Book of Abstracts

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INVITED SPEAKERS

Id-062

Electron Energy-loss Spectroscopy of Quasi-two-dimensional Crystals: One-step Simultaneous Inelastic and Elastic Scattering Theory

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Abstract: Inelastic scattering of the medium-energy (~10-100 eV) electrons underlies the method of the high-resolution electron energy-loss spectroscopy (HREELS), which has been successfully used for decades to characterize pure and adsorbate-covered surfaces of solids. With the emergence of graphene and other quasi-two-dimensional (Q2D) crystals, HREELS could be expected to become a major experimental tool to study this class of materials. We, however, identify a critical flaw in the theoretical picture of the HREELS of Q2D crystals in the context of the inelastic scattering only (``energy-loss functions'' formalism), in contrast to its justifiable use for bulk solids and surfaces. The shortcoming is the neglect of the elastic scattering, which we show is inseparable from the inelastic one, and which, affecting the spectra dramatically, must be taken into account for the meaningful interpretation of the experiment. With this motivation, we build a theory of the simultaneous inelastic and elastic electron scattering at Q2D crystals. We apply this theory to HREELS of graphene, revealing an important effect of the strongly coupled excitation of the π + σ plasmon and elastic scattering resonances. Our results open a path to the consistent and interpretable study of the elementary excitations in crystalline mesoscopic materials by means of HREELS, with its supreme resolution on the meV energy-scale, which is far beyond the capacity of the now overwhelmingly used EELS in transmission electron microscope.

Keywords: HREELS elastic inelastic scattering.

INVITED SPEAKERS

Id-063

Halogen Molecular Elimination from Halogen-containing Compounds in Atmosphere Using Cavity Ring-down Spectroscopy

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Abstract: Atmospheric halogen chemistry has drawn much attention, for the halogen atom (X) playing a catalytic role may cause severe stratospheric ozone depletion. X atomic elimination from X-containing hydrocarbons is recognized as the major primary dissociation process upon UV-light irradiation, whereas a direct elimination of X_2 product has been seldom discussed or remained as a controversial issue. This talk will cover the detection of X_2 primary products using cavity ring-down absorption spectroscopy in the photolysis at 248 nm of a variety of X-containing compounds, focusing on bromomethanes, dibromoethanes, dibromoethylenes (1,1-$C_2H_2Br_2$ and 1,2-$C_2H_2Br_2$), diiodomethane, thionyl chloride ($SOCl_2$), along with acyl bromides (BrCOCOBr and $CH_2BrCOBr$). The optical spectra, quantum yields, and vibrational population distributions of the Br_2, I_2, and Cl_2 fragments have been characterized. With the aid of ab initio calculations of potential energies and rate constants, the detailed photodissociation mechanisms may be comprehended. Such studies are fundamentally important to gain insight into the dissociation dynamics and may also practically help to assess the halogen-related environmental issues.

Keywords: Atmospheric halogen chemistry; Cavity ring-down absorption spectroscopy.

INVITED SPEAKERS

Id-064

Surface Enhanced Raman Spectroscopy Based Rapid Analysis for Food Safety

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Abstract: Food safety is a big concern in the world nowadays. For food safety analysis it is essential to rapidly and precisely analyze trace target markers from the high number of real samples on spot. Conventional food safety analyses are usually based on chromatographic and spectroscopic techniques with large instrumental scales. It still remains a big challenge to develop rapid and accurate analytical methods for trace markers from complicated food samples. Surface enhanced Raman spectrometry (SERS) is a nondestructive, sensitive, rapid and water interference-free detection technique with portable instrumental scale. Moreover, the abundant spectral fingerprint characteristics of SERS can provide crucial qualification and quantification information for real sample analysis, which makes it a potentially ideal technique for rapid on-site analysis of complicated food samples. However, there are still few works focusing on the development of rapid analytical methods for food safety purpose by SERS due to the lack of suitable enhanced substrates and sampling methods adapted to SERS. In our group, several SERS substrates such as intrinsic internal standard substrate of Au@PS-b-P4VP and Au@hydroxyl-functionalized polystyrene (Au@PS-OH) substrate and SERS adaptable sampling methods such as the portable large-volume constant-concentration (LVCC) and miniaturized thermal-assisted purge-and-trap (MTAPT) sampling methods coupling with suitable derivatization methods were developed for the rapid on-site analysis of trace food markers including formaldehyde, sulfide dioxide, ethylene, chrysoidine, etc. These SERS substrates and SERS adaptable sampling methods were well-controlled, reproducible and stable, which made the sampling result more representative and greatly improve the analytical selectivity. Good recoveries of trace target markers from real food samples suggested the proposed rapid analytical methods by SERS were reliable and suitable for food safety analysis. The works were financially supported by the National Natural Science Foundation of China (Nos. 21475154 and 21275168).

Keywords: Surface enhanced Raman spectroscopy; Rapid analysis; Food safety.

INVITED SPEAKERS

Id-068

Infrared Spectroscopy in Action: Hydrophobic Effect and Preferential Conformations of Blocked Dipeptides

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Abstract: The applicability of vibrational spectroscopy (infrared, Raman and VCD) will be demonstrated on two topics from the field of structural biochemistry and biophysics. The first issue is attributed to structural studies of blocked dipeptides ant the second one to study of physical origins of hydrophobic effect. Knowledge of the structure of the peptides, which are mainly determined by the conformational preferences of amino acids, are very important for a description of the intrinsically unordered proteins or for solving the protein folding phenomena and will significantly attribute to understanding the physical origins of the conformational preferences of amino acid residues. Moreover, it will make possible the construction of a new generation of more reliable force fields, which will produce more reliable conformations of peptides and proteins. The application of the ATR spectroscopy, the modified calculation of the absorption spectra and the parallel application of infrared and Raman spectroscopy allow precise measurements and processing of experiment data. Excellent infrared spectra of dipeptides down to 0.01 m concentrations in water were obtained by ATR spectroscopy. Through successful analyses of the amide III region we determine the ration between the P_{II}, β and α_R conformations of 19 blocked dipeptides. These bands can be found near 1313 cm^{-1} (P_{II}), near 1290 cm^{-1} (α_R) and near 1280 cm^{-1} (β) in both the infrared and Raman spectra of dipeptides in water. The population of these three conformations strongly depends on the type of the side chains. The P_{II} is the prevailing conformation in the alanine, leucine and lysine dipeptides, while β is the dominant conformation in the cysteine, threonine, valine and histidine dipeptides. The second topic of presentation is attributed to the structural study of water molecules in the vicinity of fully nonpolar solutes. We were interested in physical origin of so-called hydrophobic effect. With carefully selected infrared experiments we probed the water solutions of methane, ethane, argon and krypton in order to test whether the presence of fully nonpolar solutes enhance the strength of hydrogen bonds of the solvent in their vicinity. To prove this almost 70 years old hypothesis (”the existence of icebergs near hydrophobic molecules”) we apply mixed solvent (2.8% HDO in H_2O) and high pressures (up to 60 atm). The application of HDO prevents the saturation of OD stretching band. The low solubility of applied nonpolar solutes was increased by use of high pressures. Difference spectra between pure HDO solution in water and the same solution with added nonpolar solutes unequivocally prove the existence of enhanced water hydrogen bonds. The OD stretching frequency of these perturbed water molecules corresponds to those found in the spectra of hexagonal ice and/or clathrates.

Keywords: Infrared spectroscopy; Preferential conformations of dipeptides; Hydrophobic effect.

INVITED SPEAKERS

Id-070

Novel Fourier Phase Contrast and Fluorescence Microscopy

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Abstract: Phase contrast microscope is an extremely useful device for teaching and research. It is widely used to view images of transparent specimens such as living cells in culture and thin tissue slices. One of the major advantages is that living cells can be examined in their natural state without being fixed or stained. As a result, the dynamics of ongoing biological processes can be observed and recorded in high contrast. We developed a novel Fourier phase contrast microscopy technology which is simple, inexpensive, all optical, user-friendly and self-adaptive. It exploits monochromaticity, intensity and phase coherence characteristics of a low power laser and photo-induced birefringence in a nematic liquid crystal. Monochromaticity facilitates precisely defined deviated light and also provides well resolved Fourier plane mapping of spatial frequencies (object information). Intensity of the laser source makes object features bright and clearly visible. High degree of phase coherence preserves the phase retardation introduced by the liquid crystal. When the liquid crystal cell is placed at the Fourier plane, low spatial frequencies at the center are intense enough to induce local liquid crystal molecules into the isotropic phase where as high spatial frequencies on the edges are not so intense and remain in the anisotropic phase resulting in pi/2 phase difference between high and low spatial frequencies in real time. Moreover by using a laser line notch filter and other filter combinations, we can obtain simple bright field, phase contrast, edge enhanced brightfield and fluorescence images. Combinations of brightfield + fluorescence, phase + fluorescence and edge enhanced + fluorescence displaying sharp features of the Drosophilla embryo are imaged at once without the need for digital image registration and fusion. The phase information is obtained using the Fourier phase contrast microscopy and fluorescence is imaged using trans-fluorescence illumination. Even multiple fluorescence at different wavelengths is observed simultaneously in view of the intensity of the laser source. This comprehensive microscope has the capability of simultaneously providing both structural and functional information of biological specimens in a streamlined simplified design with a single optical path. It can potentially be used in high-throughput screening and automated microscopy. The technology is recently licensed to industry for developing the prototype and marketing the instrument.

Keywords: Fourier; Phase Contrast; Fluorescence.

INVITED SPEAKERS

Id-074

Three-dimensional Super-resolution Measurement with Digital Holographic Microscopy

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Abstract: The miniaturization of key functional components is a main trend of development in precision engineering, and the three-dimensional measurement of these micro-components is a challenging task. The digital holographic microscopy is a promising method of measuring complex shapes. A spatial light modulator (SLM) is employed to realize phase-shifting and beam separation. The anti-aliasing shift-invariant contourlet transform is developed to improve the accuracy of the reconstructed surfaces. A flexible segmentation method is developed for reliable phase unwrapping. It has advantageous capabilities of sparse and directional representations for multiscale features, as a consequence the over-segmentation problem caused by local textures and noise can be solved straightforwardly. Tilted wavefronts are generated by the SLM to compensate the slope of the small objects. The measured data associated with different viewpoints and subareas are registered, with the frequency band extended for the purpose of super-resolution measurement. Consequently small-scaled complex components can be measured by full-area coverage, and the measurement resolution limited by the optical diffraction and sensor pixels can be broken.

Keywords: Three dimensional measurement; Optical imaging; Super-resolution; Digital holography; Fresnel diffraction.

INVITED SPEAKERS

Id-075

Micro-plasma Processing in Scanning Electron Microscope

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Abstract: We have been studying on micro-plasma discharge in the specimen chamber of scanning electron microscope (SEM) for local plasma sputtering and local plasma chemical vapor deposition (CVD) with in-situ SEM observation. The prototype for experiments was equipped on a conventional SEM apparatus S-3000N (Hitachi High-Technologies). The specimen stage was specially designed for precise control of the discharge gap between a high-voltage anode and electrically grounded cathode supported by each micro-manipulator. A gas nozzle to introduce a local dense gas jet into the gap also performs the high-voltage anode. As the cathode, a cut of n-type silicon (Si) wafer was placed right in front of the gas nozzle with a gap distance of a several 100s µm. The gas nozzle was a standard 1/8 inch tubing made of SS-316 closed at one end and having a laser drilled hole around 10 µm in diameter (Lenox Laser, Inc.). As the discharge gas, Ar, N_2 or C_2H_2 was fed into the specimen chamber through the nozzle. An additional turbo molecular pomp (TMP) was installed directly on the specimen chamber to keep the high vacuum for SEM operation with introducing discharge gas. For example, by supplying 80 kPa of discharge gas pressure to the nozzle, the gas flow rate is 6.6 sccm, the pressure gage in the SEM apparatus shows below 1 Pa, and the SEM can be operated as the conventional high-vacuum mode. It was found that the gas jet appeared in the SEM image at the exit of gas nozzle. Due to the small orifice size and high applied gas pressure, the gas density is quite high at the exit of gas nozzle, possibly nearly equal to the atmospheric pressure, and the gas molecules can be ionized by the probe electron beam with resulting in electron emission form the gas jet. By using Ar micro-plasma, local plasma sputtering was performed on the Si wafer, and by using C_2H_2 micro-plasma, local plasma CVD of a symmetric mountain-shaped a:C-H film was demonstrated.

Keywords: Micro-plasma; SEM; Sputtering; CVD.

INVITED SPEAKERS

Id-076

Thin Film Magnetostriction Measurement by Using a Digital Holographic Microscope System

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Abstract: Magnetostriction (λ) is a ferromagnetic phenomenon, which describes that when an external magnetic field (H) is applied on a ferromagnetic sample, its dimensions (or shape) are changed by a small amount; usually of the order of 1-1000 ppm. In this talk, I am going to discuss a new method, i.e., by using a digital holographic microscope (DHM) system, to measure the magnetostriction of a ferromagnetic thin film. First, the sample is an 110 nm thick Fe-Co-Ga film deposited on an 100 μm thick Si(100) substrate by the magnetron sputtering technique. The film-substrate sample has to be clamped at one end of the sample length to form a cantilever. For the room temperature (RT) measurement, the DHM system includes a digital holographic microscope and an electromagnet. For the high temperature (high-T) measurement, an infrared camera, a high-T sample stage, and a vacuum chamber are integrated into the system. The DHM system maps out the 2D or 3D magnetostriction deflection profile, $\Delta z_{\parallel}$ or $\Delta z_{\perp}$, at any location (x, y) on the film plane, due to $H_{\parallel}$ (the longitudinal field) or $H_{\perp}$(the transverse field), respectively. From the longitudinal and transverse curvatures of the deflected surface, we can determine the saturated longitudinal or transverse magnetostriction, $\lambda_{s\parallel}$ or $\lambda_{s\perp}$. The saturation magnetostriction of the film is defined as: $\lambda_s = (2/3)\,(\lambda_{s\parallel} - \lambda_{s\perp})$. The following two effects need to be considered to obtain the correct temperature dependence of λ_s: the thermal expansion and the non-uniform temperature (ΔT) effects. The experimental T dependence of λ_s agrees well with the theoretical prediction in the T range from RT to 140℃.

Keywords: DHM; Magnetostriction; Magnetic film.

INVITED SPEAKERS

Id-078

Femtosecond Raman-induced Kerr Effect Spectroscopic Study on Ionic Liquids

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Abstract: Ionic liquids (ILs) are molten salts at room temperature. One of the most fascinating natures of ILs may be the property control. Chemical and physical properties of ILs are controllable by changing the combination of cation and anion. Indeed, changing the combination of cation and anion influences the intermolecular interaction in ILs. Therefore, it is essential to understand the intermolecular interaction in ILs. Intermolecular vibrational bands in liquids and solutions typically appear in the low-frequency region less than 150 cm^{-1}. Because the intermolecular vibrational band in condensed phases reflects the microscopic structure and intermolecular interaction, it is worth studying the intermolecular vibrational band in ILs. Femtosecond Raman-induced Kerr effect spectroscopy (fs-RIKES) is a time-domain Raman spectroscopy and captures the Raman active molecular motions in the low-frequency region with a great sensitivity. For example, fs-RIKES setup built in our laboratory detects in the frequency range from ca. 0.3 to 700 cm^{-1}. Therefore, fs-RIKES is a very useful spectroscopic technique to investigate condensed phases including ILs. In this talk, I will discuss results on ILs studied by fs-RIKES in our laboratory. The topics will include (i) neutral and charged aromatic rings and (ii) dication vs. monocation. The works were supported by JSPS KAKENHI (21685001 and 15K05377), the Iketani Science and Technology Foundation, the Futaba Electronics Memorial Foundation, the Iwatani Naoji Foundation, and the Tokyo Ohka Foundation for the Promotion of Science and Technology.

Keywords: Femtosecond Raman-induced Kerr Effect Spectroscopy; Ionic Liquid.

INVITED SPEAKERS

Id-079

Simultaneous UV-Visible-NIR and Long-wave Infrared Laser-induced Breakdown Spectroscopy for Rapid and Remote Chemical and Biological Sensing

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Abstract: Conventional elemental analysis via UV-Visible-NIR (UVN) Laser-induced Breakdown Spectroscopy (LIBS) has proven to be a versatile and sensitive real-time and in-situ technique used for detection of trace substances as well as the analysis of the elemental composition of materials. Our recent development of a novel MCT linear array (320 pixels) based LIBS detection system, published recently in Applied Optics, that is capable of rapidly capturing (~1-5 second) a broad spectrum of atomic and molecular LIBS emissions in the long wave infrared (LWIR) region (~5.6 to 10 μm), offers the possibility of a simultaneous UV-Visible-NIR and LWIR LIBS measurement. Intense and distinct atomic and molecular LWIR emission signatures were readily observed from wide variety of both inorganic and organic materials such as carbon allotropes, pharmaceuticals, explosives, chemical, and biological warfare agent simulants, and various thin liquid and solid films of contaminants deposited on realistic substrates such as asphalt or concrete. This report outline the attempt to combine this newly developed rapid LWIR LIBS linear array detection system to existing rapid optical analytical techniques such as conventional UVN LIBS, for chemical and biological analysis applications. Every simultaneous UVN + LWIR LIBS measurement in this work was initiated by a single laser pulse-induced micro-plasma on the target surface. This new laser based UVN+LWIR analysis shown the capabilities of acquiring complete target spectra in both UVN and LWIR spectral ranges within a few seconds and also demonstrated the capabilities of rapidly identifying a very wide range of chemical compounds from simple potassium nitrate to complex peptidoglycan of bacterial endospores. It was also observed that LWIR LIBS studies using an eye-safe pump laser reproduced atomic and molecular LWIR LIBS spectra as observed under typically used 1.064 μm laser excitation. The observed LWIR LIBS emission signatures provide complementary data to the conventional UVN LIBS signatures, thus adding robustness to identification algorithms.

