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**7th International Congress on
Microscopy & Spectroscopy
(INTERM 2020)**

October 14-20, 2020

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INVITED SPEAKERS

Id-456

Electron Microscopy and Spectroscopy in Plasmonics

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Abstract: Plasmonics deals with surface plasmon polaritons (SPP), which are collective oscillations of free electrons at metal-dielectric interfaces connected with local electromagnetic field. Localized surface plasmons (LSP) are SPP spatially restricted to a surface of a metallic nanoparticle. Such nanoparticles are often called plasmonic antennas. Fabricated antennas can be characterized using the scanning transmission electron microscopy (STEM) which includes high resolution imaging, chemical analysis by energy dispersive X-ray spectroscopy (EDS) and thickness measurement with additional chemical analysis by electron energy loss spectroscopy (EELS). LSP resonances in plasmonic antennas can be characterized with good spectral and spatial resolution by various techniques of electron beam spectroscopy. These techniques utilize an electron beam that interacts with the metallic nanoparticle and excites LSP resonances, whose typical energy lies in near infra-red, visible, and ultraviolet part of the spectra (0.1–5 eV). Such excitations can be detected, for example, by EELS and cathodoluminescence (CL). EELS measures the energy transferred from electrons to LSP and CL detects the light which LSP emit during their decay. This means that EELS detects all LSP modes, whereas CL detects only LSP modes radiating light. In my invited talk, I will introduce the possibilities of electron microscopy and spectroscopy in plasmonics illustrated on silver amalgam nanoparticles including the influence of experimental conditions of STEM-EELS on signal to background ratio. Further, I will present a comparative study of LSP resonances in gold nanodiscs fabricated by focused ion beam (FIB) lithography and electron beam lithography (EBL). Finally, I will discuss Babinet's principle of complementarity for disc-shaped antennas and plasmonic antennas with electric and magnetic hot spots based on Babinet's complementary (bow-tie and diabolos antennas in the form of particles and apertures). I will show a possibility to map the magnetic field by EELS by mapping the electric field of Babinet-complementary antenna.

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Keywords: EELS, Cathodoluminescence, Plasmonic Antennas, Babinet's Principle.

INVITED SPEAKERS

Id-459

**Recombinant Protein Production in Biofilms: Opportunities, Challenges
and How Microscopy Can Help**

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Abstract: Biofilms can be described as structured communities of microbial cells attached to surfaces and enclosed in a self-produced matrix of extracellular polymeric substances. Bacterial biofilms are often regarded as a problem in industrial and biomedical settings since their formation entails high costs and health risks. However, they can also be used advantageously in engineered systems such as in the production of industrial chemicals and in wastewater treatment since biofilm reactors show many advantages over suspended cell reactors, especially in their higher biomass density and operational stability. For recombinant protein production, *Escherichia coli* biofilms have great potential since this organism has been one of the preferred hosts for the past decades and it has been successfully used in metabolic engineering for the synthesis of high-value products. Despite this success, recombinant protein production using *E. coli* biofilms has been scarcely investigated for the past 25 years. Our group has recently demonstrated that biofilm systems can be considered as an alternative form of high cell density cultivation and that even without optimization of the operational variables, *E. coli* biofilm cells were able to express the model green fluorescent protein (GFP) at a level which was 30 fold higher than their planktonic counterparts. This presentation addresses the advantages and concerns of using biofilm reactors for the production of recombinant proteins and highlights the importance of microscopy in unraveling key factors regarding cell adhesion, biofilm architecture and production performance.

Keywords: Recombinant Protein Expression, Biofilms, Microscopy.

