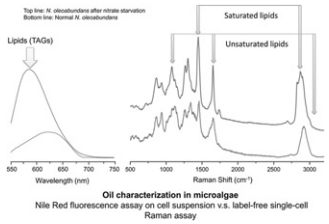
and allows a deeper insight into structural rearrangements during the first isomerization step.

3869-Pos
Effects of Hydration Levels on the Bandwidth of Microwave Resonant Absorption Induced by Confined Acoustic Vibrations
Tzu-Ming Liu, Hung-Pin Chen, Shih-Chia Yeh, Chih-Yu Wu, Chung-Hsiung Wang, Tang-Nian Luo, Yi-Jan Chen, Shen-Iuan Liu, Chi-Kuang Sun.
National Taiwan University, Taipei, Taiwan.
The vibration modes of molecules can be revealed by infrared absorption spectroscopy if their displacements change the dipole moments of molecules. Depending on the bonding strength, the mass of atoms, and the types of vibrations, the resonant absorption frequencies of molecules range from hundreds of terahertz (THz) to several THz. For collective vibrations of macromolecules like proteins or virions, the corresponding resonant frequencies will be around THz and could be probed by the THz or microwave absorption spectroscopy. However, in this frequency range, the periods of vibrations are close to or above the persistence time of hydrogen bonding of water molecules. If the surface to volume ratio of macromolecules is large, surrounding water molecules will overdamp the vibrations and smear the resonant absorption feature. Recently, we demonstrated that confined acoustic vibrations (CAV) of viruses can modify dipole moments and result in microwave resonant absorption (MRA) (Liu *et al.*, 2009). The resonant absorption frequencies correspond to those of dipolar active [SPH, *l*=1] modes. The activation of the resonant coupling relies on the core-shell charge structures, which are inherent on the capsid surfaces. Such characteristic absorption peak is rarely found in THz spectroscopy on solvated proteins and the actual mechanism worth a further investigation.
In this study, by decreasing the pH value of solution dwon to 5.2 or inactivating viruses, we enhanced the surface hydrophilicity and increased the magnitude of surface potentials. Both of these surface manipulations raised the surface affinity to water molecules, provide better acoustic confinements, and narrowed the bandwidths of CAV-induced MRA. Our results indicate that the viscoelastic transition of hydration shells play a critical role in the THz or microwave vibration spectroscopy.
T.-M. Liu *et al.*, *Appl. Phys. Lett.* **94**, 043902 (2009).

3870-Pos
Raman Spectroscopic Detection of an Optically Trapped Single DNA Molecule
Satish K. Rao, Saurabh Raj, Dmitri Petrov.
ICFO - The Institute of Photonic Sciences, Castelldefels, Spain.
Optical trapping has opened up a number of biophysical fields because of its ability to hold and manipulate single cells and molecules. In addition, the force sensitivity of an optical trap has allowed for a number of studies in to the mechanics of the most basic biological systems such as DNA. However, the majority of these experiments are based on a measured force correlated to a detected displacement or extension of the molecule in question. Due to the low optical cross section of a single DNA molecule, for example, interacting light directly with the structure, in order to obtain a detailed spectrum, has not been possible.
In this work, we present a measurement of a Raman spectrum from a single DNA molecule that is attached to two optically trapped dielectric microspheres. The scattering cross section in this instance is enhanced by the injection of nanosized silver colloids to the solution that adsorb on to the DNA. A near-infrared beam is used for excitation and Raman bands of DNA are obtained that agree with those from previous studies of DNA-metal colloid solutions. The presence of just one DNA molecule is verified by measuring the well-established force-extension curve. The adsorbed nanometer sized silver structures do not greatly affect the overall elasticity of the DNA, however the mechanical response at low to medium range forces seems to be altered. The addition of Raman spectroscopy to existing force spectroscopy methods could provide new information about the mechanochemical makeup of a structure through a correlation of the two methods.