Keywords: Laser spectroscopy infrared emission.

INVITED SPEAKERS

Id-083

Identification of Fungal Phytopathogens Using Infrared Microscopy Spectroscopy and Multivariate Analysis

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Abstract: Early diagnosis of phyto-pathogens is of a great importance since it could save large economical losses due to crops damaged by fungi diseases. Moreover, it could prevent unnecessary soil fumigation or the use of fungicides and bactericides and thus prevent considerable environmental pollution. Taxonomic classification of these phyto-pathogens by standard physiological methods, based on cultivation, is very difficult especially between closely related species and strains. In addition, visual and microscopic observations are usually time consuming and not always very specific. Molecular biology and immunological methods are very sensitive and rapid relative to physiological methods for the identification of pathogens; however, their use is still limited due to their availability for only a small number of fungal pathogens. Developing such methods for different fungi strains is complicated, costly and not always possible. Therefore, the use of these methods is expected to be limited and not available for the screening of large numbers of different fungal strains samples. In this study, three different fungi genera; Colletotrichum coccodes, Verticillium dahliae and Fusarium oxysporum were investigated. All those fungi are pathogens that attack crops (including cotton, tomatoes, potatoes, eggplants and peppers) leading to large economical losses. Our main goal was to differentiate these fungi samples on the level of isolates, based on their infrared absorption spectra obtained using the Fourier transform infrared (FTIR) spectroscopic technique. Supervised and unsupervised machine learning algorithms, cluster analysis, principle component analysis (PCA) and linear discriminant analysis (LDA) were used for the classification manner. Our results showed significant spectral differences between the various examined fungi genera. The use of PCA and LDA has achieved a 99.7% success rate.

Keywords: E. coli; Multivariate analysis; Infrared microscopy; Bacterial resistivity to antibiotics; Support vector machines (SVM).

INVITED SPEAKERS

Id-086

The Application of Transmission Electron Microscopy to the Analysis of Powder Coatings Deposited on Metal Substrates by Plasma Method

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Abstract: The multi-purpose methods of Thermal Coating Spraying have become popular all over the world lately. The main purpose of thermal sprayed coatings is protection of surfaces of parts operating under conditions of increased friction, temperatures and aggressive environments: cylinder liners, shafts, etc. The method of microplasma spraying of hydroxyapatite and titanium powders onto metals surface is also used for applying biocompatible coatings to medical implants. When applying powder coatings with plasma spraying, it is very important to provide a controlled structure of coatings, such parameters as homogeneity of the coating, the desired structural-phase composition, the required porosity, etc. The main method of analyzing the structural-phase composition of coatings is the method of transmission electron microscopy (TEM). The purpose of this paper is to consider the advantages and challenges of application of TEM method for analyzing the structure of thick (up to 300 μm) powder coatings deposited by plasma spraying methods onto metal substrates. The main results of this work are the following: The methods of preparation of the samples of thick powder plasma spraying coatings for the TEM investigation have been developed; The laboratory samples with protective and biocompatible powder coatings deposited by microplasma according to the recommended modes onto metal substrates have been obtained; it was established by TEM that the coatings have the desired structure and phase composition. We proposed an alternative approach to the choice of modes of the coatings' additional processing by plasma treatment. The use of relatively low power densities of the plasma source in our study allowed the hardening of the coating due to the phase transformations which take place at lower temperatures; By the experience of TEM investigation of a number of coatings obtained by the methods of microplasma spraying and plasma detonation of powders on metal substrates the advantages and challenges of application of TEM method for analyzing the structure of thick plasma sprayed powder coatings have been established. These results are of significance due to the following: the new approaches and techniques developed during this study can be useful for a wide range of researchers involved in the development and implementation of plasma technologies. The study has been conducted with the financial support of the Science Committee of RK MES in the framework of the target financing program for the 2017-2019 biennium within the program 0006/PTF-17 "Production of titanium products for further use in medicine".

Keywords: TEM; Plasma spraying; Powder coatings; Structural-phase composition.

INVITED SPEAKERS

Id-087

A Theoretical Modeling of $L_{2,3}$ Absorption Spectra: From Single Molecules to 2D Complex Systems

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Abstract: Near edge X-ray absorption fine structure (NEXAFS) spectroscopy of transition metal (TM) complexes is unanimously recognized as a tool able to probe, site-selectively, the empty frontier MOs, the TM coordinative environment, the ligand -field splitting, the oxidation states and , in general the nature and the strength of the TM–L (L = ligand) bonding in TM complexes. Its versatility has been extensively exploited to investigate not only single molecules, but also solids and metalloproteins. Although huge, the chemical information achievable from $L_{2,3}$-edges XA spectra needs a detailed theoretical analysis. The $L_{2,3}$-edges XA simulated spectra herein presented have been obtained by using the Restricted Open shell Configuration Interaction with Singles (DFT/ROCIS) method, which includes spin orbit coupling in a molecular Russell-Saunders fashion by employing the ORCA program package. The resolution of the identity approximation has been used with the def-TZVP/J basis set, while the zeroth order regular approximation has been adopted to treat scalar relativistic effects; moreover, the reliability of diverse exchange-correlation (XC) functionals in reproducing geometrical and electronic properties has been tested. DFT/ROCIS numerical experiments have been carried out to investigate the $^{TM}L_{2,3}$-edges spectra of the following systems: $TM(acac)_2$ (TM = Mn, Co), $^{TM}L_{2,3}$-edges of $TM(acac)_3$ (TM = Cr, Mn, Fe), $^{V}L_{2,3}$-edges of PcV and PcVX systems (X = O, I) and the $^{Fe}L_{2,3}$-edges of a 2D complex system consisting of PcFe and PcFe(η^2-O_2) both of them contributing to the XA spectrum. As far as donor atoms' K-edge spectra are concerned, they have been modeled by running Time Dependent Density Functional Theory (TD-DFT) calculations and by using the ADF program package. Numerical experiments with different XC functionals on the ^{O}K-edge of $TM(acac)_3$ (TM = Cr, Mn, Fe), ^{N}K-edge of PcV, PcVO, PcVI and the ^{N,C,F}K-edge of CuTPP/CuTPP(F) have been performed. The final goal of this systematic study was that of revealing the role played by the central TM, by its oxidation state and its environment in determining the spectral features. Theoretical outcomes highlighted the important role played by the TM oxidation state in determining the overall spectrum appearance. Moreover, a common trend of the $^{TM}L_{2,3}$-edge spectra is the presence at the lowest excitation energies (EE) of the L_3-edge of states generated by TM-based 2p → 3d electronic excitations, having the same ground state spin multiplicity; at higher EEs, electronic states associated to metal-to-ligand-charge-transfer transitions and involving empty L-based orbitals are systematically present.

Keywords: Transition metal complexes; $L_{2,3}$-edge spectra; Metal to ligand charge transfer transitions; DFT/ROCIS calculations.

INVITED SPEAKERS

Id-088

Solvatochromic Studies, Molecular Docking and ct-DNA Binding Studies of a Chalcone

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Abstract: Chalcone, 3-(4-methoxyphenyl)-1-(4-(pyrrolidin-1-yl) phenyl) prop-2-en-1-one (MPPP) is a low molecular weight compound, easy to prepare in a cost effective manner, thus having potential to be developed as drug candidate. In this work we have done interaction studies using molecular docking, fluorescence spectroscopy and the absorption and steady state emission properties of a chemically synthesized chalcone, containing asymmetrical donor and acceptor groups have been investigated both experimentally and theoretically. Computational molecular docking approach has been utilized for intercalation of chalcone derivatives with double helical mammalian DNA studies. In this chalcone derivatives are used as ligand and DNA octamer d(GAAGCTTC)2 as a target. It is an octomer with an oft repeat sequence of CT-DNA and it is retrieved from Protein Data Bank in form of complex with Actinomycin. As reflected from the absorption spectra in pure non polar, polar and neutral solvents the ground state, MPPP has a significant intramolecular charge transfer (ICT) character and a great sensitivity to the hydrogen bond donating ability of the medium. On the other hand, its excited singlet state exhibits high ICT characters as exhibited by the drastic solvatochromic effects and these results are consistent with the data. Intramolecular charge (ICT) transfer took place from pyrrolidine nitrogen to α, β unsaturated carbonyl in the ground state.This study depicts that with increasing polarity of solvents, absorption spectra of the compound MPPP undergoes minor changes and in both position and fluorescence quantum yields ,a distinct bathochromic shift is experienced by fluorescence spectra. It increases and reaches a maximum before it decreases with increasing solvent effects. Using Austin Model 1 (AM1) magnitude of change in the dipole moment was calculated.This study provided evidence about the intramolecular charge transfer in the emitting singlet state of this compound. This study also suggested solvent dependence of quantum yields of MPPP and it was interpreted on the basis of positive and negative solvatokinetic as well as hydrogen bonding effects. By participation of hydrogen bonds with guanine and cytosine, chalcone derivatives act as intercalating agent is suggested by computational docking studies study and validated by fluorescence spectroscopy on CT-DNA-chalcone adduc.

Keywords: Molecular docking, ct DNA; Fluorescence spectroscopy; Absorption spectroscopy.

INVITED SPEAKERS

Id-089

Super-resolution Fluorescence Microscopy Imaging from a Chemometrician's Perspective

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Abstract: Super-resolution wide-field fluorescence microscopy can provide structural information at the nanoscale and dynamic insight about biological processes in live cell samples. In general, the available information in super-resolution images is related to the density of emitters, with more emitters leading to more information. One of the strategies for obtaining a high spatial resolution is based on the sequential imaging and localization of sparse subsets of blinking fluorophores distributed over thousands of frames. However, to obtain a high spatial resolution on short time sampling, and potentially probe dynamic processes in live cells, this principle must be extended to the analysis of high-density of emitters distributed over a few tens of frames. In such frames, many emitters are simultaneously active and their emissions strongly overlap. Since single-emitter fitting methods collapse when applied to high-density super-resolution data, the development of new methods and image processing algorithms remains a topical and challenging issue for dynamic imaging and faster super-resolution experiments. The cornerstone of high-density super-resolution single-molecule fluorescence imaging is data analysis and involves several steps ranging from pre-processing, e.g. image background handling, to post-processing, e.g. image rendering, clustering, etc. The core of our approach is the SParse Image DEconvolution and Reconstruction (SPIDER) algorithm. This approach tackles the image deconvolution problem in a penalized regression framework with multiple penalties (the L_0-norm provides spatial sparsity, while a penalty on successive frames ensures the continuity of the emitters in the time mode). Additionally, the high density of the active fluorophores often results in the presence of significant auto-fluorescence (endogenous fluorescence) of the cells and strong bleaching of the fluorophores, masking the blinking of the exogenous fluorophores. In this talk, we present the processing and analysis approaches developed. We combine spatial and temporal pre-processing to eliminate the non-specific fluorescence from the data, with as a result a clearer image and a better resolution. We illustrate the result obtained on different data obtained by fluorescence microscopy imaging, including protein-labeled mitochondria in a HEK293-T cell. On this example we demonstrate a resolution of 50 nm over a time sampling as short as 0.5 s. On overall, we show that we can work with higher densities of active emitters, for faster imaging, allowing the investigation of highly dynamic structural and morphological changes that membrane organelles undergo to accommodate the continuous processes occurring in live cells.

Keywords: Chemometrics; Super-resolution; High-density.

INVITED SPEAKERS

Id-091

Saturated Absorption by Cavity Enhanced Absorption Spectroscopy, CRDS and Beyond

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Abstract: Cavity Ring Down spectroscopy is an attractive technique for several reasons, i) it simple to implement, ii) it takes advantage of the long effective absorption length, providing high sensitivity, iii) it is self calibrated, and iv) it is insensitive to the intensity fluctuation of the source. It can be implemented by using pulsed, or continuous mode sources. In addition, the analysis of the temporal response carrying the absorption losses, is deemed to be simple. If the medium under study responds linearly, the conversion of the ring-down time to the absorption coefficient is trivial. However, because cavity with a high-finesse can be reached, it may be relatively easy to reach nonlinear absorption regimes, because the intensity of the electromagnetic field (EMF) trapped inside the cavity is proportional to the cavity finesse. Saturated absorption has been demonstrated at the end of the 90s but it is still barely exploited. In the strong saturation regime, during the decay time of the cavity EMF, the medium can exhibits very low absorption at the very beginning of the decay (i.e., when the EMF is strong), and a linear absorption at the end of the decay (i.e., when the EMF is weak). In the regime of intermediate saturation, the analysis of the nonexponential decay gives access simultaneously to the number density, and to the absorption crossection. This is a great advantage compared with the linear regime. In addition, the scan of the Doppler-broadened transition can reveal narrow Lamb-dip featuring the nonlinear absorption at the line center. This saturated absorption technique is a way to reach metrology accuracy, typically when the laser source is coupled against an Optical Frequency Comb. However, the nonlinear regime requires a fine control on the intracavity power density which may be not trivial. An alternative, or complementary CEAS technique can be investigated to accurately control the trapped density of photons. The so-called NICE-OHMS (Noise-Immune Cavity-Enhanced Optical Heterodyne Molecular Spectroscopy) technique, based on a radio-frequency phase modulation, has been first setup at NIST in the late 90s by J. Hall. It has been demonstrated that it can challenge the photon-shot-noise. NICE-OHMS has a high potential in gas metrology because even relatively weak transitions can be saturated. However, under low pressure conditions, transit-time broadening play a major role since the molecules are not submitted to the any collisional relaxation. In addition, in the Infrared range, there is a lack of spontaneous radiative emission. Hence, the saturated absorption can be an ideal technique to probe the relaxation processes under low pressure conditions, and to challenge the collisional mechanisms like velocity changing collision (VCC). Nevertheless, the regime of the saturated absorption, when the transit-time becomes a major parameter, accurate modeling and simulations are required. Such a model has been developed to detect both, the usual absorption (like in CRDS), and the in-phase and in-quadrature modulation.

Keywords: Saturated Absorption; CRDS; FMS; CEAS; NICE-OHMS.

INVITED SPEAKERS

Id-092

Muscle Fiber Types Identification by Synchrotron Fluorescence Microspectroscopy

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Abstract: The skeletal muscle consists of three to four pure types of muscle cells (also called muscle fibers) identified as type I, type IIA, type IIX and/or type IIB in different proportions depending on the muscle function. They differ in their contraction speeds and metabolic pathways. Type I fibers are slow-twitch while type II fibers are fast twitch. The energy required to maintain cell homeostasis and muscle contraction is provided by the hydrolysis of ATP. The fibers IIX and IIB regenerate ATP by anaerobic glycolysis with lactate production. Fibers I and IIA favor cellular respiration (glycolysis + Krebs cycle). The latter are rich in mitochondria, where the cell respiration take place, and in myoglobin which carries oxygen to mitochondria. The intracellular composition of fibers therefore depends on their metabolic and contractile characteristics. The objective of our work was to study the impact of these slight differences in composition on the optical properties of muscle cells. Our hypothesis was, in part, based on the autofluorescence detection of NADH which is more concentrated in the mitochondria and thus in the oxidative metabolism fibers. Therefore, we studied the impact of cell type on the fluorescent response following excitation in deep UV. Rat soleus muscle consisting of I and IIA fibers and extensor digitorum longus (EDL) muscle consisting of I, IIA, IIX and IIB fibers were used as models. On each muscle, fibers, previously identified on their cell types by immunohistofluorescence, were analyzed by synchrotron fluorescence microspectroscopy on stain-free serial muscle cross sections. Muscle fibers excited at 275 nm showed differences in fluorescence emission intensity among fiber types at 302 (assigned to tyrosine fluorescence), 325, 346 (both assigned to tryptophan fluorescence) and 410 nm (assigned to NADH fluorescence). The 410/325 ratio decreased significantly with contractile and metabolic features in EDL muscle, ranked I>IIA>IIX>IIB fibers ($p<0.01$). Compared to type I fibers, the 346/302 ratio of IIA fibers decreased significantly in both EDL and soleus muscles ($p < 0.01$). In a subsequent experiment, we acquired autofluorescence images for fast fiber types discrimination on label free histological sections. Computer processing of the images allowed us to improve the contrast and identify the metabolic types of fibers with a fairly good reliability. These studies highlight the usefulness of autofluorescence signals to characterize histological cross section of muscle fibers with no staining chemicals.

Keywords: Fluorescence microspectroscopy; Skeletal muscle fiber type; Synchrotron radiation; Histology.