INVITED SPEAKERS

Id-470

**Application of Scanning Electron Microscopy Reveals
the Nematode Microbiota**

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Abstract: The invention of a microscope was an immense step forward for science making visible objects undetectable to the naked eye. A microscope at that time, could view objects in micrometers. Nowadays, an electron microscope with its powerful magnification and resolution allows observation of organisms or their parts in nanometer scale. This is particularly important for small organisms such as nematodes. The nematodes are generally microscopic invertebrates that inhabit terrestrial and aquatic areas and can be found as parasites on or inside other organisms. The electron microscope enables research on such tiny organisms and their parasites that are even more difficult to observe. While the transmission electron microscope (TEM) demands small and thin cross sections of samples, cut with microtome, in the scanning electron microscope (SEM) samples should be conductive enough enabling the electron beam to scan the surface. A group of nematodes known as cyst nematodes are important plant parasites causing economic loss of millions of dollars annually. The potato cyst nematodes (PCN): *Globodera pallida* (Stone) Behrens and *G. rostochiensis* (Wollenweber) Behrens are recognized quarantine organisms worldwide. Both the external and internal side of a cyst may contain bacterial and fungal antagonists as part of cyst microbiota that can cause cyst death and decline of population. Such antagonists can be used as biocontrol agents against harmful organisms. This research demonstrates the application of scanning electron microscopy in the studies of potato cyst nematodes, enabling insights into their microbiota. Results indicate that the microbiota of PCN consist of a great variety of organisms such as bacteria, actinobacteria, fungi, etc. The isolated bacteria belong to the following genera: *Bacillus*, *Paenibacillus*, *Arthrobacter*, *Devosia*, *Psichrobacillus* etc. The antagonistic fungal genera were as follows: *Fusarium*, *Oxyporus*, *Botrytis*, *Aspergillus*, and *Geotrichum*. Unexpectedly, the SEM revealed a presence of an organism not detected by the optical microscope. The organism can be a predator of eggs or juveniles within the cyst, suggesting that the list of possible biocontrol agents

should be extended with microscopic invertebrates that are often overlooked by standard techniques.

Keywords: SEM, Nematodes, Microbiota.

INVITED SPEAKERS

Id-473

Study of Organogelators by Freeze-Fracture TEM

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Abstract: Organogelators are small molecules able to self assemble to form fibrillar aggregates in solvents. The fibrils form a 3D network and lead to the formation of a gel. These fibers have widths of few tens of nm and lengths of several micrometers. It is important to know their structure to understand better the mechanism of formation and the impact on the rheological properties. Transmission electron microscopy (TEM) can characterize such fibrillar objects. However, gels are usually uneasy to observe by TEM: they are too viscous to form thin films for cryoTEM observation, and they are too soft to be sliced easily into microsections. Upon high dilution, the studied compounds form solutions, but the shapes and dimensions of the aggregates are not the same as in the gels, as it will be shown on some examples. For these reason, it is preferable to observe the natives, aggregates embedded and solvates in their solvent, at the nominal concentration. Therefore, in order to unveil the structures in organogels, our group has implemented freeze fracture to organic solvents. We will show which that this technique of preparation is efficient for organogels and allows the observation of the aggregates in their native form. Organogels made of objects with well-defined shapes and dimensions have been studied by freeze-fracture TEM and by small angle scattering. The measurements gained by both techniques were compared to assess the validity of the distances measurements in freeze-fractured samples. More generally those studies enable to explore the strengths and limitations of freeze-fracture in the study of such self-assemblies. In addition, the organogels can be transformed into aerogel and mesoporous materials. This derived materials represent can be considered as replica of the organogels; their study by SEM or tomography to provide information at higher scale, like the distribution of the aggregates or the presence of heterogeneities.

Keywords: Organogels, Freeze fracture, TEM, SANS.