3871-Pos
Single-Cell Diesel Mining on Microalgae: Direct and Quantitative Monitoring of Microalgal Oil Production In Vivo by Raman Spectroscopy
Huawen Wu, Joanne V. Volponi, Seema Singh.
Sandia National Laboratories, Livermore, CA, USA.
Microalgae, known for their rapid growth and high lipid content, became a promising candidate for the next generation feedstocks for liquid biofuels. Traditionally, instead of living organisms, they were treated as lifeless biomass in bulky, lyophilized or extracted forms, making it difficult to understand the fundamental biological processes in play. Labeling algae with fluorescent probes can be a potential high-throughput method but it provides little chemical information and is limited by impermeability, toxicity and specificity. In this work, we focus on in situ, in vivo and label-free Raman characterizations of single living green algae of several species. Our study has demonstrated that single-cell laser-trapping confocal Raman spectroscopy can directly obtain quantitative information of the lipids produced inside individual algae. Information critically related with the quality of derived biodiesel, such as lipid unsaturation and melting temperature can be obtained at single-cell level. Meanwhile, lipid triggering effect by nitrate starvation was characterized in vivo on single cells. Our real-time in vivo "diesel mining" on individual microalgae cells enables the possibility of researching and engineering of the best conditions and species for algal oil production.



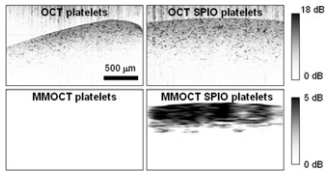
Imaging & Optical Microscopy IV

3872-Pos
How to use Confocal Microscopy in Search of a Highly Resolved Hologram
Aura I. Moreno-Vega[1], Carlos Saldaña[2], Veronica Morales-Tlalpan[3], Mauricio Diaz-Muñoz[4], Victor M.Castaño Meneses[5].
[1]Centre of Applied Physics and Advanced Technology Universidad Nacional Autónoma de México , Campus, Juriquilla, Querétaro 76230, QRO, México., Queretaro, Mexico, [2]Biomedicine Department, Universidad Autónoma de Querétaro. Qro-México., Queretaro, Mexico, [3]High Specialty Regional Hospital of the Bajio. León Guanajuato, México, Leon, Guanajuato, Mexico, [4]Department of Molecular and Celular Neurobiology, UNAM, Campus, Juriquilla, Querétaro 76230, QRO, México., Queretaro, Mexico, [5]Centre of Applied Physics and Advanced Technology) Universidad Nacional Autónoma de México, Campus, Juriquilla, Querétaro 76230, QRO, México., Queretaro, Mexico.
Objective: To apply confocal microscopy and non-conventional holographic techniques, for the three-dimensional reconstruction of cancer cell endomembranes.
Both confocal microscopy (CM) and holography (H) allow the capture of high quality images for their 3D reconstruction, while each technique varies in the way light is captured and processed. Combining both techniques with electron microscope grids of different sizes will hence allow a 3D reconstruction of higher quality and fidelity.
We hypothesize that, by placing grids of differently sized holes in our cell preparations, they will acts as multiple pinholes, increasing image resolution for its 3D construction as a digital hologram. The hologram produced would have higher spatial precision, due to wave optics phenomena.
Preliminary results of images captured with grids of differently sized holes (100, 50, 40 & 30μm) have shown a differential pattern in the fluorescence intensity. Additionally, image resolution distributes itself as a Gaussian. This may be due to the bar thickness of the grid interfering with the capturing of light. So far, these results show two important aspects: 1) The fluorescence intensity obtained is not proportional to the mesh size and 2) Image resolution behaves in a normally distributed way against the grid hole size.
Our prospects are therefore to use grids with specific characteristics (hole size and bar thickness) to create higher quality images and so more precise 3D reconstructions.

3873-Pos
Imaging Contrast and Biomechanics using Optical Coherence Tomography to Sense Superparamagnetic Iron Oxide Labeled Platelets
Amy L. Oldenburg, Thomas H. Fischer, Caterina M. Gallippi.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
Optical coherence tomography (OCT) provides 3D tissue imaging by contrasting light backscattering. OCT also senses nanoscale motions from optical phase shifts. We employ temporally modulated magnetic field gradients to mechanically displace superparamagnetic iron oxide nanoparticles (SPIOs). By locking in to the modulation frequency, SPIOs are contrasted in OCT, dubbed magnetomotive OCT (MMOCT).

