Stuttgart, Germany.
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
Yali Yang[1], Lindsay M. Leone[2], Laura J. Kaufman[1].
[1]Columbia University, New York, NY, USA, [2]Washington and Jefferson College, Washington, PA, USA.
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