INVITED SPEAKERS

Id-474

**Sparsity-Based Spectral Unmixing of Hyperspectral Imaging Data Using
Kronecker Least Angle Regression**

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Abstract: Hyperspectral imaging (HSI) systems acquire images using hundreds of contiguous wavelengths in some electromagnetic band. Hyperspectral cameras typically have low spatial resolutions, so they acquire spectral pixels as mixtures of spectra of pure materials, known as endmembers, present in the scene. Spectral unmixing refers to the process of separating these spectral pixels into their individual endmembers, along with estimating their fractional ratios known as abundances. Each spectral pixel in a given hyperspectral data cube could be modeled as a linear mixture of unknown endmembers, with unknown abundances subject to nonnegativity and sum-to-one constraints. This problem of unsupervised spectral unmixing could be considered a Blind Source Separation (BSS) problem that could be solved using a coordinate descent optimization method. This method alternates between solving a basis pursuit minimization problem to estimate the unknown endmembers that are assumed to be sparse in some dictionary and solving a constrained least-squares problem to estimate their abundances. The basis pursuit problem could be solved using an iterative thresholding method, but it would be inefficient for HSI data, as it only solves the basis pursuit problem for a single value of a required regularization parameter, typically obtained using trial and error. Least Angle Regression (LARS) could efficiently solve the basis pursuit minimization problem for all values of this regularization parameter. However, the LARS algorithm is not practically applicable to multichannel data, e.g., HSI data, as data vectorization would be necessary, and would result in very large arrays that would be extremely challenging to store and process. In this work, we exploit the properties of Kronecker products to extend the LARS algorithm to handle multichannel data without the need to construct or store very large arrays, thereby making the application of LARS to solve the HSI spectral unmixing problem practical and efficient. We refer to our extended LARS method as the Kronecker LARS (K-LARS) algorithm. We demonstrate the validity of K-LARS by using to efficiently spectrally unmix synthetic and AVIRIS

hyperspectral data, without the need for any trial and error to obtain the regularization parameter value.

Keywords: Hyperspectral Imaging, Spectral Unmixing, Least Angle Regression.

INVITED SPEAKERS

Id-479

Using SEM to Select Optimal Parameters for Microplasma Spraying and Characterize Biocompatible Coatings

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Abstract: Currently, along with widely used Titanium and its alloys, the most promising materials for the production of medical implants are Tantalum, Zirconium and Niobium. To increase the biocompatibility of medical endoprostheses that grow into the patient's bone, various surface treatment methods for these implants are used, including coatings made of biocompatible materials applied by plasma spraying. Fotovvati have compared the results of obtaining biocompatible coatings by cold and thermal spraying in favor of thermal spraying. However, despite the advantages and relative cost effectiveness of the method of thermal plasma spraying, its use for the manufacture of medical implants has not yet become as widespread. This is mainly due to the high heating temperatures of the bulk resulted from the thermal spraying process. The microplasma spraying (MPS) avoids the issue of overheating. It allows obtaining coatings from materials with a high melting point, such as Ti, Ta, Zr. MPS introduces a very small thermal impact into the substrate. The use of robotic MPS could be considered promising for the production of patient specific implants. The studies proved that it was possible to obtain coatings from biocompatible materials with the desired level of porosity and satisfactory adhesion to the substrate by microplasma spraying. A robotic assisted microplasma spraying of coatings from biocompatible materials of Titanium and Hydroxyapatite onto titanium implants has been implemented. The objective of this work is to consider the advantages and challenges of using robotic MPS for applying Zirconium coatings to medical implants using scanning electron microscopy (SEM) to analyze the effect of various MPS modes on the morphology and structure of Zr coating and its fractions. The research has been carried out at a pilot production site with an industrial complex for plasma processing of materials on the basis of Kawasaki RS-010LA, an industrial robot (Kawasaki Robotics, Japan). The robot's

arm is equipped with “MPN-004” microplasmatron produced by Paton Institute of Electric Welding (Ukraine) for microplasma spraying of wire or powder coatings. The main results of this work are the following: 1) it has been established that the main parameters controlling the size of the sprayed particles and the porosity of the coatings are the electric arc current and the plasma gas flow rate. The parameters of microplasma spraying of Zi-wire for the formation of porous coatings with rough surface have been established. 2) The advantages of applying SEM for analysis of the structure of microplasma sprayed Zirconium coatings have been shown. The analysis has been successful in assessment of the relation of the morphology and structure of coatings on the processing parameters of the MPS. The results of the research are of significance for a wide range of researchers developing the plasma spray technologies of biocompatible coatings manufacturing. The study has been conducted with the financial support of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan by the project AP05130525 “The intelligent robotic system for plasma processing and cutting of large-size products of complex shape”.

Keywords: Microplasma Spraying, Zirconium Coatings, SEM, Splats, Porosity.