INVITED SPEAKERS

Id-096

Application of Infrared Spectroscopy and Chemometric for Determination of Alkaloid in Medicinal Plant Extract

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Abstract: Infrared Spectroscopy combined with chemometric has been developed for simple determination of alkaloid in the extract of medicinal plant leaves. Alkaloid was extracted from medicinal plant leaves by ultrasonication and maceration. NIR and FTIR spectras of selected medicinal plant extract were correlated with alkaloid content using chemometric. The chemometric method that used for calibration analysis were Partial Least Square (PLS), Principal Component Regression (PCR), dan Support Vector Machines Regression (SVMR) and for classification analysis were Linear Discriminant Analysis (LDA), Soft Independent Modelling of Class Analogies (SIMCA), and Support Vector Machines Classification (SVMC). The results shown that the PLS of NIR model give the best calibration model with the R2 and RMSEC value were 0,9941739 and 1,8891197 respectively. In addition, the R2 of leave one out cross validation (LOOCV) and 2-Fold-Cross-Validation of the model were 0,9885431 and 0,9957413, respectively. The LDA of NIR model shown good results of classification analysis with 100% accuracy. The PLS and LDA of NIR models were then applicated into real samples. The significance and t value of total alkaloid content measured by NIR and UV-Vis spectroscopy were evaluated with paired samples T-test. In conclusion, total phenol content measured with both methods gave no significant difference.

Keywords: Alkaloid; Chemometric; Medicinal plant; Infrared spectroscopy.

INVITED SPEAKERS

Id-098

Observation of Electrical Potential Distribution by Active Voltage Contrast Imaging Using Helium Ion Microscopy

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Abstract: Demand is growing for measurement techniques for observing electrical potential distributions with high spatial resolution as the size of devices continues to decrease. So far, the electrical potential distribution of semiconductors has been measured by using scanning electron microscopy (SEM) and kelvin probe force microscopy (KPFM). Helium ion microscopy (HIM), developed by Ward et al., is a imaging technique. The HIM has higher spatial resolution (i.e., nanometer) than SEM and KPFM. A lot of researches using HIM have been done. In this presentation, I will introduce the new electrical potential observation technique by active voltage contrast (AVC) imaging using HIM. The specimens were inorganic solid multilayer ceramic capacitors (MLCCs) with Ni internal electrodes and $BaTiO_3$ dielectric between the internal electrodes. MLCCs were cut, and the cross-sectional surfaces were mechanically polished. The cross-sectional surface of the MLCCs with and without voltages applied to the internal electrodes was observed using HIM. Voltages were applied by using a compact rechargeable all-solid-state battery and an in situ voltage-application system. The former method enabled the sample to be rotated, and the latter method enabled the applied voltage to be changed. When no voltage was applied, the SE intensity of the internal electrode region was stronger than that of the dielectric region (Material contrast). The electrical potential difference between the grounded and the positively biased (positive voltage applied) internal electrodes affected the contrast in the SE images (voltage contrast) when voltages were applied. The grayscale value of the positively biased internal electrodes decreased as a result of applying the voltage. The electrical field between the Everhart–Thornley (ET) detector and the sample surface at the positively biased internal electrodes weakened because the electrical potential of the positively biased internal electrodes became high. This means the efficiency with which an SE reached the ET detector from the positively internal electrodes decreased. The grayscale value decreased linearly from the grounded to the positively biased internal electrode when the applied voltage was less than or equal to 1 V. We found that the AVC corresponding to the electrical potential in the SE images can be observed when the applied voltage is less than or equal to 1 V. In the future, nanometer-scale electrical potential distributions of several devices (solar cells and Li-ion rechargeable batteries, etc.) will be observed with this technique. This work was supported by the MEXT Program for Development of Environmental Technology using Nanotechnology and the Japan Society for the Promotion of Science (KAKENHI; 16K14104).

Keywords: Helium ion microscopy; Active voltage contrast; Electrical potential distribution.

INVITED SPEAKERS

Id-100

Cytochemical Identification of Programmed Cell Death in Plants

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Abstract: Programmed cell death (PCD) is a genetically controlled process which originates during development and under stress conditions in all living organisms. According to the last biochemical and molecular studies PCD has been classified in three main groups: Apoptosis, autophagy and necrosis. Considering the ancestral form of PCD, plants are expected to have evolved their own pathways to manage the plant specific characteristics such as the presence of cell walls that interfering apoptotic bodies with being phagocytosed by neighboring cells. Besides, molecular evidences suggest that plants are lack of homologs of animal apoptosis-related genes. Plants share some characteristic features with animals during cell death process such as cell and chromatin condensation, DNA fragmentation and proteolytic enzyme activities. During programmed cell death the cellular alterations can be monitored by microscopic analyses. The dying and living cells can be monitored by acridine orange and ethidium bromide staining. Acridine orange is also used to trap the acidic vesicular organelles in autophagic cell death. The pycnotic nucleus can be characterized both chromogenic and fluorescence staining such as hematoxylin, toluidine blue and DAPI. Moreover TUNEL reaction as a sensitive method for detecting DNA fragmentation by labeling the 3′- hydroxyl termini in the nucleus is frequently used. Vacuolization and breakdown of cellular contents are best described by electron microscopy. Ultrastructural results reveal ER dilatation, organelle disruption, cell membrane, nuclear membrane and tonoplast rupture apparently. According to used microscopic techniques containing cytochemistry programmed cell death can be identified and classified in plant cells.

Keywords: Cytocemistry; Nucleus; Plant, Programmed cell death.

INVITED SPEAKERS

Id-101

Photodissociation of Small Molecules in Solution Probed by Time-resolved Vibrational Spectroscopy

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Abstract: Photodissociation dynamics of CF_2ICF_2I and CF_2I_2 in solution was investigated by probing the C–F stretching mode using femtosecond infrared spectroscopy after excitation with a UV photon. Dissociation of the first I atom was faster than 0.3 ps after the UV absorption. All of the resulting haloethyl radicals undergo the secondary dissociation with two time constants; the radical from the anti-CF_2ICF_2I in CCl_4 or in acetonitrile solution dissociates with time constant of 180 ps or 45 ps, respectively. The radical from gauche-CF_2ICF_2I in both solutions dissociates with the same time constant of 4 ns or 3 ns, respectively. Photodissociation of CF_2I_2 produces CF_2I and CF_2 radicals. The CF_2I radical undergoes further secondary dissociation, producing CF_2 radical, too. Two CF_2 radical eventually combines to be CF_2CF_2. Detailed photodissociation dynamics of CF_2I_2 will be presented. A photodissociation mechanism that explains the conformer-dependent dissociation rate as well as solvent-dependent dissociation rate will also be presented based on a potential energy surface of CF_2ICF_2 radical calculated CASSCF.

Keywords: Photodissociation; IR spectroscopy.

INVITED SPEAKERS

Id-103

Multiphoton Microscopy and Photoacoustic Microscopy in our Tissue Engineering Study

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Abstract: Reconstruction of tissues or organs remains a major clinical challenge during tissue engineering, which usually due to unsatisfactory angiogenesis in scaffolds and neo-tissues. Angiogenesis in tissue-engineering scaffolds is a prerequisite for supplying oxygen and nutrients to the cells, removing waste products, and ultimately survival and integration of implanted scaffolds with original tissue. In out lab, we have investigated angiogenesis in 3D scaffolds by in vivo multiphoton microscopy (MPM) and photoacoustic microscopy (PAM) during the bone formation process in a murine calvarial critical bone defect model. The scaffolds used in experiments were genetically modified by preloaded bioactive lentiviral vectors containing platelet-derived growth factor-b (pdgfb) gene. Continuously released bioactive lentiviral vectors (LV-pdgfb) from the scaffolds would transfect adjacent cells and express PDGFB protein, to facilitate angiogenesis and enhancing bone regeneration. Both MPM and PAM are novel imaging modality that can acquire volumetric data in a non-invasive manner. Theoretically, both methods can visualize vasculature without contrast agents administrated in advance, but depend on the primary protein in red blood cells, hemoglobin, to excite autofluorescence signal or acoustic signal which could be used to image blood vessels. However, contrast agent-free imaging by these microscopic methods is only suitable for vasculature imaging in superficial tissues. In most cases, necessary contrast agents are important to get higher SNR images. In our studies, we firstly used MPM and PAM methods for investigating neovascularization in 3D printed bone scaffolds. In current stage of these methods, we can use them to evaluate and determine if a certain scaffold and its materials are beneficial to neovascularization in tissue engineering. In future, these methods would be satisfactorily used in clinical non-invasive imaging.

Keywords: Tissue engineering; Angiogenesis; Multiphoton microscopy; Photoacoustic microscopy; 3D printing.

INVITED SPEAKERS

Id-108

Confocal Surface Plasmon Microscopy for Surface Plasmon Wave Vector Measurement

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Abstract: In this talk, we will discuss how a confocal microscope can be modified for localized measurement of surface plasmons (SPs) wave to measure the complex wave vector (complex k-vector) of the surface wave. The SP wave is a guided surface wave along the surface of a noble metal, such as, gold (Au), silver (Ag) and copper (Cu). The SP resonance phenomena have been a prime standard for protein binding kinetic measurements since the SP wave is sensitive to a change in refractive index of the region within 200nm from the metal surface. The SP wave is a leaky wave, which its k-vector is a complex number: $k_{sp}=k_{sp}'+k_{sp}''i$. It has been very well established that the sensitivity of SPR sensor depends on the accuracy of the position of the SPR dip or k_{sp}' and the imaginary part k_{sp}'' plays a crucial role in imaging performance. Here, we discuss a development of our modified confocal microscope that operates as an embedded interferometer. This allows to accurately measure and quantify the real part k_{sp}' and the imaginary part k_{sp}'' of the SPR wave k-vector. We will also discuss the current advancement in our optical design, where we have two spatial light modulators (SLM) integrated in conjugate planes of the back focal plane and the image plane of the microscope objective lens. This enables us to capture an SPR image through a time-coded widefield confocal microscope.

Keywords: Confocal Microscopy; Surface Plasmons; Surface Plasmon Microscopy.

INVITED SPEAKERS

Id-109

Positron Annihilation Lifetime Spectroscopy in Nanomaterials Engineering and Science: From Phenomenological-mathematical Algorithms to Practical Implementation

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Abstract: Recent progress in the engineering of modern nanomaterials developed for attractive practical application in different fields of nanobiotechnology relies, to a great extent, on characterization possibilities of experimental probes sensitive to atomic and atomic-deficient structural arrangement at the levels ranging deeply beyond subnanometer (angstrom) length-scales. One of such probes is grounded on space-time continuum determination for electron interaction with its antiparticle (the positron), i.e. physical phenomenon of positron-electron annihilation in lifetime measuring mode. The positron annihilation lifetime (PAL) spectroscopy is high informative indirect characterization tool to study atomistic imperfections like extended free-volume defects (vacancies, vacancy-like clusters, voids and void agglomerates, pores, micro- and macroscopic cracks, etc.) in solids whatever their structural organization (crystals or glasses, fine- or coarse-grained powders, ceramics or bulk alloys, etc.). The PAL spectrum consisting of histogram of measured annihilation times of individual positrons after successful deconvolution with instrumental resolution of the detecting system and fitting with a theoretical model governed the corresponding regularization strategies in data parameterization, carries a rich information on atomic- and molecular-scale positron and Ps trapping free-volume imperfections in substances. Typically such imperfections evolve sub-nanometer low electron density spaces stretching far below character sizes a few interatomic bonds, a level which is far beyond measuring possibilities of direct probes, such as intrusion porosimetry and optical/electron microscopy. In application to nanostructurized materials, the possibilities of PAL spectroscopy treated within x3-term fitting-mathematical route are critically considered to parameterize the underlying free-volume sub-atomistic void-evolution processes. Unlike conventional x3-term analysis based on admixed positron trapping and positronium (Ps, bound positron-electron state) decaying, the nanostructurization is considered as occurring due to conversion from preferential Ps decaying in a *host* matrix to positron trapping in modified *host-guest* matrix. The developed approach referred to as x3-x2-CDA (i.e. coupling decomposition algorithm) allows estimation defect-free bulk and defect-specific positron lifetimes of different free-volume (atomic-deficient) elements responsible for nanostructurization.

Keywords: Positron Annihilation Lifetime Spectroscopy.

INVITED SPEAKERS

Id-110

How does the Force Spectroscopy Shine Light on Heparin-induced Thrombocytopenia?

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Abstract: Heparin-induced thrombocytopenia (HIT) is a severe side effect of adverse drug reaction when patients receive heparin (H) as an anticoagulant. Due to charge differences, heparin can easily form ultra large antigenic complexes with small protein platelet factor 4 (PF4). The human immune system creates antibodies against these PF4/H antigens, forming multiple complexes which then bridge and activate platelets via FcɣRIIa receptors. The extreme manifestation of the immune response is life-threatening autoimmune HIT. Although HIT is widely investigated, little is known about the detailed binding characteristics of these multimolecular interactions. By using single-molecule force spectroscopy (SMFS) and some other biophysical techniques, we found the boundary between antigenic and non-antigenic heparins between 8- and 12-mers, and introduced a model to differentiate short from long heparins. Even though short heparins have been applying to patients, HIT still occurs. We recently found an additional factor involving in HIT, i.e., a subset of HIT antibodies can self-cluster multiple PF4 molecules in the same way long heparins do. The resulting ultra large PF4/antibody complexes allow binding of other antibodies in blood flow. As a result, these multiple complexes bridge and activate platelets even faster and stronger than long heparins do. Our results revealed that SMFS allow answering some important questions in medicine and findings of the new binding mechanism of a subset of HIT antibodies may be generalizable to other diseases. To reduce HIT, we should not only focus on the development of ultra-short heparins but also find the way to inhibit these HIT antibodies. We could also identify a buffer condition that allows differentiating platelet activating HIT antibodies from non-platelet activating ones, and found that depending on the binding substrate, the PF4/H antigen exposes different epitopes for antibody binding. Finally, we will show our new set-up that allows for the first time to measure the interaction between single platelets by SMFS.

Keywords: Force spectroscopy.

INVITED SPEAKERS

Id-111

Four-dimensional Speckle-interferometric Microscopy of Thin Biological Objects

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Abstract: At present to study phenomena occurring in the cells of living systems, a variety of techniques including interference microscopy was successfully used. In particular, the methods and devices to determine the distribution of the refractive index n(x,y,z) within cells was developed. The value of the refractive index averaged in a certain region of space and time required for carrying out sensing of the object. However, the logic of optics and the needs of practice aim to determine not only average values but also deviations from the average value and the relaxation time τ (velocity of change) of refractive index. In the report, the four-dimensionality considered in the analysis of the n dependence not only on the spatial coordinates but also in time. The bases for this analysis are the results obtained by the author earlier. The report briefly discusses the theory, experimental and metrological justification speckle interference technique to study processes in thin biological object. Some of its capabilities for practical use discuss. It is shown that the method establishes communication between a) the average value, dispersion, relaxation time difference of the refractive index in a small region of the object and b) the average value of the radiation intensity in speckle picture of this region, time and coefficient of correlation of this intensity. The disadvantage of this method is the averaging of the data on the thickness of the object and main purpose of the report is a theoretical justification of the ability to determine the distribution referred to in paragraph a) values. The model of transparent (phase) thin object located near the fixed transparent diffuser is considered. The path of the probing wave is divided into M parts. It is assumed that the parameters characterizing the change of the refractive index in each part are 1) independent and (or) 2) uncorrelated random variables. Based on these assumptions, the author obtains expressions for the time average of the radiation intensity in a certain point of the image plane of the object and the temporal autocorrelation function of this intensity. These expressions are transcendental functions of the parameters. It is shown that if the difference of optical paths of pairs of waves is small compared to the wavelength, then the resulting equations are linear functions of the unknown values. In this case, the solution of the problem of four-dimensional imaging is greatly simplified. If the difference of optical paths of pairs of waves is not small compared to wavelength, it is necessary to solve a system of transcendental equations. A simple experiment set for testing the considered approach is discussed. On two-layer physical model of the cells changes in the standard deviation of the difference of the refractive index at a distance of 2 μm in the cytoplasm and in the nucleus is studied. Unsolved issues that require further study is discussed.

Keywords: Speckle-interferometric Microscopy; Thin biological objects.

INVITED SPEAKERS

Id-117

Study on Quenching and Binding of Rhodamine B – Pluronic F-127 Complex in Presence of Salts and Gold Nanoparticles Using Optical Spectroscopy Methods for Exploring Their Development as Sensors

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Abstract: The fluorophore Rhodamine with xanthene scaffold has received considerable attention because of its rich photo physical properties viz. high fluorescence quantum yield, high absorption coefficient and high photo stability. Also, it is nontoxic and has good water solubility. Additionally it is capable of functioning at physiological pH. Hence there has been recent attempts to introduce mechanisms to modify the sites on the Rhodamine B dye scaffold, through either quenching or fluorescence enhancement, with a view to enabling it to be utilized as a sensor. Objective of the present study is to evaluate the quenching effect of a combination of factors viz. surfactant (Pluronic F-127) and salt; and surfactant and gold nanoparticles on Rhodamine B dye (flurophore) for exploring their development as sensors. The quenching effect of different salts on Rhodamin B, both in the presence and in the absence of pluronic F127 surfactant, commonly used for enhancing solubility of multiple drugs, was studied. Among the salts two series of cations viz,. (a)$CdSO_4$, $MnSO_4$, $ZnSO_4$, $FeSO_4$, and $CuSO_4$ and (b) $SrCl_2$, $CaCl_2$, $ZnCl_2$, $CuCl_2$and $CoCl_2$; and two series of anions viz. (a) KCl, KBr, KI and KNO_3 and (b) NaCl, NaOAc, NaOH and NaI were used. Additionally, in another set of studies, gold nanoparticles were used in place of salt in the above mentioned manner. The characterization before and after quenching were carried out by using UV-Visible Spectro photometry, Dynamic Light Scattering, Fluorescence studies, Lifetime measurements and SEM images. Among the cationic salts Cu^{2+} and out of the the anionic salts I- were found to be the best quenchers. It was also observed that the probe and the quencher were bound more strongly in presence of Pluronic F127 surfactant. In the studies with the gold nanoparticles it was observed that there is strong binding ability between the probe and gold nanohybrid in the cavity. There is static nature of quenching operating in all the cases and there is better energy transfer efficiency of fluorescent dye in presence of pluronic than in absence of it. The significance of the present study lies in the suggestion that the formation of stable Pluronic F127 - salts and Pluronic F127 - gold nanohybrid complexes with strong binding to the Rhodamine B fluorescent dye molecules has introduced a platform for further exploration of their possible application in sensing and pharmacology.