Rehydrated lyophilized human platelets (''RL platelets''), are chemically stabilized infusion hemostatic agents retaining viability. We investigate similar dried platelets with intracellular SPIOs (''SPIO platelets'') as imaging therapeutics. SPIOs are uptaken into the surface connected open canalicular system of platelets where they form clusters. Platelets are then stabilized and dried with the same methods as RL platelets. These SPIO platelets retain the primary hemostatic functions of RL and fresh platelets.

We found that MMOCT provides highly specific contrast to SPIO platelets at 1.5e6/μL in 1% agarose scaffolding (see figure). Furthermore, by sweeping the modulation frequency, a mechanical frequency spectrum is obtained, and resonance peaks are associated with the sample elasticity. This has potential for imaging sites of vascular damage and monitoring the local mechanical microenvironment to provide more detailed information about vascular pathologies.



3874-Pos

Imaging on Nano-Resolution Scale of Carrier Modifications Caused by Therapeutics & Diagnostics by Freeze-Fracture TEM

Brigitte Papahadjopoulos-Sternberg.
NanoAnalytical Laboratory, San Francisco, CA, USA.

The potency of nano- and micro-particles, loaded with therapeutic and/or diagnostics is frequently depending upon their morphology adopted in biological relevant environments. Freeze-fracture transmission electron microscopy (ff-TEM) as a cryo-fixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, antimicrobial- and imaging molecules[1]. At a 2 nm resolution limit we are able to study structural modifications of such carriers related to their pay load, application milieu, and during cell interaction.

Using ff-TEM we studied the morphology of a wide variety of nano- and micro particles suitable as carriers for diagnostics as well as therapeutics including quantum dots (coupled to drug-loaded immunoliposomes)[2], gold nanoparticles, superparamagnetic iron oxide nano-particles loaded in polymeric immunomicelles[3], micelles (spherical-, disc-, and worm-type micelles)[4,5], small unilamellar liposome[6], multilamellar liposome, niosomes, cationic liposome/DNA complexes, integrin-targeted lipopolyplexes[7], polymer- or lipid-stabilized gas bubbles[8], cochleate cylinder, depofoam particles, and drug crystals. Recently we explored liposome-, virosome-, and virus-based vaccines, including measles vaccine powders, by ff-TEM. Furthermore, we explored structural modifications within bilayers such as domain-formation[1] but also transformations to non-bilayer structures such as hexagonal and cubic phases.

References

[1] B. Papahadjopoulos-Sternberg, in:Liposomes Methods and Protocols, Human Press, 2, 22 (2009).
[2] K.C. Weng et al. Nano Lett., published online: 08/20/2008.
[3] R.M. Sawant et al. J. Nanopart Res published online: 08/03/2009.
[4] V.P. Torchilin et al. PNAS (2003) 100 (4) 1972.
[5] Y.T. Ko et al. J. Controlled Release, available online 10/07/2008.
[6] V. P. Torchilin et al. PNAS (2003) 100 (10) 603.
[7] P.C. Bell, et al. Biochem. 46 (2007) 12930.
[8] C. Brancewicz et al. J. Disp. Sci. & Techn. 27 (2006) 761.

3875-Pos

Synchrotron X-Ray Fluorescent Imaging and Spectroscopy Studies of the Role of Copper in the Stem Cell Niche Architecture of Adult Neural Stem Cells

Yulia Pushkar.
Purdue University, West Lafayette, IN, USA.

Improvements in sensitivity and spatial resolution of X-ray fluorescent (XRF) imaging and spectroscopy allowed us to study the distribution of metals in brain tissues. We discovered the specific Cu enrichment in cells in the subventricular zone (SVZ) of the lateral ventricle. This area in the brain contains adult neural stem cells (NSCs). NSCs niche architecture enables to continuously generate functional neurons in specific brain regions throughout life. Knowledge about the mechanisms controlling NSCs self-renewal, proliferation and differentiation is of critical importance for future therapeutic interventions of major brain disorders.