POSTER SESSIONS

Id-495

Structure, Infrared Spectra and Spectroscopic Properties of Derivatives of Monosaccharides: Experimental Data, Theoretical Modeling, Results

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Abstract: Vibrational spectroscopy occupies a priority place in the investigation of the structure and properties of carbohydrates. It makes it possible to establish spectral-structural correlations that enable one to solve problems of identification of compounds and to develop methods of monitoring and diagnostics of the state of substances in technological processes and those of evaluation of the quality of finished products. In this work the effectiveness of coupled using of theoretical vibrational spectroscopy and quantum chemistry is demonstrated when applied for solving the problems of spectral-structural correlations in the studies of methyl- and nitro-derivatives of crystalline glucopyranosides and sugar epoxides. We have carried out complete calculations of vibration frequencies and intensities of a set of compounds of selectively substituted nitrates of methylglucopyranosides and epoxyranosides with a gradually complicated molecular structure (methyl- β -D-glucopyranoside, 4-O-methyl- β -methyl-D-glucopyranoside, 2,3- , 2,6- , and 3,6-di-O-nitro-methyl- β -D-glucopyranoside, 4-O-methyl-2,3-di- and 4-O-methyl-2,3,6-tri-O-nitro-methyl- β -D-glucopyranoside, methyl 2,3-anhydro-4-deoxy- α -D-ribo- hexopyranosides and methyl 3,4-anhydro- α -D- talo-hexopyranoside). Our original program technique was used combining the classical calculation of frequencies and the potential energy distributions of normal vibrations of complex molecules with the quantum-chemical intensity estimation. On the basis of a good agreement achieved with experiment (both in frequencies and intensities) a detailed interpretation of the IR spectra of compounds investigated has been performed. The absorption spectra sensitivity to the O-methyl substitution of hydroxyl groups has been discovered in details. The results of the calculations have enable us to reveal a characteristic components of diffuse bands in the 1500-1200 cm^{-1} region and to establish the regularities of formation of the structure of complex band in the region of 1150-950 cm^{-1} on O-methyl substitution of hydroxyl groups in the β -D-glucose molecule. It was studied whether the spectral features of the oxymethyl group in methyl- β -D-glucopyranoside with

additional oxymethyl and nitro substituents are characteristic. The possibilities of using these features in spectral analysis have been also investigated. The effect of the epoxy group on the bands characteristic of the pyranose ring has been analyzed. The characteristic of spectral features of oxymethyl substitution for the hydroxyl group and the oxirane ring has been studied. The IR spectrum of 4-O-methyl-2,3,6-tri-O-nitro-methyl- β -D-glucopyranoside has been interpreted in detail for the first time basing on a coupling complete calculation of the normal vibration frequencies and absolute IR band intensities as well as on their comparison with the corresponding experimental data. The results indicate that the location of nitrogroups in cellulose nitrates can be determined experimentally from separate components of complicated absorption bands in the ranges 1700 – 1600 and 900 – 800 cm^{-1} of the IR spectrum. The calculations performed have allowed to establish the analytically important IR bands for study spectra-structure correlations for methyl-, nitro- and epoxy- derivatives of glucopyranosides. The results of these calculations are in good agreement with experimental data, have high predicting possibilities and made it possible to obtain spectrum-structural dependences necessary in practice.

Keywords: Monosaccharides.

ALL SUBMISSIONS & TOPICS

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Applications of Microscopy & Spectroscopy in the Biological Sciences	Id 459 - Recombinant Protein Production in Biofilms: Opportunities, Challenges and How Microscopy Can Help
	Id 470 - Application of Scanning Electron Microscopy Reveals the Nematode Microbiota
Applications of Microscopy & Spectroscopy in the Physical/Chemical Sciences, at All Dimensional Scales	Id 473 - Study of Organogelators by Freeze-fracture TEM
Surfaces/Films/Coatings	Id 479 - Using SEM to Select Optimal Parameters for Microplasma Spraying and Characterize Biocompatible Coatings
Electron Energy Loss Spectroscopy	Id 456 - Electron Microscopy and Spectroscopy in Plasmonics
Hyperspectral Imaging	Id 474 - Sparsity-based Spectral Unmixing of Hyperspectral Imaging Data Using Kronecker Least Angle Regression
Vibrational Spectroscopy	Id 495 - Structure, Infrared Spectra and Spectroscopic Properties of Derivatives of Monosaccharides: Experimental Data, Theoretical Modeling, Results