Keywords: Rhodamine B; Pluronic F127; Nanoparticles; Optical spectroscopy; Quenchers.

INVITED SPEAKERS

Id-119

Precise Cavity Ring-down Spectroscopy Referenced to Atomic Frequency Standards

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Abstract: Cavity ring-down spectroscopy (CRDS), which relies on the determination of the photons lifetime inside the optical cavity, is one of the most accurate spectroscopic techniques. This well-established method is widely used for the measurements of very weak spectral lines as well as trace gas detection and was considered ideal to provide reference data for the satellite based monitoring systems. We present molecular spectroscopy with spectra frequency axis referenced via the optical frequency comb (OFC) to the atomic frequency standard: either an ^{88}Sr optical atomic clock or the hydrogen maser implementing UTC(AOS) (Coordinated Universal Time from the Astro-Geodynamic Observatory in Borowiec, Poland) reference. High accuracy and precision line-shape study of weak transitions of the oxygen B band occurring near 689 nm, which are particularly important in atmospheric research, is presented. We used the OFC-linked, Pound-Drever-Hall locked, frequency stabilized cavity ring-down spectrometer (PDH-locked FS-CRDS). We demonstrate the first experiment in which the optical atomic clock is used as a frequency reference in molecular spectroscopy. With Doppler-limited spectroscopy we achieved the relative standard uncertainty of 2×10^{-11} on O_2 line position, typical for Doppler-free techniques. We also present the comparison of CRDS with two other sensitive cavity-enhanced spectroscopic techniques. First method is complementary to CRDS cavity mode-width spectroscopy (CMWS) based on the measurement of the width of cavity resonance to determine the sample absorption coefficient. The other one is the cavity mode-dispersion spectroscopy (CMDS) based on the measurement of dispersive shift of the cavity resonances. It is based solely on the measurement of the frequency, i.e. the physical quantity currently measured the most accurately, and has the potential to become the most accurate of all spectroscopic methods. Contrary to any absorption method CMDS is the first spectroscopic technique that is one-dimensional. This method is insensitive to systematic instrumental errors of the detection system, which cannot easily be eliminated in the case of absorption measurements. We present absolute frequencies of unperturbed $^{12}C^{16}O$ transitions from the near-infrared (3-0) band measured with accuracy at the level of 10^{-10}. We used a unique experimental setup enabling the simultaneous measurement of the spectra by these three cavity-enhanced techniques. The frequency axis of spectra was linked to the primary frequency standard – UTC(AOS) having long-term relative stability of 10^{-15}.

Keywords: Cavity ring-down spectroscopy; Atomic frequency standards; Line shapes.

INVITED SPEAKERS

Id-122

Tunable Thin-film Structures of Lipophilic Nucleoside Derivatives

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Abstract: Due to their specific binding and molecular recognition properties, DNA nucleosides are very appealing as building blocks for construction of the lab-on-a-chip systems. Active surface films required in such systems can be conveniently fabricated by the Langmuir-Blodgett (LB) technique. We investigate molecular assembly of lipophilic nucleoside derivatives in Langmuir monolayers at the air-water interface and in the LB films deposited from the air-water interface onto various solid substrates. The emphasis is on exploration of different mechanisms that can provide regulation of the surface assembly and consequently enable construction of surface structures with predetermined properties. The investigations are based on measurements of surface pressure versus area isotherms, Brewster angle microscopy (BAM), spectroscopic ellipsometry and atomic force microscopy (AFM). Our observations reveal that films from guanosine derivatives exhibit very distinctive intermolecular organization with respect to the films from analogous derivatives containing other nucleobases. The number of lipophilic chains attached to the sugar hydroxyl group affects the assembly much stronger than the length of the chains. Very important factors in surface organization are the type and concentration of cations and anions present in the water subphase. We demonstrate that modifications of intermolecular organization can be generated also by optical irradiation. For this purpose we use azo-functionalised guanosine derivatives in which the *trans-cis* isomerisation can be induced by irradiation with UV light. We examine selective binding of these derivatives to derivatives with other nucleobases. This is a starting step in development of thin film configurations whose specific binding to selected target molecules can be regulated by optical addressing. This work was supported by Slovenian research program P1-0192.

Keywords: DNA oligonucleosides; LB films; Structural regulation; Light-sensitive materials.

INVITED SPEAKERS

Id-125

Quantitative Nanoscale Magnetic Imaging for Fe/Ti Containing Oxides: Lorentz Microscopy and Other Related Techniques

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Abstract: Transmission electron microscopy (TEM) is a microscopy technique whereby the electron beam that has high-energy electrons is transmitted through a thin specimen, interacting with the specimen as it passes through. Therefore TEM enables us to study internal microstructures at high spatial resolution and is used in various research fields. Beside conventional TEM techniques such as structural and compositional analyses, TEM also allows magnetic and electrostatic properties in specimens to be imaged at the nanometer scale. One of the most commonly used magnetic imaging techniques in TEM mode is “Lorentz microscopy”, which utilizes the phenomenon that electrons are deflected by magnetic force during they travel through a magnetic thin film. Although there are several modes of Lorentz microscopy, the most well-known method is “Fresnel” imaging. The Fresnel mode visualizes the positions of magnetic domain walls by defocusing the image largely (typically at defocus values of µm to mm). The advantage of the Fresnel mode is to provide the opportunity relatively easily to perform *in-situ* magnetization reversal experiments by the application of external magnetic fields. In scanning TEM Lorentz imaging, differential phase contrast (DPC) imaging is often used. The local Lorentz deflection off the centered probe (in diffraction mode) is detected by a segmented detector and is used to image magnetic domains at sub-10 nm resolution. Another known magnetic imaging technique (which is not Lorentz microscopy) is off-axis electron holography, which allows magnetic and electric nanoscale fields in materials to be imaged quantitatively. Fe/Ti containing oxides have been used in various magnetic applications (e.g. ferrite) and are good magnetic field recorders in Earth and other planets. Magnetite (Fe_3O_4) is found in some biological systems such as magnetotactic bacteria and birds. Here we use Lorentz microscopy and the other magnetic imaging techniques mentioned above to understand fundamental magnetic properties of Fe/Ti oxides and show how these imaging techniques work complementarily. Lorentz microscopy of an interface magnetism at antiferromagnetic hematite (Fe_2O_3) and paramagnetic ilmenite ($FeTiO_3$) interfaces. Asymmetrical Lorentz image contrast observed at their interfaces was simulated to reveal the presence of the significant magnetization. Lorentz microscopy and electron holography of ferrimagnetic/ferroelastic domain interactions in low-temperature magnetite below 120 K. In-situ magnetization reversal Lorentz microscopy was used to study the motion of an unusual magnetic domain wall, which was pinned at a ferroelastic twin wall.

Keywords: TEM; Magnetic imaging; Oxides.

INVITED SPEAKERS

Id-126

Digital Holographic Microscopy for Analyzing 3D Particle Dynamics of Non-spherical Particles

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Abstract: A particle subjected to a shear flow exhibits various dynamic behaviors. Among them, the dynamic motions of non-spherical particles suspended in microscale flows are essential phenomena in both the scientific and engineering fields, because most cells and particles in nature possess a nonspherical shape. These dynamic motions can be accurately measured by using three-dimensional (3D) quantitative digital holographic microscopy (DHM) technique. DHM is employed to measure the 3D position and orientation information of transparent ellipsoidal particles. Information about in-plane and out-plane positions and orientations is obtained by analyzing the distinctive light scattering of micro-sized ellipsoidal particles. The performance of the measurement method is experimentally verified for ellipsoidal particles seeded in a planar surface and a microtube. In addition, 3D rotational behavior of ellipsoidal particles in a circular tube is investigated to show its potential. This DHM method can be used to extract the essential information of ellipsoids, and therefore it has great potential in particle dynamic analysis.

Keywords: Digital holographic microscopy; Particle dynamics.

INVITED SPEAKERS

Id-128

Anisotropic Exchange and Effective Crystal Field Parameters for Low Dimensional Systems from EPR Data

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Abstract: Low dimensional magnets are fascinating testing grounds for the fundamental understanding of quantum physics. A particularly rich example of a frustrated low dimensional quantum antiferromagnet are the spin-1/2 system $LiCu_2O_2$ and Cs_2CuCl_4. Electron paramagnetic resonance (EPR) signal of this system consists of a single exchange-narrowed resonance line with linewidth near 1000 Oe. EPR is a convenient method to probe anisotropic spin-spin interactions. It has been shown that in the case of sufficiently strong isotropic exchange interaction the EPR spectrum is narrowed into a single Lorentz line. The EPR linewidth displays the average amplitude of the fluctuating field on the magnetic ion, which can be directly related to the parameters of the anisotropic spin-spin interactions. In the general case the anisotropic Hamiltonian contains the anisotropic exchange interactions, dipole-dipole interaction, and anisotropic Zeeman term and crystal fields for S>1/2. We calculated theoretically the second and fourth moments of the EPR line for a given spin Hamiltonian. Here we focus mainly on the problem how to extract the spin-Hamiltonian parameters from the angular dependencies of the EPR resonance fields and the linewidth jn three compounds $LiCu_2O_2$, $Cs_2Cu_{1-x}Br_xCl_4$ and Mn_2BO_4 together with the analysis of structural data and with the values of the isotropic exchange interaction J derived from neutron scattering data or calculated from first principles. As it has been shown for other Cu based spin-chain compounds like $CuGeO_3$, $LiCuVO_4$ or $KCuF_3$ the anisotropic exchange interactions are usually dominant as compared to the dipole-dipole interaction. A similar situation is realized in Cs_2CuCl_4 as well. Following conventional estimations the contribution to the linewidth related to the dipole-dipole interaction is about 1 Oe only, because the distance between nearest neighbor Cu-ions is quite large 6.3Å. If there are two magnetically nonequivalent positions on neighboring chains in the unit cell, we additionally investigated the contribution to the linewidth due to the anisotropic Zeeman interaction. As a rule it is can be seen from the analysis of the structural data, where one finds that the unit cell contains two magnetically nonequivalent ligands surrounding the paramagnetic ions producing an EPR signal. Since the EPR spectrum exhibits only one line, its effective g-factor is the average of the g-factors of two magnetic centers located at the nonequivalent positions. If the EPR linewidth along one axis at a different frequency is greater than its value in the X-band, i.e. it depends on the field as H^2; this effect originates from the difference between the individual g-factors of interacting spins. This work was supported by the program of the Presidium of the Russian Academy of Sciences [Program number 5P].

Keywords: EPR; Anisotropic exchange interaction.

INVITED SPEAKERS

Id-129

Signatures of the Water-lipid and Lipid-lipid Interactions in Phospholipids Bilayers by Infrared Spectroscopy and Increasing Hydration

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Abstract: Fast thermal fluctuations and low frequency phonon modes are believed to play a part in the dynamic mechanisms of many important biological functions in cell membranes. Recently we have investigated the vibrational collective dynamics of lipid bilayers by MD simulations and showed that these molecules support several low energy optical-like phonon modes in a spectral region below 300 cm^{-1}. These optical vibrational modes are potentially involved in the vibrational energy transfer and in the hydrogen-bond dynamics at the membrane-water interfaces. Here we report the results of a detailed far-infrared study of the molecular subpicosecond motions of phospholipid bilayers at various hydrations. We show that these systems have a rich spectrum of low frequency collective modes and confirm the MD predictions. We deduce that these low frequency modes arise from vibrations of different lipids interacting through intermolecular van der Waals forces. Furthermore we observe that the low frequency vibrations of lipid membrane have strong similarities with the subpicosecond motions of liquid water and suggest that resonance mechanisms are key elements to the dynamics coupling between membranes and their hydration water.

Keywords: Phospholipid bilayer; Lipid membrane; FT-IR; Far-Infrared.

INVITED SPEAKERS

Id-130

Application of Infrared Spectroscopy in the Identification of Ewing Sarcoma

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Abstract: Ewing sarcoma (ES) is the second most common malignant bone tumor in children and adolescents. The final results of applied treatments stay still unsatisfactory. This is why introducing of new diagnostic tools like Fourier Transform Infrared (FTIR) spectroscopy into ES research is so important. The aim of our studies was to compare the spectra of ES bone samples and the spectra of normal bone tissues, analyzed before and after induction chemotherapy, by means of FTIR spectroscopy and to establish the possibility of FTIR spectra detection from ES bone samples without preceding deparaffinization. FTIR spectroscopy (range 1100 cm^{-1} - 3500 cm^{-1}) (Vertex 70 Bruker spectrometer) was used for the specimen analysis. In the FTIR spectra we observed a shift in the wave number of the phosphate ion (from 3 to 26 [cm^{-1}]) associated with the presence of tumor tissue. After chemotherapy, a change of the FTIR spectrum was associated with the ES's histopathological response. In patients with a high ratio of the necrotic cells in the tumor (>90% of cells) after chemotherapy, we showed a shift of the peak \absorption bands to the higher wave numbers. In contrast, in patients with a poor chemotherapy response (<30% of necrotic cells in the tumor), we observed a decline in the peak absorption bands to the lower wave numbers. The presence of wax does not affect the surface area, absorbance, half-width or peak position observed in the spectrum peaks in the every analyzed sample. However, in the case of peak at 1234 cm-1 in deparaffinized cancerous tissues the statistically significant differences in the value of absorbance, surface area and half-width (FWHM) compared to paraffin cancerous tissues were observed but without significant impact on the final interpretation. The analysis function of the dielectric imaginary $\mathcal{E}i$ (ω) confirm that the Lorentz function parameters for the individual peaks are the same so they do not depend from the sample preparation. The results showed that analysis of the spectrum changes of tissue specimens in ES can be helpful in the assessment of clinical response to cancer therapy. It seems that FTIR spectroscopy is a valuable tool for his purpose. The spectra obtained from paraffin and deparaffinized bone tissues samples do not differ significantly.

Keywords: Ewing sarcoma; Fourier transform infrared spectroscopy FTIR; Chemotherapy; Bone cancer.

INVITED SPEAKERS

Id-132

Mayan Inspired Nanocomposite Materials: An Overview

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Abstract: Maya Blue is a famous blue pigment, used by the ancient Mayas in Central America (especially in the Yucatàn peninsula, Mexico) from the V to the XVI Century AD, mostly for mural paintings and decorations on ceramics and statues. Once re-discovered in modern times (circa 1930), it quickly grabbed the interest of the Scientific Community due to its amazing stability: this pigment, in fact, is known to resist the attack of acids, alkalis and solvents, with no fading in colour nor loss of structural features. Around the turn of the millennium, it was acknowledged – following seminal studies – that Maya Blue forms after to a heating-induced encapsulation and bonding of indigo – an organic dye – into the channels that permeate the structure of palygorskite and sepiolite – microporous clay minerals. As a matter of fact, Maya Blue can therefore 'legally' be considered the ancestor of modern nanocomposite materials (though the Mayans hardly knew that!). The incorporation and shielding of the guest indigo dye in the host palygorskite framework de facto stabilizes the organic molecule – frail and decomposable when isolated – thus gifting the resulting hybrid nanocomposite with its renowned invulnerability. These achievements raised new aspirations: "Mayas made it Blue; what if someone else makes it … any other colour?" To do that, several dye molecules were ground and heated with palygorskite, so to check whether one of the ensuing compounds might have represented an innovative nanocomposite material, endowed by the very same stability of its renowned blue predecessor. Curiously, a stable red 'Mayan-inspired' hybrid composite was obtained by 'marrying' palygorskite with methyl red – an azo dye used as an indicator, whose colour changes after pH fluctuations (red at pH ≤ 4.4, yellow at pH ≥6.2 and orange in between). Once fixated on palygorskite, however, methyl red undergoes no colour change consequent to pH variation, and the resulting palygorskite@methyl red hybrid composite maintains a vivid and brilliant red-purplish hue in spite of severe acid or alkali attacks, with no appreciable fading. The fixation of methyl red inside the palygorskite framework and the nature of the mutual host/guest interactions were investigated with a multi-analytical approach, including both molecular mechanics simulations and experimental techniques (i.e., UV-visible, FT-IR, SER and FT-Raman spectroscopies, synchrotron XRPD and TGA coupled with GC-MS). As well as in Maya Blue, the palygorskite@methyl red composite forms after incorporation of the guest dye inside the tunnels that cross the host framework.