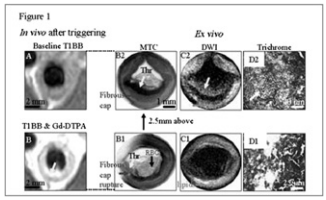
XRF-imaging with sub-cellular (200 nm) resolution on rat brains demonstrated that sub-population of cells in the SVZ builds up sub-cellular Cu accumulations. The Cu concentration inside these structures is as high as 50 mM. It is well established that SVZ has four types of cells: ependymal cells, type B progenitors, type C transit amplifying cells and type A migrating neuroblasts. Imaging the Br signal in brains of BrdU treated rats, we found that actively dividing cells are largely depleted of Cu accumulations. However, cells surrounding actively dividing cells, which are assigned to type B progenitors, demonstrate significant Cu accumulations. Cu K-edge micro-XANES demonstrated that Cu is in Cu(I) form with and spectrum has shape characteristic of a Cu(I)-thiolate multimetallic cluster. The Cu co-localizes with increased sulfur signal which allows quantitation of the Cu/S content.Copper is known to play an important role in the brain's development and function. However, the role of Cu in the viability and control of the NSCs is presently unknown. Our study is a first attempt to look at the role of Cu in mechanisms controlling the NSCs.

3876-Pos

MRI of Thrombus Propogation after Plaque Rupture

James A. Hamilton, Alkystis Phinikaridou.
Boston University School of Medicine, Boston, MA, USA.

Atherosclerotic plaque disruption and subsequent thrombosis is the leading cause of acute cardiovascular events. We have used a rabbit model of controlled atherothrombosis and combined *in vivo* and *ex vivo* MRI and histology to study whether thrombus organization and composition could be detected based on its biophysical properties. In vivo MRI at the site of plaque rupture without (Figure 1A) and with gadolinium (Figure 1B) showed the luminal thrombus. Ex vivo MRI of disrupted aortic plaques revealed that thrombi propagated both anti-parallel **(Figure 1)** and parallel to blood flow. Examination of disrupted aortic plaques revealed that the % magnetization transfer was much lower and the apparent diffusion coefficient much higher in platelet-rich thrombi compared to organized, fibrin-rich thrombi. This permitted distinction of the thrombus at the site of plaque rupture (Figure 1B1 and C1) from the subsequently propagated thrombus (Figures 1B2 and C2).The conclusions drawn from MRI were validated by histology (Figures 1D1-D2).



3877-Pos

Development of an arganotypic System to Image Metastasis of Carcinoid Tumors in Situ

Sasi Arunachalam[1], Riaz Nasim[2], **David Giovannucci**[1].
[1]University of Toledo College of Medicine, Toledo, OH, USA, [2]Khyber Medical College, Peshawar, Pakistan.

Patients with neuroendocrine tumors of the small or large bowel commonly called carcinoid often develop liver metastases. Hepatic portal circulation is a predicted route for establishing liver metastases of carcinoid tumors or following intra-splenic injection of cancer cells. However, the cellular and molecular events that mediate this site-specific metastasis are not well understood. We developed a system to study extravasation, migration, invasion and proliferation of a human carcinoid cell line using mouse liver organotypic slice culture as a tractable preparation that more closely resembles the three-dimensional, multi-cellular tumor microenvironment than does a dispersed cell culture system. BON cells stably transfected with GFP were introduced to liver by portal vein injection. Organotypic slices obtained from the liver were monitored using fluorescence macroscopy and confocal/multi-photon microscopy. The seeded cancer cells adopted an elongated morphology and arrested in the lumens of red fluorescently-labeled venules. Seeded cells in the liver slices were monitored out to 14 days in culture. Although liver slices generally remained viable over the experiment, there was a general reduction in the number of seeded cells with the greatest reduction occurring by the 24 hr or 48 hr time points. After 72 hr to 96 hr, there was a detectable increase in GFP-labeled cells indicating that subsets of the remaining seeded cells were proliferating. These cells formed small ''tumorlet'' or spheroid structures that extended beyond the vasculature and into or on the surface of the parenchymal tissue. These tumorlets continued to increase in size to a maximum diameter of about 300 μm. We provide evidence that this organotypic slice/xenograft model is a promising tool with considerable potential as a means to probe the early events mediating metastasic tumor growth in the liver.