Keywords: Hybrid nanocomposite; Palygorskite; Methyl red; Pigment; Maya Blue.

INVITED SPEAKERS

Id-133

Electrical Characterization of Semiconductor Nanostructutes by Kelvin Probe Force Microscopy and Conductive Atomic Force Microscopy

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Abstract: Besides morphological characterization, atomic-force microscopy (AFM) based techniques can also successfully be employed to study electrical and optoelectronic properties on the nanometer scale via conductive atomic-force microscopy (C-AFM) and Kelvin Probe Force Microscopy. This will be demonstrated for dielectric thin films as well as for of individual semiconductor nanostructures like upright standing ZnO nanorods polycrystalline ZnO multilayer varistors, Ge nanodomes, radial junction Si solar cells, and nanostructured graphene flakes. In particular, we also demonstrate C-AFM and KPFM measurements under simultaneous illumination under white or monochromatic light which are called photoconductive AFM (PC-AFM) and photo-assisted KPFM (PA-KPFM). Work has been performed in collaboration with S. Kremmer, A. Andreev, I. Beinik, A. Nevosad, M. Mirkowska, M. Kratzer, K. Gradwohl, A. Matković, M. Hofstätter, P. Supancic (Leoben), Y. Kozyrev, S. Kondratenko (Kiev), M. Müller, A. Hývl, A. Vetushka, M. Ledinský, A. Fejfar (Prague), and B. Vasić, R. Gajić (Belgrade).

Keywords: Kelvin Probe Force Microscopy; Conductive AFM; Photoconductive AFM.

INVITED SPEAKERS

Id-135

Mapping of Localized Plasmons in Metallic Particles with Electron Energy Loss Spectroscopy and Cathodoluminescence

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Abstract: Localised surface-plasmon polaritons (LSP) are collective oscillation of free electron gas in metallic nanostructures strongly coupled to the near electric field, localised in deeply subwavelength region. They allow for extreme light concentration (tens of nm or less) and light manipulation below the free space diffraction limit. To study a spatial distribution of the LSP near field, imaging methods are available including electron beam microscopy (EBM) and spectroscopy, near-field optical microscopy, and far-field optical microscopy. Of those, electron beam spectroscopy offers the best spatial resolution below 1 nm. Two most common EBM techniques used for imaging of LSP are electron energy loss spectroscopy (EELS) and cathodoluminescence (CL). To measure EELS, scanning transmission electron microscope (STEM) equipped with a spectrometer is used. A focused beam of relativistic electrons (60 – 300 keV) is scanned over the sample in a form of a thin membrane to allow for the transmission of electrons. There is a probability (about 10^{-4} or less) that the transmitted electron excites a LSP resonance and decreases its energy correspondingly. From the spectra and spatial maps of the transmitted-electron counts it is possible to reconstruct the LSP near field. Spectral resolution of the technique is determined by spectral width of impinging electrons and for monochromatized electron beam it reads 0.1 eV. In CL, the excitation mechanism is similar to EELS but light emitted by decaying LSP is recorded. CL has better spectral resolution (0.01 eV) but can detect only optically active LSP modes while EELS is sensitive also to dark LSP modes. Both techniques therefore provide complementary information. We present EELS and CL mapping of various plasmonic structures. (i) We studied by EELS plasmonic modes in crescent-shaped gold nanoantennas that exhibit rich structure of LSP modes. Up to four distinct modes and a multimodal peak were resolved in individual antennas. With the help of numeric simulations, the near electric and magnetic field for each mode was restored. (ii) Next, we focus on the Babinet principle in plasmonics.

Keywords: Plasmonic antennas; Localised surface-plasmon polartion; Electron energy loss spectroscopy; Cathodoluminescence.

INVITED SPEAKERS

Id-143

In Situ Study on the Environmental Behaviors of Typical PAHs in Mangrove Plants by Fluorimetry and Microscopic Fluorimetry

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Abstract: A serious of in situ fluorimetry, laser-induced time-resolved nanosecond fluorescence spectroscopy (LITRF) and a microscopic fluorescence spectral analysis method were established in the lab. With these methods established, environmental behaviors of some typical polycyclic aromatic hydrocarbons (PAHs) in mangrove have been studied. A self-designed multipurpose fluorescence colorimetric cell was employed to establish a simple in situ method for the determination of phenanthrene (Phe) adsorbed on leaf surfaces of Kandelia obovata (Ko), Aegiceras corniculatum (Ac) and Avicennia marina (Am). The detection limit and RSD of the established method were superior to solid surface optical fiber fluorimetry while the linear range of these two methods were in the same order of magnitude. A dual-wavelength fiber-optic fluorimetry for in situ simultaneous determinations of fluorene (Flu), Phe and pyrene (Pyr) adsorbed onto the leaf surfaces of living Am seedling were developed and used to study the depuration kinetics of the three PAHs, adsorbed individually or mixed together, onto living Am leaf surfaces. The depuration kinetics of the three selected PAHs were divided into rapid and slow phases; both phases followed the same first-order kinetics with relative clearance rates of Flu > Phe > Pyr during the rapid phase, and a clearance rate order of Pyr > Flu > Phe during the slow phase. For the three PAHs coadsorbed on living Am leaf surfaces, a significant synergistic effect was detected during the rapid phase clearance; conversely, an antagonistic effect was observed during the slow phase. A novel method for in situ determination of the PAHs adsorbed onto the root surface of Ko seedlings was established using LITRF.

Keywords: In Situ; Environmental Behaviors; PAHs; Mangrove Plants; Fluorimetry.

INVITED SPEAKERS

Id-145

Athlete's Response to Exercise in Normobaric Hypoxia-serum Fluorescence Study

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Abstract: Exposure to reduced oxygen levels (altitude or hypoxia) is a challenge to the human body because oxygen is the primary source of energy for our cells. Stimulation of red blood cell production to compensate the lover amount of oxygen in people living in the high mountains is well known. On the other hand the hypoxia may be used as an integral component of modern athletic performance. In this work the usefulness of serum fluorescence spectroscopy to study the impact normobaric hypoxia exercise training is considered. The effect of normobaric hypoxia exercise training on fluorescence of blood serum has been evaluated in a group of 12 athletes (6 male and 6 female). The exercise test was carried out in: normoxic conditions - 0 m above the sea level (ASL), and simulated normobaric hypoxia – 3000 m, 4000 and 5000 m ASL. Blood samples were drawn at rest and 3 min, 1 h and 24 h after of passive recovery. Fluorescence study were carried out for water diluted 1:20 samples and undiluted serum. Data are presented as means ± SD. The level of statistical significance was set at $p < 0.05$, results with $p < 0.1$ were interpreted as tendencies. Excitation emission matrix were considered in two parts: the emission in the ultraviolet and visible regions which discloses the complex nature of human blood serum autofluorescence. The emission in the ultraviolet region may be identified as fluorescence resulting from aromatic amino acids emission included in blood serum proteins, mainly from tryptophan (Trp) and tyrosine (Tyr). The emission in the visible region may be attributed emissions from reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H), the Schiff base of pyridoxal phosphate (PLP), riboflavin (vitamin B2) and flavins. Emission observed in the ultraviolet region originated from Tyr and Trp showed higher level of fluorescence caused normobaric hypoxia than training. Changes in serum fluorescence power between 0 ASL and 3000 and 5000 were statistically significant. Decrease tendency of Tyr fluorescence due to exercise training was obtained unlike Trp emission. More marked changes in serum fluorescence associated with normobaric hypoxia conditions as well as training were obtained in visible range of excitation. Spectra in visible region showed two maxima at about 450 nm and 505 nm. These peaks can be attributed to fluorescence derived from (NAD(P)H) and flavins, respectively. The fluorescence intensity at about 450 nm is increased as with an increase in altitude.

Keywords: Serum; Fluorescence; Normobaric hypoxia; Athletes; Exercise training.

INVITED SPEAKERS

Id-147

An Array of Microcopy Techniques to Assess Recombinant Protein Production in Escherichia Coli Biofilms

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Abstract: Biofilms can be described as communities of microbial cells attached to a surface embedded in a self-produced matrix of extracellular polymeric substances. Microbial adhesion and subsequent biofilm development can be affected by several factors such as hydrodynamic conditions, nutrient requirements and surface properties. Biofilms are mostly known for their negative effects on human health and industrial productivity. However, they can be used in the production of industrial chemicals like ethanol, butanol and lactic acid, and in wastewater treatment. In the last years, the growth of the biotechnology industry led to improvements in recombinant processes. Using a strategy that combines the production of recombinant proteins with bacterial biofilms can be advantageous for different biotechnology industries. Microscopy has traditionally been used for biofilm studies and techniques such as confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), atomic force microscopy (AFM) and epifluorescence microscopy (FM) have provided valuable information to understand these microbial communities. In the current work, microscopic and image analysis techniques were used to evaluate the surface material and the physiology of Escherichia coli biofilms used for expression of a model recombinant protein (enhanced green fluorescent protein, eGFP). The topography of polyvinyl chloride (PVC) was characterized by AFM, which revealed that this material is smooth (average roughness of 32 nm). Regarding recombinant protein production, eGFP fluorescence (assayed by FM) showed that bulk production increased during the first 4 days of biofilm cultivation but decreased abruptly (about 61%) after that. The fraction of viable cells was determined by Live-Dead staining and FM showed that from day 4, the number of viable cells also decreased (by 45%), which seems to indicate that the reduction in the specific fluorescence intensity resulted from the loss of pre-existing eGFP molecules due to cell lysis. eGFP fluorescence and FM also enabled single-cell scale analysis of the biofilms, showing that protein expression is highly heterogeneous and that this heterogeneity increased with time. Observation of E. coli biofilms using CLSM confirmed a marked spatial heterogeneity of eGFP expression. The morphology of biofilm cells was further observed by SEM, which showed that eGFP-producing cells have filamentous forms when compared to non-producing cells. We conclude that combining different microscopy techniques can be very useful for monitoring protein expression in biofilm cells and may help to find the best processing conditions for recombinant protein production in this type of cells.

Keywords: Biofilms; CLSM; AFM; FM.

INVITED SPEAKERS

Id-148

Monitoring of Methamphetamine Induced Apoptosis of Live Central Nervous System Cell Cultures by Digital Holographic Phase Imaging and Raman Spectroscopy

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Abstract: Apoptosis inducing effects of Methamphetamine were examined in real time in live glial cells by digital holographic microscopy and Raman spectroscopy. While digital holographic microscopy measurements provide information about cellular volume changes, which indicate the alterations in cell cycle and initiation of cell death mechanisms, Raman offers complementary information about cellular chemistry, such as protein and nucleic acid content, and structural changes in DNA. Our work confirms that Methamphetamine induces cellular apoptosis, which produces apoptotic volume decrease. In this work, differences in dynamic chemical changes in Methamphetamine induced apoptosis versus the apoptosis induced by Doxorubicin were observed, which accompanied (and in some cases, preceded) apoptotic volume decrease.

Keywords: Live cell imaging; Digital Holographic Microscopy; Raman Spectrosscopy.

INVITED SPEAKERS

Id-149

Ultrasensitive SERS Detection in Catechin Solution

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Abstract: Traditional methods for the analysis of catechins, in tea beverages for example, are solvent-bound, time-consuming and insensitive. We report here a solvent-free, speedy and highly sensitive Raman method that allows detection at various concentrations. The utility of the method is demonstrated with an actual example of application. Using an argon laser for excitation, surface-enhanced Raman scattering was achieved with the aid of citrate-capped Ag nanoparticles. It successfully detected catechin at a concentration as low as 10^{-18} M. In application, green tea bags were soaked in hot water. Catechin molecules released were adsorbed on Ag surface and subjected to SERS measurements. This method proved to be practical, efficient, and highly sensitive for catechin analysis.

Keywords: Surface-enhanced Raman scattering; Catechins; Green tea brew; Ag nanoparticles.

INVITED SPEAKERS

Id-151

Thermal Lens Spectrometry and Microscopy: Recent Progress and Applications in Chemical Analysis and Biomedical Diagnostics

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Abstract: After over 30 years of intensive research and development, thermal lens spectrometry (TLS) has reached maturity and has already contributed to important scientific discoveries. Among these, one cannot overlook the confirmation of endogenous free bilirubin in vascular endothelial cells, which was found at 0.9-1.5 pmol/mg protein levels in arterial and venous rat endothelium. This finding opens possibilities for modulation of bilirubin by up-regulation of heme oxygenase-1, which is important for intracellular antioxidant activity, since bilirubin is a strong antioxidant. Such low levels of bilirubin were only accessible after previous development of a novel ultra-sensitive analytical technique based on liquid chromatography hyphenated with TLS detection (HPLC-TLS). This enabled determination of bilirubin, as well as its oxidative product biliverdin at levels of 1 nM or lower. With HPLC-TLS we established new frontiers for ultra-sensitive simultaneous analysis of bilirubin and biliverdin, and were able to determine for the first time the concentrations of unbound biliverdin in human serum, which are in the 2-3 nM concentration range, with bilirubin-biliverdin ratios of 2.5 to 4.5. Recent advances in TLS instrumentation and methodologies have furthermore focused on development of thermal lens microscopy (TLM), with particular interest in vanguard analytical methods. Combination of TLM with various microfluidic platforms enabled development of rapid and high throughput screening methods for chemical analysis and biomedical diagnostics. Utilization of colorimetric reactions and flow injection analysis (FIA) in microfluidic systems with TLM detection enables, for example, determination of Cr(VI) at ng/mL levels in sub μL samples with throughputs of up to 20 samples/min. The achieved limits of detection (LOD = 0.6 ng/mL) are over one order of magnitude lower compared to those achievable by spectrophotometry, despite over 100-times shorter optical interaction lengths in case of μFIA-TLM. Microfluidic systems offer particular advantage in case of biological macromolecules by reducing molecular diffusion times due to short diffusion distances. This significantly reduces reaction times and improves sample throughputs as it was shown for determination of neutrophil gelatinase associated lipocalin (NGAL) - a biomarker of acute kidney injury. By immobilizing NGAL specific antibodies onto SiO_2-coated magnetic nanobeads, which are held in a microfluidic channel by magnetic field, the entire ELISA based assay for NGAL in human plasma can be completed in only 35 minutes, compared to four hours, required for a commercial assay in a microtiter plate, while offering four times lower LODs (2.3 pg/mL).

Keywords: Thermal lens spectrometry; Microscopy; Chemical analysis; Microfluidics; Biomedical diagnostics.

INVITED SPEAKERS

Id-152

Combining Spectral and Spatial Information for Classification, Example with a Biological Study Case

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Abstract: Vibrational spectroscopy imaging can characterize the molecular structure of the measured entity in a label-free manner. For this reason, Raman spectroscopy has become more and more popular in biology to analyze living cells or tissues. Previous studies have been aiming to identify cells or diagnose samples, which is often done by averaging the spectral information of cells. However, since spectral wavelength can be used to reconstruct images, spectral images contains multiplex spatial information providing unique information about the spatial structures of studied entities. In this work, we showcase how Raman spectral images processed by an image classifier can perform to identify the cell type in biological samples. We collected the spectral images of several cell types, including hepatocytes, mesenchymal stem cells, and neuronal cells, and reconstructed images from the molecular information obtained by spectroscopy. Processed by eleven machine learning algorithms, 1025 discriminant features can be extracted from the spatial information of each image, which, when analyzed by multivariate analyses, allowed to discriminate cells with a good accuracy (70% or more).

Keywords: Bioimaging; Raman spectroscopy; Machine learning; Classification.

INVITED SPEAKERS

Id-153

Single-particle FRET Microscopy of Structural Rearrangements in Nucleosomes Induced by Some Nuclear Proteins

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Abstract: Various proteins are involved in the controlled re-organization of nucleosomal DNA in the course of transcription, replication and DNA repair. Structure of complexes between nucleosome and such proteins requires detailed investigation, which can be conducted combining a mononucleosome experimental system and an advanced spFRET (single particle Förster resonance energy transfer) microscopy technique. Mononucleosomes are assembled from core histones and short DNA containing a strong nucleosome-positioning sequence. They are nanoparticles of 10×5 nm size. Application of the spFRET microscopy requires fluorescent labeling of a nucleosome with a donor-acceptor pair of fluorophores. When these fluorophores are closely situated in the neighboring gyres of a nucleosomal DNA, alterations in DNA packing result in FRET efficiency changes. Using highly diluted solutions of labelled nucleosomes, FRET efficiency can be measured for individual nucleosomal nanoparticles that cross the focal volume of a confocal microscope in the course of a free diffusion. Here we present and summarize main principles of spFRET microscopy of freely diffusing single particles and our recent results obtained in the course of studies of interactions between nucleosomes and two functionally important nuclear proteins: poly-(ADP-ribose)-polymerase 1 (PARP1) and the protein complex abbreviated as FACT (FAacilitates Chromatin Transcription). Results of our experiments clearly demonstrate that spFRET microscopy has important advantages in the investigations of complex heterogeneous systems. Using spFRET microscopy, structural aspects of nucleosome-protein interactions can be clarified, and, moreover, structurally different subpopulations of nucleosomes and (or) their complexes can be revealed and characterized. PARP1 studies were supported by RFBR (grant 17-54-33045). FACT studies were supported by Russian Science Foundation (grant 14-24-00031).

Keywords: Nucleosome; Single molecule; FRET; PARP1; FACT.

INVITED SPEAKERS

Id-154

Discrete Fourier Transform Spectroscopy Using Precisely Periodic THz Pulse Train

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Abstract: Fourier transform spectroscopy (FTS) is a spectroscopic technique where spectra are obtained by measuring a temporal waveform or interferogram of electromagnetic radiation, or other types of radiation, and calculating its Fourier transform (FT). FTS possesses inherent advantages over conventional dispersive spectrometers, such as a high signal-to-noise ratio, simultaneous acquisition of signals in a broad spectrum, and versatility for different radiation sources. Therefore, various types of FTS, including terahertz (THz) time-domain spectroscopy (THz-TDS), have been widely used in fields of research, industry, and medicine. When the temporal waveform of a phenomenon is measured, the spectral resolution is simply determined by the inverse of the measurement time window size during which the temporal waveform is observed. Therefore, as the time window size is extended, the spectral resolution is enhanced. However, when the majority of the signal components are temporally localized, excessive extension of the window size increases the noise contribution as well as the acquisition time. Furthermore, the travel range of a translation stage used for time-delay scanning practically limits the spectral resolution. On the other hand, when the phenomenon repeats, it is generally accepted that the achievable spectral resolution is limited to its repetition frequency because the maximum window size is restricted to a single repetition period to avoid the coexistence of multiple signals. If the spectral resolution of FTS can be improved beyond the repetition frequency, the scope of application of FTS will be further extended. In this paper, we demonstrate a significant spectral resolution improvement over the time window size limitation by using discrete Fourier transform spectroscopy (dFTS) with the mode-locked THz pulse train. We confirmed that the time window size did not limit the spectral resolution in discrete Fourier transform spectroscopy using precisely periodic THz pulsed radiation only when the time window size is matched to the repetition period.

Keywords: Fourier transform spectroscopy; Terahertz time-domain spectroscopy.

INVITED SPEAKERS

Id-155

Soft X-ray Spectromicroscopy: New Techniques and Applications to Fuel Cell Materials

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Abstract: Soft X-ray scanning transmission X-ray microscopy (STXM) is a powerful materials analysis tool, which can provide quantitative chemical speciation and mapping in 2D and 3D, with a spatial resolution better than 30 nm. This presentation will give an overview of current capabilities of STXM, and show how the spatial resolution is being dramatically extended into the sub-10 nm range using coherent diffraction imaging with ptychography. Examples will be given from recent studies on biological samples (magnetic bacteria), catalytic naomaterials (ZnO coated alumina aerogels), and biomaterials (Ti implants). A major part of the talk will deal with our studies of polymer electrolyte membrane fuel cell (PEM-FC catalyst layers) aimed at optimizing formulations and processing to improve performance and reliability by optimizing distributions of the proton conducting perfluorosulfonic acid (PFSA) ionomer. Research performed using the ambient-STXM on BL 10ID1 at the Canadian Light Source and STXMs on BLs 5.3.2.1, 5.3.2.2 and 11.0.2 at the Advanced Light Source. Research supported by AFCC, NSERC and the Catalyst Research for Polymer Electrolyte Fuel Cells (CaRPE-FC, Canada) network.

Keywords: Spectromicroscopy; Soft X-ray; Ptychography; Fuel cells; Tomography.

INVITED SPEAKERS

Id-158

New Approach in Auger RSF Calculation for Precise Quantitative Analysis Using Correlation with TEM-EDS Analysis by Extrapolation Method Based on Pure Elements

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Abstract: In semiconductor power devices, the backside of the wafer is often used as drain contact, then, in order to ensure a good contact, a stack of Ti-NiV-Ag is deposited. Moreover, this stack is important because it is the connection between device and package. For these reasons the control of surface appears crucial to avoid metallurgical bonding issues. In fact a well know failure mechanism consist in the Ni migration on the Ag surface followed by an oxidation of the Ni that damages the metal surface and inhibits the correct bonding. For this reason it is very important during the process of product qualification, to control the Ni segregation on Ag surface in back metal layers under stress; in particular for the power devices in automotive applications. The stress is a High Thermal Stress that is performed in order to verify the stability of the contact designated at the die attach. Auger Electron Spectroscopy (AES) has been proved to be a valuable analysis technique to check for possible migration of Ni on the silver surface. Unfortunately, its results are qualitative. To obtain quantitative results in Auger analysis, a reference sample with known concentration of the unknown element in a quite similar matrix to that one under analysis, must be available, in order to calculate the correct Relative Sensitivity Factor (RSF). Alternatively the tabulated RSF can be used, but a noticeable error could be made. The other technique, often used in failure analysis, is Energy Dispersive X-ray Spectrometry (EDS), even if it is not as sensitive as Auger spectroscopy. Even in this case, however, the extrapolation of a quantitative result appears difficult. In fact, in the case of massive samples, the matrix (ZAF) correction must be estimated before. Using Transmission Electron Microscopy (TEM) EDS, the analysis seems easier but the condition called thin-foil criterion must be evaluated and the correction due to the effect of secondary fluorescence should be calculated. The Cliff-Lorimer method is effective in the case of binary alloys, but, even in this case, the use of a reference sample to compute the Cliff-Lorimer coefficient, k_{AB}, is mandatory. Recently, a method to determine this coefficient by extrapolation was proposed, using samples constituted by multi-layers of pure elements. In this work, we applied this method on samples prepared on the tool used in a semiconductor factory for back metal deposition. A new strategy to prepare TEM samples and to acquire the EDS spectra has been proposed. Finally the k_{AB} was calculated. This coefficient was used to perform an EDS quantitative analysis in a sample in which Ni migration was stimulated by thermal budget.

Keywords: TEM-EDS; AES; Back metal.

INVITED SPEAKERS

Id-162

Drop-Coating Deposition Raman Spectroscopy in Protein Research

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Abstract: Raman spectroscopy can be a powerful probe of structure and function of biomolecules. However, its usage, particularly in biochemistry and medicine, is often limited by amount and concentration of samples. A drop coating deposition Raman (DCDR) method based on a "coffee ring effect" has been reported. It enables measurements of solutions with concentrations of biomolecules down to 1 μM (ca. 0.01 mg/mL). The method is based on a deposition of a small amount of sample solution (typically units of μL) on a hydrophobic surface (usually Teflon-coated stainless steel or CaF_2) that enables drying of a sample. It has been demonstrated that even after drying its solution structure is preserved and Raman spectra taken from the dried deposit and (concentrated) solution are virtually identical. Nevertheless, little discrepancies have been reported in the case of proteins. Here we present the overview of our applications of DCDR spectroscopy of different kinds of protein structural studies where the advantage of low sample concentration could be crucial. This is illustrated by the case of RNase L dimerization upon binding 2'-5' adenosine tetramer and its analogs or by structural analysis of natural killer cell receptor protein 1. The low limit of detection of biomolecular samples in DCDR spectroscopy might be problematic with respect to SERS for SERS–active substrates. In this case, DCDR spectra can be mistaken for SERS spectra if the solution sample is dried by the “coffee ring effect” on the solid SERS-substrate. This can play a role especially in amino acid or peptide studies. Our work adverted to subtle differences corresponding to the glass-like phase of deposited samples. Thus, DCDR protein samples represent a "transition phase" between saturated protein solutions and crystals. This enables to distinguish spectral differences given by the density of molecules in crystals from those caused by protein crystal artifacts. We illustrate the applicability of the method for distinguishing these differences and improving x-ray protein structures by molecular modeling based on information from Raman spectroscopy showing the example of the PsbP protein of photosystem II. The potential applicability of such a remarkable experimental technique in medical diagnostics of neurodegenerative diseases, focused on low concentrated misfolded proteins in cerebrospinal fluid, is discussed as well. The Czech Science Foundation is gratefully acknowledged for support (No. P205/12/G118).

Keywords: Raman spectroscopy; Proteins.

INVITED SPEAKERS

Id-164

Novel Developments in Image Analysis for Iron Ore Textural Characterisation

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Abstract: The traditional trading of iron ores is based on particle size and chemical specifications. However, microstructural characterisation has been increasingly recognized as an important tool since it provides mineralogical and textural information that can improve iron ore beneficiation and agglomeration operations, and subsequent steelmaking processes. Therefore, the industry is moving from the qualitative evaluation on the optical microscope towards quantitative and automatic methods based on digital microscopy and image analysis. Automatic image analysis systems can easily recognize the most common iron-bearing minerals (hematite, magnetite and goethite) by their characteristic colors on suitable images acquired on a reflected light microscope in bright field mode. On the other hand, the use of polarized light makes the crystals of hematite present different brightness and colors according to their crystalline orientation, revealing the hematite textures. This talk describes the current state of our system for iron ore characterisation and presents the novel developments that were incorporated to improve the textural analysis. The system is fully automatic. It employs a motorized and computer-controlled reflected light microscope in a correlative approach that combines bright field and circular polarization modes. Mosaic images covering large areas of polished sections are acquired to image thousands of particles. Mineral phases and hematite grains are segmented. Different supervised classifiers with overall success rates above 90% discriminate polycrystalline and monocrystalline particles, and recognize five textural classes of hematite (granular, lamellar, lobular, microcrystalline, and martite). Then, the mineral and textural phases are quantified.

Keywords: Iron ore; Texture; Characterization; Correlative microscopy; Image analysis.

INVITED SPEAKERS

Id-170

Recent Advances in the Application of Digital Holographic Microscopy

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Abstract: Digital holographic microscopy is a very advanced imaging technique because it yields a 3D volume image from a single image capture. Holography is a technique by which a wavefront can be recorded and subsequently recon- structed in the absence of the original wavefront. In conventional holography, invented by Gabor, the holograms are photographically recorded and optically reconstructed. Both the amplitude and phase information of the light wave are recorded in a hologram. Because the holographic image retains the phase as well as the amplitude information, a variety of interference experiments can be performed, and this is the basis of many interferometric applications in metrology. Digital holography does not require wet chemical processing of a photographic plate, although at some expense of resolution. However, once the amplitude and phase of the light wave are recorded numerically, one can easily subject these data to a variety of manipulations, and so digital holography offers capabilities not available in conventional holography. The remarkable aspect of the digital reconstruction – its possibility to refocus at different depths inside a transparent object, depending on the reconstruction distance, makes this technique very suitable for biological cells studies and could have many applications in life sciences. The objective of the work is a comprehensive review of the recent advances in the application of digital holographic microscopy. The research carried out at the Agricultural University of Plovdiv in the area of digital holographic microscopy is presented. One of the significant results reported is the development of a new method for observation of the domain structure in transparent ferroelectrics.

Keywords: Digital holographic microscopy.

INVITED SPEAKERS

Id-176

Plasmonic 2D Transition Metal Dichalcogenides/Graphene van der Waals Heterostructures for High-sensitivity Surface-enhanced Raman Spectroscopy

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Abstract: van der Waals (vdW) heterostructures consisting of two-dimensional transition metal dichalcogenides (TMDC), such as MoS_2 and WS_2, and graphene integrate the superior light-solid interaction of TMDC and charge mobility in graphene, enabling high-efficiency charge transfer for surface-enhanced Raman spectroscopy (SERS). Density functional theory suggests promoted electric dipole moments and dipole-dipole interaction through the TMDC/graphene interface, yielding an effective means to facilitate an external electrostatic perturbation on the graphene surface and a primarily chemical (charge transfer) and a possible electrical contribution to SERS. As a proof of concept, we developed a transfer-free process for fabrication of the TMDC/graphene vdW heterostructures on SiO_2/Si. Using fluorescent Rhodamine 6G (R6G) as probe molecules, SERS signatures of R6G were compared on the MoS_2/graphene vdW heterostructures, single-layer MoS_2 and graphene substrates. Significantly stronger SERS signals of R6G were observed on the MoS_2/graphene vdW heterostructure substrates with high SERS sensitivity up to 5×10^{-8} M using non-resonance 633 nm Raman excitation, which is one order of magnitude higher than that obtained on the plasmonic Au nanoparicle/graphene SERS substrates, and three orders of magnitude higher than that of single-layer semiconductor MoS_2 or graphene substrate. The SERS sensitivity was shown to further increase when the MoS_2/graphene vdW heterostructure was decorated with plasmonic Au nanoparticles. This result illustrates the plasmonic TMDC/graphene vdW heterostructure as a promising high-sensitivity SERS susbtrates that are scalable for commercial applications.

Keywords: 2D transition metal dichalcogenides/graphene van der Waals heterostructures; Plasmonic Au nanoparticle; Surface-enhanced Raman scattering; Rhodamine 6G; Biosensing.

INVITED SPEAKERS

Id-178

The Measurement of Trace Moisture in High-purity Gas Using Cavity Ring-down Spectroscopy

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Abstract: The measurement of trace amounts of water vapor (trace moisture) in gases is a challenging issue that needs to be addressed for various high-tech industries that use high-purity gases. The most well-known industry is the semiconductor industry. For example, in the semiconductor industry so-called bulk gases such as N_2, Ar, O_2, H_2, and He are used in large amounts, for instance, as purge gases to remove contaminants in the manufacturing processes. Therefore, these gases must have the highest purity, and any residual moisture needs to be controlled to less than 10 nmol/mol (10 ppb) in amount-of-substance fraction (mole fraction). Reliable measurement of trace moisture is essential to enable these moisture controls, but measurement of trace moisture in the range less than 10 nmol/mol is difficult. A primary reason behind this is that only a few types of trace-moisture analyzers have sufficient sensitivity in this range. Cavity ring-down spectroscopy (CRDS) is a highly sensitive technique that enables the measurement of trace species in gases even at parts-part-trillion (ppt) levels, which is expected to be necessary in the semiconductor industry in the near future. In this work, we developed at National Metrology Institute of Japan (NMIJ) a simple, robust measurement system based on CRDS that can reliably measure trace moisture at the ppt level. The performance of the system was evaluated using a primary trace-moisture standard in N_2 generated by the NMIJ magnetic-suspension balance/diffusion-tube humidity generator (MSB/DTG).

Keywords: High sensitivity; Metrological traceability; Parts-part-trillion; Humidity standard.

INVITED SPEAKERS

Id-179

Thermoreflectance Microscope and Applications

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Abstract: More powerful and faster semiconductor devices mean hotter devices, which can lead to a decrease in performance and lifetime of devices. Thus, thermal characterization of devices, such as surface temperature profile measurements and localized heat generation detection under their operating conditions, have become an important factor in the development of devices. Several thermal imaging and analysis techniques, such as scanning thermal microscopy, micro-Raman thermography, and infrared micro-thermography, have been developed to investigate thermal properties in micro- and nano-scale devices. In particular, thermoreflectance microscopy (TRM) is an optical imaging technique that provides a two-dimensional thermal image of devices with high spatial and thermal resolution. In this presentation, we will demonstrate the various types of TRM systems, we have developed, including wide-field TRM, spectroscopic TRM, and laser scanning confocal TRM. Several applications of TRM, such as quantitative measurement of the surface and sub-surface temperature distribution of semiconductor devices, hot spot detection for failure analysis of semiconductor devices and integrated circuits, and detection of defects in thin films, will be presented. This work was supported by the KBSI grant (D37615) and Ministry of SMEs and Startups grant (S2410822).

Keywords: Thermal microscopy; Thermoreflectance.

INVITED SPEAKERS

Id-185

Phase Identification by Energy Dispersive Spectroscopy Combined with Electron Back Scattered Diffraction and Transmission Kikuchi Diffraction Technique

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Abstract: Energy Dispersive Spectroscopy (EDS) is the one of the most frequently using technique for the phase identification of materials in scanning electron microscopy (SEM). But it also has a limitation in allotrope identification as well as relatively coarse resolution of μm scale. To overcome these limitation, a diffraction technique such as Electron Back Scattered Diffraction (EBSD) is adapted for the phase identification. It uses Kikuchi map based on the crystallographic information and the actual resolution is around 100nm. But it also has a limitation of ambiguity in phase identification of materials with similar crystal structure even though some refinement techniques are developed by several EBSD makers. To compensate the limitations of both technique, a combination of spectroscopy and diffraction is adapted. To acquire the maximum resolution, an EBSD pattern is used as a base identification determinant and the EDS spectrum acquired at the same time is used as the assistant classifier. For the phase map, the acquisition speed is another important factor for the users to adapt a new analysis technique. To increase the mapping speed, it is required to use the EDS spectrum only for the classifier and it means that only the key elements are selected for the consideration. People who want to achieve the better resolution for the spectrum/diffraction combination technique adapted the Transmission Kikuchi Diffraction (TKD) technique for the combination partner of the EDS technique. With TKD, several tens of nm resolution can be acquired. In this presentation, the application of spectrum/diffraction combination techniques in oxide layers of heat resistant steels and Ni base superalloys will be introduced.

Keywords: Phase ID; EDS; EBSD; TKD.

INVITED SPEAKERS

Id-187

Low Wavenumber Raman Scattering on Semiconductor and Metal Oxide Nanoparticles

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Abstract: Raman scattering is one of the most important non-destructive techniques to provide information on vibrational states of nanostructured materials. The intention of this talk is to give basic understanding on the low wavenuber Raman Spectroscopy (LWRS) on semiconductor and metal oxide nanoparticles. A short theoretical introduction of the Lamb theory[1] on the vibrations of homogenous elastic spheres will be described for the free nanoparticles as well as for the nanoparticles embedded in matrix with the proper account on the boundary conditions. The observed LWRS peaks of diffferent kind of nanoparticles were interpreted with symmetric and quadrupolar acoustic vibrations. Also, the methodology for the determination of a nanosized particle distribution by LWRS is described. It is based on a v^{-1} dependence of the Raman light of the vibration coupling coefficient and on the fact that each nanocrystalite of diameter D vibrates with its eigen-weavenumber $v \sim D^{-1}$. The effect of the particle vibrational lifetime on the shape of the distribution is analyzed for the free as well as for the embedded nanoparticles in matrix. The application of the Lamb theory will be illustrated by the LWRS measurements on different powder samples: TiO_2, SnO_2, ZnO and CdS. The effects of sintering of nanoparticles will be described with the ZrO_2 nanoparticles doped with Sn. The Raman scattering spectra of the nanoparticles embedded in glass matrix are analyzed in a model of confined acoustical vibrations. The model-calculation considered homogenous broadening of the confined acoustical modes due to interaction of the particles with matrix and inhomogeneous broadening due to the contribution of the Raman scattering from particles of different sizes. Polarized and depolarized scattering from confined acoustic phonons was distinctly resolved near the laser line and polarized inner particle modes were observed. The material-dependent generalized form of this analysis enables one to use it for any given combination of particle and matrix materials. A good agreement between the experimental and the theoretical results will be illustrated by Si, Ge, TiO_2, CdS_xSe_{1-x}, and HfO_2 nanoparticles in glass matrix. The nanoparticle sizes and even sizes distribution obtained from Raman scattering agree well with those obtained from transmission electron microscope proving the LWRS to be a simple, fast and reliable method for the size distribution measurements. By inverse procedure, starting from the Raman spectra and known particles size distribution, the mean sound velocities of longitudinal and transverse phonons of nanoparticles could be deduced, providing LFRS to be unique technique for determination of the sound velocities in nanoparticles.

Keywords: Raman Scattering; Semiconductor; Metal Oxide; Nanoparticles.

INVITED SPEAKERS

Id-188

Molecular Recognitions in a Dendrimer Assembly

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Abstract: Novel macromolecules dendrimers with a globular protein like architecture have widespread applications in pharmaceutical industries. PAMAM dendrimer molecules possess four water filled peripheral cavities along with a small ethylenediamine (EDA) hydrophobic core. In our recent reports, we show that an extremely hydrophilic dye coumarin 153 (C153), instead of binding with hydrophobic EDA core, it partitioned into the water filled cavities of dendrimer, which is indeed an unusual observation. Fast water like rotational diffusion, in conjunction with a slow translational diffusion of C153 when complexed with PAMAM dendrimer substantiate the fact that C153 resides within the water filled cavities of dendrimer molecules. In a recent study using isothermal calometric titration we show a negatively charged surfactant sodium dodecyl sulfate (SDS) forms encapsulation complex with positively charged PAMAM dendrimer at SDS-to-dendrimer mole ratio ~3-5. Within these encapsulation complexes, one SDS monomer occupies one cavity of dendrimer, by replacing water molecules from the cavity. Electrostatic, hydrophobic and hydrogen bond interactions all together reinforce the structural stability of dendrimer-surfactant complex. A maximum heat release of 6.3 kJ/mole-of-SDS binding was recorded for this complex. We observed that a dendrimer-surfactant complex, possessing more number of hydrophobic binding sites than dendrimer alone, offers exceptionally strong binding to hydrophobic C153 molecules.

Keywords: Dendrimer; Molecular Recognitions.

INVITED SPEAKERS

Id-193

Calcium Signaling in Inner Hair Cells and Non-sensory Cells of the Developing Mouse Cochlea Investigated by Multiphoton Microscopy

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Abstract: In the immature auditory system, the firing of Ca^{2+}-dependent action potentials in the primary sensory inner hair cells (IHCs) of the cochlea is thought to modulate the refinement of the peripheral and central auditory circuitry. However, it is currently uncertain whether action potentials in immature IHCs are intrinsic to the cells or solely due to ATP released from connexin hemichannels in cochlear non-sensory cells. Using a combination of 2-photon imaging and cell-attached patch clamp recordings performed at body temperature, we show that spontaneous firing activity is present in immature IHCs in the absence of ATP-dependent intercellular Ca^{2+} signaling from the non-sensory cells in the greater epithelial ridge (GER). This spontaneous firing baseline in IHCs can generate, but also be modulated by, Ca^{2+} transients in non-sensory cells. IHCs use this bidirectional communication with non-sensory cells to synchronize their electrical activity, a mechanism that is likely to occur in parallel to that provided by spontaneous Ca^{2+} signaling from the GER. This work was supported by CNR Progetto di Interesse Invecchiamento (grant DSB.AD009.001.004/INVECCHIAMENTO-IBCN to FM), Fondazione Theleton (grant GGP13114 to FM) and The Wellcome Trust (grant 102892 to WM). FC was supported by a junior post-doctoral fellowship from the University of Padova (grant CPDR132235 to FM). SLJ is a Royal Society URF.

Keywords: Hearing; Connexins; ATP release.

INVITED SPEAKERS

Id-196

X-ray Absoprtion Spectroscopy and Dynamical Lattice Distortions

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Abstract: We discuss the basic principles of the process of x-ray absorption in condensed systems, including the inelastic scattering of high-energy photoelectrons. It is shown how x-ray absorption spectroscopy (XAS) can be used to determine the local atomic structure in materials, it being sensitive to atomic distortions that are dynamic. We show the relation between x-ray absorption and the dynamical structure factor, contrasting the information that can be obtained by elastic techniques as x-ray and neutron diffraction with that provided by XAS. We discuss the use of XAS in materials, where the interaction between electrons and ions produces changes in the local atomic structure as a result of electronic changes. As specific examples, we discuss the case the observation of polaronic signatures in high-temperature superconductors and nickelates.

Keywords: XANES; EXAFS; Inelastic x-ray scattering.

INVITED SPEAKERS

Id-197

Local Thermal Conductivity of a Super Insulator Nanoporous Silica Aerogel Investigated by Thermal Microscopy

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Abstract: Nanoporous aerogels have gained significant attention in the biomedical field as potential candidates for use in drug release formulations and biocompatible materials. Such biomedical features result from the exceptionally high porosity and specific surface area of these materials which consequently exhibit, among other properties, an extremely low thermal conductivity value. De facto, a thermal analysis may provide useful information about structural and functional properties. Besides, the thermal parameters also depends on processing temperature during material formation and drying during the synthetic process. Thus, the possibility to control or tune thermal conductivity seems possible and would be of great interest but this required predictive numerical computation means to simulate heat transfer behaviors. Today, the local thermal properties data needed for modelling are not available. So, we report here our local thermal conductivity measurements made at a microscopic level on nanoporous Silica aerogel granules by using a Wollaston probe tip associated to a thermal microscope (2990 MicroTA from TA Instrument). The challenge was to determine a local thermal conductivity lower than that of the open air, the feasibility of the measurement being not demonstrated to this day. We propose a method of data processing based on the identification of an effective thermal exchange area at the probe-sample interface and more specifically an effective thermal contact radius *vs* the thermal conductivity. The method was applied at different average probe temperatures (between 20°C and 150°C) in order to investigate the influence of the water meniscus at the contact interface. As a main result, the apparent local thermal conductivity of Silica aerogel exhibits a strong variation at temperature conditions close to the ambient. Then it follows the temperature increase of the probe apex, starting from 10 $mW \cdot m^{-1} \cdot K^{-1}$ up to a nearby constant value of about 23 $mW \cdot m^{-1} \cdot K^{-1}$ that is related to dry condition above an average value of probe temperature of 150°C. As a matter of fact, the confined free water at probe sample interface is acting as a coupling fluid combined with surface asperities of the materials and might play a major role in the definition of the measured local heat flow at room temperature. However, the estimated values obtained for average tip temperatures above 40°C are in agreement with mesoscopic measurements found in literature. This study opens new perspectives for super insulator characterizations at a local level and potentially for surface investigations of water film.

Keywords: Nanoporous aerogel; Super insulator; Microthermal; Local thermal conductivity; Wollaston probe.

INVITED SPEAKERS

Id-199

Scanning Thermal Microscopy (SThM): State of the Art and Main Challenges

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Abstract: The Scanning Thermal Microscopy (SThM) has been due to its high spatial resolution applied in various fields of science and engineering since the 1990s. Its main applications are in the characterization of heat conduction in nanostructures, thermal analysis of active components of microelectronic materials and polymer science. Either way, many scientific bottlenecks remain for a complete understanding of the technique. The thermal interaction between a nanotip and the surface of the object to characterize such raises many questions. Several vectors of heat transfer contribute to the thermal interaction between the tip and the surface of a sample: the solid-solid micro and nano-contacts, water molecules adsorbed at probe and sample surface, surrounding gas and radiation. Each of these vectors of heat transfer is a full theme of research. Their dependence on the geometry of the tip, the surface chemistry and other factors such as surface roughness is poorly known due to the lack of sensitivity of the current tips and descriptions too simplified of heat transport between and within the tip and sample. The research activity of our group covers not only the application of the technique to various materials and systems but also these aspects through the implementation of dedicated experiences and modelling. This includes not only the analysis of the measurement in various environments but also the development of reference samples and new probes. These actions are not only motivated by the understanding of technique SThM for its improvement and its qualification as a nanometrology validated but also to transpose the knowledge produced in other systems putting in interaction two objects at the micro and nanoscales. Through this talk, our current vision of the State of the art in SThM will be initially presented. The contribution of our group to the development of the technique will be given. Areas where progress could be made in the future to develop new opportunities for SThM will then be discussed.

Keywords: Scanning thermal microscopy; Micro and nanoscale heat transfer.

INVITED SPEAKERS

Id-201

Global Estimation of Experimental Parameters and Material Thermal Properties in Scanning Thermal Microscopy

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Abstract: In this work we propose to identify simultaneously the contact conditions (thermal contact resistance and contact area radius) as well as investigated thermal properties of the investigated device within the scanning thermal microscopy experiment (SThM). We implemented the SThM experiment in the 3□ mode that allowed us to measure the amplitude and the phase at each point of the investigated area by the probe. The parameters identification method is based on the minimization of the quadratic gap between the experimental and theoretical phase according to the frequency of the heat flux generated within the probe. The minimization rests on the Levenberg-Marquardt algorithm and the reliability of the method stands on the sensitivity analysis that ensures the identification of each parameter. An application is given that is devoted to a new technology in non-volatile memories that have been developed for several years based on phase-change alloys whom the most known is the $Ge_2Sb_2Te_5$. Huge work has been done already to investigate the thermophysical properties of this material deposited as a thin film with sub-micrometric thickness. Memory devices have been produced since 2009 and work is in progress to make the unit cell yet scalable in order to reach even more fast read-write process and higher memory storage. In order to achieve those objectives, the thermal investigation of the cell itself must be done since the heat transfer is the main limiting aspect for the optimal functioning of the device. The results lead to understand the 3D heat diffusion in the cell as well as the role of the vertical and horizontal interfaces. The heat transfer model is based on a system identification regardin the probe whereas the finite element method is used to simulate the g-heat transfer within the cell. Another application on phase change nanowires is presented that shows how the SThM technique leads to identify the anisotropic thermal conductivity along the a-plane and c-axis. In that configuration, both analytical and discrete techniques are used in order to simulated the heat transfer within the nanowire.

Keywords: Scanning thermal microscopy; Inverse methods; Phase change material; Memory devices; Nanowire.

INVITED SPEAKERS

Id-203

Nanoimaging of Buried Structures Using SubSurface Ultrasonic Resonance Force Microscopy

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Abstract: Nondestructive Imaging of nanostructures buried under several layers is an extremely challenging task, but is also considered extremely important in a wide variety of fields. From fundamental research into the way living cells are built up and how they react to external stimuli to process control in semiconductor manufacturing would all benefit from the capability to image nanoscale structures through arbitrary covering layers. Combining Scanning Probe Microscopy (SPM) with ultrasound wave has been shown a promising technology to enable such imaging in various configurations. Here we report the development of an alternative method of combining SPM with ultrasound which we call SubSurface Ultrasonic Resonance Force Microscopy (SSURFM) and which is based on a combination of the two most common variants described in literature, which each have their specific strong points: Ultrasonic Force Microscopy (UFM) and Contact Resonance AFM (CR-AFM). We show the excellent performance of this combination on a number of samples designed specifically to mimic relevant conditions for the application as an in-line inspection technique in the semiconductor manufacturing process. We also discuss the physics of the contrast mechanism which is based on sensing visco-elastic properties of the sample through generating large indentations into the surface. Understanding the contrast mechanism allows us to optimize the image contrast and depth sensitivity of our method. In summary, the experimental results presented here provide possible new industrial metrology and inspection solutions for nanostructures buried below arbitrary covering layers with excellent resolution and depth sensitivity.

Keywords: Subsurface AFM; Subsurface Ultrasonic Resonance force microscopy; Subsurface nanoimagine.

INVITED SPEAKERS

Id-204

Multiphoton Tomography

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Abstract: We report on the Prism Award winning clinical multiphoton tomograph MPTflex-CARS based on femtosecond near infrared laser pulses and a flexible 360° scan head for small animal imaging as well as in vivo human skin imaging. Optical biopsies with submicron resolution were obtained with more information than conventional stained and sliced biopsies. In particular, time-resolved two-photon cellular autofluorescence (NADPH, flavins, elastin, melanin, keratin), second harmonic generation (SHG) from collagen, and CARS signals from lipids and intratissue water were obtained with single-photon sensitivity. The clinical TCSPC-FLIM tomograph has been used to distinguish fluorophores and to control therapeutical effects on patients suffering from dermatitis, psoriasis, wounds, and skin/brain cancer. Furthermore, MPT is employed for a quality check for human corneas prior transplantation. The device with its flexible arm is also employed for small animal research such as stem cell tracking in hair follicles as well as "real-time monitoring" of bacteria-induced cancer cell killing in living transgenic mice.

Keywords: Clinical multiphoton tomograph.

INVITED SPEAKERS

Id-205

Study of the Local Redox Processes on Transition Metal Oxides Surfaces Using Kelvin Probe Force Microscopy

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Abstract: Reduction-oxidation (redox) processes involve all reactions where oxidation state of atoms changes. It needs the transfer of electrons between species present in the system. When reduction occurs, the electron is gained (oxidation state decreases) and otherwise for oxidation. Focusing on the field of nanotechnology, redox reactions are widely investigated for bio applications, interfaces and molecular redox films but are most crucial for the oxide materials. In their case, vast majority of structural, chemical and physical properties are governed by the oxygen concentration and, subsequently, cation oxidation state. The global cation stoichiometry changes can be easily tracked by use of averaging techniques like X-ray Photoemission Spectroscopy (XPS) or Secondary Ion Mass Spectroscopy (SIMS). However, the only way to determine the chemical composition of the surface at the scale of single nanometers is to use Kelvin Probe Force Microscopy (KPFM), where the surface potential is directly mapped. In this paper an overview of KPFM principles followed by details on AFM instrumentation will be presented. In particular, the frequency modulation method of the non-contact AFM (NC-AFM) used in ultra-high vacuum conditions will be explained in details. Then, applications of KPFM for nanometer-scale characterisation of electronic properties of transition metal oxides surfaces (i.e. TiO_2(110) and $SrTiO_3$(100)) due to high-temperature (up to 1000ºC) annealing under reducing conditions and in situ oxidation will be discussed.

Keywords: Local Redox Processes; Transition Metal Oxides Surfaces; Kelvin Probe Force Microscopy.

INVITED SPEAKERS

Id-208

Optofluidic Techniques for Directed Evolution of Enzymes

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Abstract: Enzymes are highly versatile and ubiquitous biological catalysts. They can greatly accelerate large variety of reactions, while ensuring appropriate catalytic activity and high selectivity. These properties make enzymes attractive biocatalysts for a wide range of industrial and biomedical applications. Over the last two decades, directed evolution of enzymes has transformed the field of protein engineering. We have devised microfluidic systems for directed evolution of haloalkane dehalogenases in emulsion droplets. In such a device, individual bacterial cells producing mutated variants of the same enzyme are encapsulated in microdroplets and supplied with a substrate. The conversion of a substrate by the enzyme produced by a single bacterium changes the pH in the droplet which is signalized by pH dependent fluorescence probe. The droplets with the highest enzymatic activity can be separated directly on the chip by dielectrophoresis and the resultant cell lineage can be used for enzyme production or for further rounds of directed evolution. The developed platform is applicable for ultra-fast screening of large libraries in directed evolution experiments requiring mutagenesis at multiple sites of a protein structure. In a system for evaluatoin of the enzyme kinetics of the dehalogenase reactions, we use Raman microspectroscopy and surface enhanced Raman spectroscopy (SERS) in microfluidic systems for detection of the reaction substrate and metabolites. This research is supported by Czech Science Foundation (CSF) 16-07965S, infrastructure was supported by MEYS (LO1212, LM2015055) and EC (CZ.1.05/2.1.00/01.0017).

Keywords: Fluorescence spectroscopy; Raman; SERS; Enzymes; Biotechnology.

ORAL SESSIONS

Id-077

Mechanical Alloying Effect on Structural, Thermal and Mechanical Properties of Sn-Bi Alloy

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Abstract: Fine powder of high purity (99.999) BDH from both Sn and Bi of 200 micrometer were used to prepare the soldering alloy of combination having Sn40-Bi 60 Wt.%. Mechanical alloying by using FRETCH600 (German) milling machine were done at 600speed under static air and for different milling time ranging from 2, 4 and 6 hours respectively. St- St., boll mill were used with certain volume calculate according to the vial volume and the mass of powder we used. XRD were indicated the formation of alloy. The best crustal structure was achieved under milling time of 6 hours. Annealing for all alloys got were also done under 100C, under static air. The best pattern was indicated after the annealing process. DSC were investigated to get the best thermal behavior after the mechanical alloying. SEM and mechanical properties were studied and identify remarkable results after mechanical alloying.

Keywords: Mechanical alloy; Structure; Thermal; Mechanical; Sn-Bi alloy.

ORAL SESSIONS

Id-090

Adsorption Characteristics of MOF-5, MOF-199 and ZIF-8 Synthesized Using Palm Oil Derived Fatty Alcohols as Template

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Abstract: In this study, metal-organic framework materials namely MOF-5, MOF-199 and ZIF-8 were successfully synthesized using plant-based polymer as the porous template. The mixing of metal oxide and organic linker precursors was done at room temperature followed by low temperature aging process. The synthesis approach applied was environmental-friendly by eliminating the use of organic solvents and commercial surfactants. We substitute organic solvents with water and surfactants with fatty alcohols derived from palm oil (PODFA). These resulted in a sol-gel formulation of known as surfactant-free microemulsions (SFME). X-ray diffraction patterns of MOF-199 exhibited prominent reflection peaks assigned to octahedral structure with small peak shift while MOF-5 exhibited truncated and full cubic morphology. On the other hand, ZIF-8 has similar structure to MOF-5 framework with the exception of bigger 3-dimensional extensions exhibited crystal structure analogous to that of zeolite. PODFA with longer aliphatic carbon chain exhibited higher porosity and higher pore volume. It was found that the addition of PODFA increased the homogeneity of the cubic and octahedral single crystals typically observed in MOF-5 and MOF-199 morphology. This was due to the increased in *immiscibility* of both precursors of metal oxide and linker hence allowing reactions to occur from all directions. PODFA molecules were suggested to form self-assemblies at the air-water interface governed by surface tension auto-oscillations and formed clusters on which metal oxide and organic linker precursors bind with. The water adsorption isotherms of MOF-5 and MOF-199 prepared in this study exhibited “gate-opening” effect at lower relative pressure not observed in other nanoporous framework structure made up of two-components such as organosilica materials.

Keywords: Metal-organic framework; MOF-5; MOF-199; ZIF-8; Palm oil derived fatty alcohols.

ORAL SESSIONS

Id-099

Determination of Antibiotic in Tablet Using Near Infrared Spectrosopy and Chemometric

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Abstract: Near Infrared (NIR) spectroscopy combined with chemometric has been developed for the determination of antibiotic namely amoxicillin and ciprofloxacin in tablet dosage form. NIR spectra of selected antibiotic tablets were correlated with antibiotic content using chemometrics. Chemometrics method that used for calibration analysis was Partial Least Square (PLS) and for classification analysis, was Linear Discriminant Analysis (LDA). In this study, PLS of calibration of NIR model showed best results with the R2 and RMSEC value were 0.990 and 0.413 respectively for amoxicillin and were 0.990 and 0.634 respectively for ciprofloxacin. The accuracy of LDA models was 100%. PLS and LDA of NIR models were further used to predict unknown amoxicillin and ciprofloxacin content in commercial tablets. Using these model, the significance of amoxicillin and ciprofloxacin that have been measured by NIR and UV-Vis spectroscopy was evaluated with paired samples t-test. The amoxicillin and ciprofloxacin content that have been measured with both methods gave no significant difference.

Keywords: Chemometric; Amoxicillin; Ciprofloxacin; Infrared spectroscopy.

ORAL SESSIONS

Id-191

Single-platelet Force Spectroscopy

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Abstract: Platelet aggregation may lead to the formation of a hemostatic plug at the site of endothelial damage which eventually results in blood vessel closure.[1] An important issue for biotechnological applications in medicine such as microfluidic devices, micro- and nanoparticulate drug delivery, and intravascular devices is modulation of platelet surface-activation. However, this process is not well understood as platelets are difficult to handle because they activate immediately after short contact with non-physiological surfaces. We could recently minimize platelet surface-activation by introducing a thin and soft gel on solid substrates. Interestingly, this gel also allows platelet adhere strongly on an atomic force microscopy cantilever. We applied these findings to establish a 'single-platelet force spectroscopy' setup to measure the rupture forces between two single platelets exposing different degree activation.[2] The rupture force was found to increase proportionally to the degree of platelet activation, but reduced with blockade of specific platelet receptors. Quantification of single platelet-platelet interaction provides major perspectives for testing and improving the biocompatibility of new materials; quantifying the effect of drugs on platelet function, and assessing the mechanical characteristics of acquired/inherited platelet defects.

Keywords: Single platelets; Surface-activation; Rupture force; Atomic force microscopy.

POSTER SESSIONS

Id-065

Optical Addition to X-ray Fluorescence Analysis of Mineral Fertilizers

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Abstract: Nowadays modern industry of phosphorus fertilizers strives to simplify chemical and physical analyses and reach quality control automation of manufactured products. This paper describes low-cost, informative and quite simple optical control method with big data exploration. The proposed method can be used as supplement to the method of X-ray fluorescence (XRF) which strongly depends on physical condition of samples or as an independent quality control system. About 7 grades of fertilizer and 270 samples of industrially produced complex mineral fertilizer was described in order to allocate their physical properties that can effect to XRF analysis, such as fraction, grade or moisture. Each object was prepared by pressing of three types of samples: granules, powder with fraction less than 500 μm and powder with fraction less than 100 μm. Furthermore, algorithm of analytical information obtaining from the RGB (red green blue) matrix of pixels is described. It contains several stages: preliminary allocation of surface, preparation of image (differentiation and smoothing by a median filter) and anomaly detection by matching squares methods. Thus, with obtained properties, map of linear Pearson correlation was build. Moreover, based on the obtained information, possibility of fraction, grade and moisture classification of fertilizers with the use of the random forest algorithm was showed. Obtained results revealed that the proposed method is suitable as an independent, express and low cost method of physical properties determination and as additional source of information to XRF method.

Keywords: Optical recognition; Big data exploration; Mineral fertilizers; X-ray fluorescence.

POSTER SESSIONS

Id-114

Fluorescence Spectroscopy in Structural Studies of Plant Cell Walls

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Abstract: Plant cell walls represent the most abundant, renewable and biodegradable composite on Earth. Its highly complex structure consists mainly of three organic compounds: cellulose, hemicelluloses, and lignin. Cell walls have wide applications in different industries, especially for biofuels and biomaterials. Fluorescence spectroscopy is the method allowing investigation of cell wall structure thought monitoring of lignin autoflorescence and thus interactions of lignin with the other cell wall constituents. Deconvolution of fluorescence spectra reveals the number and location of spectral component peaks by calculation of the approximation of the probability density (APD) of component positions. A characteristic of complex CW fluorescence is that the emission spectrum contains multiple log–normal components originating from different fluorophores, shorter wavelengths corresponding to phenolic structures and longer wavelengths to conjugated structures in lignin. Fluorescence spectroscopy has been used for fast screening of the cell wall properties from plants of different origin (hardwood, softwood and herbaceous plant), that may be important for selection of plants for possible applications. Fluorescence spectroscopy may be applicable in the investigation of the effect of stress on the cell wall. Lignin fluorescence emission spectra, peak intensities and shifts in the positions of the long-wavelength spectral components may be indicators of changes in cell wall structure during the stress. There is an increasing application of quantum dots (QDs) in plant science, as fluorescent markers. The isolated cell wall is an appropriate object for study of the interactions with nanoparticles. The results of different physico-chemical techniques including fluorescence spectroscopy combined with spectral deconvolution, show that in the cell walls, CdSe QDs predominantly bind to cellulose, via OH groups, and to lignin, via the conjugated C=C/C–C chains. Variability of bond types in lignin is related to the involvement of this polymer in plant response to various types of stress, by introducing local structural modifications in the cell wall. Different lignin model compounds have been used in order to reveal spectroscopic properties of lignin. Lignin model polymers were synthesized from three monomers, coniferyl alcohol, ferulic acid and *p*-coumaric acid mixed in various ratios, simulating lignin synthesis in the real cell walls. Further, by using fluorescence spectroscopy and appropriate mathematical methods, it is possible to get deeper insight into the structural characteristics of the molecule. Future investigations will be based on synthetic cell walls and on variation in a portion of all three main components: cellulose, hemicelluloses and lignin, also having in mind results of fine structural modifications in lignin model compounds.

Keywords: Fluorescence spectroscopy; Spectral deconvolution; Cell wall; Lignin.

POSTER SESSIONS

Id-146

Interlaminar Fracture Toughness of Micro and Nano Composites

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Abstract: Effect of micro and nano SiO_2 (2,4,6,8 and 10) %wt on the Interlaminar fracture toughness of 16-plies of woven roving glass fiber /epoxy composites prepared by hand lay – up technique, the specimens were tested using DCB test, mode I. The results computed using area method shows that, the Interlaminar fracture toughness was increased with the filler content, the main failure in micro and nano composites was delamination in the layers. The delamination reduced with the increasing in the filler content.

Keywords: Interlaminar Fracture Toughness; Micro; Nano; Composites.

POSTER SESSIONS

Id-161

Damage Dynamics and Dose-dependent Effects of Gamma Irradiation on Vegetable Tanned Leather by an Integrated Analytical Approach

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Abstract: The use of gamma irradiation as a method for mass decontamination of organic historical and archaeological items is highly desirable since ionizing radiation is universal biocide and assure large-scale and volume decontamination due to almost unlimited penetration. No residues, no induced radioactivity, no chemical and heat dependence are further advantages. The main drawback concerns with the possibility of irreversible effects on materials properties, i.e. colour, opacity, transparency, mechanical properties and chemical alterations, i.e. depolymerization, hydrolysis, crosslinking. The gamma radiation effect on vegetable tanned leather is yet to be established. The aim of our study was to evaluate both the short-term and long-term dose-dependent effects of gamma irradiation on the structure, thermal stability and physical-chemical properties of vegetable tanned leather. New leather samples prepared from calf, sheep and goat hides using mimosa-bark, chestnut-wood and quebracho-wood tannins were exposed to increasing doses of ^{60}Co gamma rays: 10, 25, 50 and 100 kGy. The doses were high so that the changes taking place could be more readily detected. Since alterations can be observed throughout the collagen's hierarchical structure we used an integrated analytical approach based targeting the molecular (ATR-FTIR, Py-GC/MS), nanoscopic (NMR MOUSE), mesoscopic (SEM and TG/MS), microscopic (imageMHT thermal microscopy) and macroscopic (contact angle measurement) levels of collagen. This approach has allowed us to integrate the data from different techniques and overcome the intrinsic limitation of each single technique. The results indicated that the effects depend on the radiation dose and are influenced by the collagen-tannin complex (e.g. tannin type and collagen species). In particular, a trend change was observed at 25 kGy for most measured physical parameters such as shrinkage T_s and denaturation T_d temperature, proton spin-lattice relaxation times T_1 and effective spin-spin relaxation times T_{2eff}. Surface properties, i.e. collagen fibers morphology and average contact angle values, showed significant variations at 50 kGy, while variations of both the amide I and amide II ratio and amide bands relative position were observed at 100 kGy dose only.

Keywords: NMR; Vegetable tanned leather; Gamma irradiation; Thermal microscopy.

POSTER SESSIONS

Id-163

General Applicability of Drop-coating Deposition Raman Spectroscopy

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Abstract: Raman spectroscopy represents a widely used vibrational technique for study of various molecules and molecular complexes beneficial for molecular biology, biochemistry or medicine. Its using is often limited by small amount and low concentration of sample. A drop coating deposition Raman (DCDR) method, partially surpassing these limitations. This method is based on a deposition of a small amount of solution (units of μL) on a hydrophobic substrate (e.g. Teflon-coated stainless steel or CaF_2) with its subsequent drying. The measurement from dried pattern (obviously subjected to the "coffee ring effect") can be done from solutions of initial concentration down to 1 μM (ca. 0.01 mg/mL). The hydrophobic surface of the substrate together with a highly refractive layer strongly increase the Raman signal. The thiol-modified Au coated glass is one of the possible examples for a low-cost substrate. Moreover, it has been demonstrated that even after drying of the sample the structure is preserved. Nevertheless, little discrepances have been reported in the case of proteins. Here we present an overview of using the DCDR spectroscopy, starting from small molecules and ending with proteins. The measurements can be often complicated by limited formation of dried pattern with "coffee ring effect" that is influenced by surface wettability as well as by concentration of the sample in the droplet. The DCDR measurements on porphyrins and lipids were reported and subsequently used as a basis for membrane interaction studies of liposome/porphyrin complexes. The advantage of possibility to measure the sample at low initial concentration is widely used in protein studies, e.g., of RNase L dimerization or NKR-P1 structural studies. Another example of law concentrated samples is demonstrated by the resonance DCDR spectroscopy of protoporphyrin IX, a marker in diagnostics of cancer, where Raman spectra were obtained from ~10 nM aqueous solutions. Since, the dried DCDR samples can correspond to the glass-like phase, the case of protein samples represents a "transition phase" between saturated protein solutions and crystals. This enables to distinguish spectral differences given by the density of molecules in crystals from those caused by protein crystal artifacts as we illustrated on the PsbP protein. The potential applicability of DCDR technique in medical diagnostics of neurodegenerative diseases, focused on misfolded proteins in cerebrospinal fluid, is discussed as well.

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Keywords: DCDR; Proteins; Lipids.

POSTER SESSIONS

Id-184

Evaluation on Exothermic Fault in Inhomogeneous Structures Using Lock-in Thermography: An Experimental and Numerical Approach

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Abstract: Experimental and numerical estimation of the depth of thermally active buried heating source in silicon wafer stacked die architectures was performed by both the phase image obtained from infrared microscopic sensor and FE simulation. Highly sensitive infrared images were measured and post-processed by using a lock-in method. By applying the lock-in method to infrared images, the detection sensitivity and signal to noise ratio were enhanced by the phase-sensitive narrow-band filtering effect. Operational principle of lock-in method concerning the thermal wave propagation through the different materials of multi-layers was discussed and it was demonstrated that the phase information of thermal emission from silicon wafer stacked heat source chip (SSHSC) sample can provides the good metrics for the depth estimation of heat source. In addition, the coupled electro-thermal simulations were implemented to evaluate the behavior of thermal waves from multi-stacked silicon wafer samples by comparing a calculated depth with real one. Results showed that the both infrared microscopic sensor with lock-in method and FE simulation have a good potential in the application of the three-dimensional fault isolation of the exothermic faults and their depth estimation for the multi-layered structures, especially in the packaged semiconductor.

Keywords: Infrared thermal microscopy; Lock-in method; Nondestructive test; Finite element simulation; Silicon wafer stacked heat source chip.

POSTER SESSIONS

Id-207

Digital Holographic Microscopy for Characterization of Fabiana Imbricata Ruiz & Pav. Cell Suspesion Cultures

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Abstract: Present report illustrates for the first time an application of digital holographic microscopy (DHM) for measuring the size of cell clusters in cell suspension cultures in vitro. Undifferentiated plant cell cultures are widely used for basic and applied research, for elucidation of biosynthetical pathways, production of secondary metabolites and as screening tools for biotechnology in pharmacy, food technology and agriculture. A digital in-line holographic microscope (DIHM) was developed at the Agricultural University of Plovdiv. The light source is 20 mW He-Ne laser. The emerging spherical wave illuminates the object, and the hologram is recorded on a CCD sensor and stored in a computer. DIHM was applied to visualise three different cell suspensions of Fabiana imbricata Ruiz & Pav. named A, D and MSD. Digital reconstruction of the recorded interference patterns was performed using the "HoloVision 2.2.1" software by Oysen Skotheim and Vegard L Tuft. The reconstructed intensities represent the cell clusters in the suspensions under observation. Cell suspension cultures of Fabiana imbricata Ruiz & Pav. consists of cell aggregates dispersed and growing in moving liquid media. Small cell aggregates having dimensions between 120 and 180 μm have been observed in all suspensions. The cell aggregates in suspension D have the smallest dimensions (120-150 μm) while the biggest cell aggregates are characteristic for suspension A (140-180 μm). Because the plant cell walls have a natural tendency to adhere, the obtaining of homogenious cell lines that consist only of single cells is problematic. Some progress has been made in selecting cell suspensuions with increased cell segregation. The proportion and the size of the cell aggregates is genotype dependent and influenced by the growth regulators in the culture. The accurate, fast, and reliable determination of cell growth is of critical importance in plant cell and tissue culture. The measurement of growth parameters in different cultures introduce diverse problems that must be addressed by using a specific methodology for each type of callus and cell suspension cultures. The attractive features of DHM are: non contact, non destructive, marker free in vivo imaging and quantifying biological cells and tissues. DHM is capable of label free morphology analysis of cells and label free studies of cell division and migration. It is a very useful technique for application in biological research and in the agricultural science.

Keywords: Digital holographic microscopy; Plant cell cultures.

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