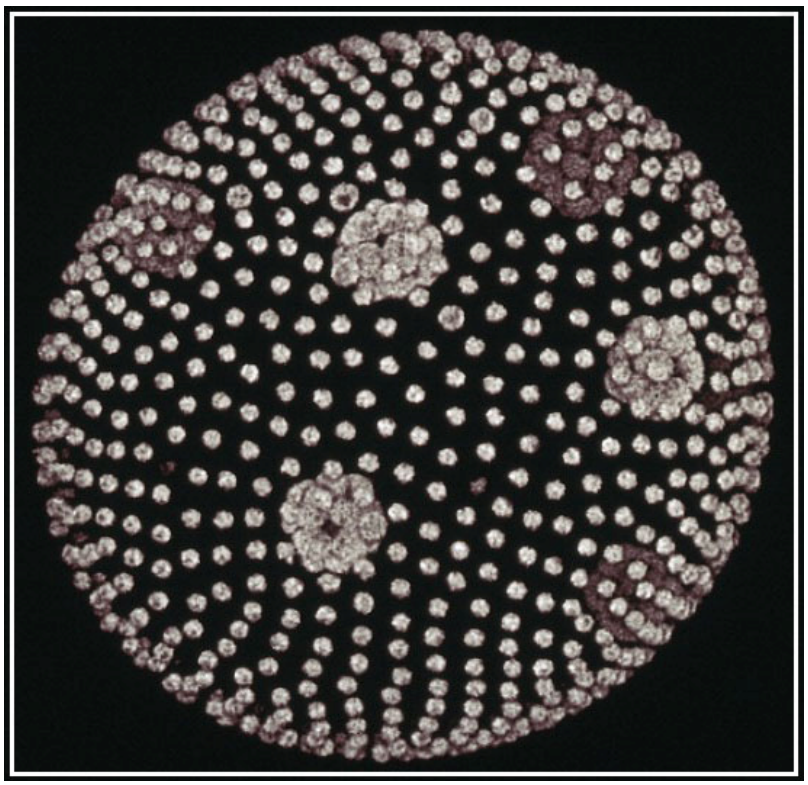
Mikroskopie 2010



Československá mikroskopická společnost

Hotel SKI, Nové Město na Moravě,

17. – 18. února 2010

Mikroskopie 2010

Pořádá:
Československá mikroskopická společnost
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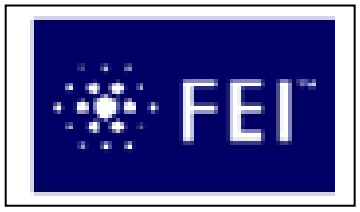
Hotel SKI, Nové Město na Moravě

17. – 18. února 2010

Sponzorují:


FEI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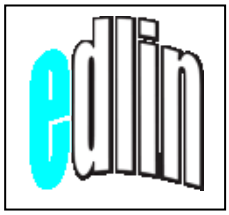
DIGITAL MICROSCOPY IMAGING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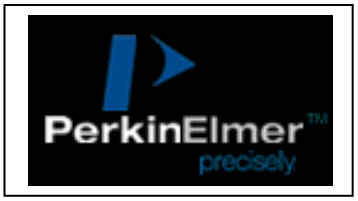
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Program

Středa 17. února

10:00 -12:00 **registrace**

12.00 -13.00 **oběd**

13:00 -13:10 **zahájení** – Pavel Hozák, předseda ČSMS

13:10 -13:20 **vyhlášení ceny ČSMS pro rok 2009 za zásluhy v mikroskopii:**
cenu získala Jana Nebesářová z Biologického centra AV ČR v Českých Budějovicích za celoživotní působení v oboru biologické elektronové mikroskopie

13:20 -13:50 **přednáška laureáta:**
Nebesářová Jana: Současné trendy v přípravě biologických preparátů pro elektronovou mikroskopii

13:50 -14:00 **vyhlášení ceny ČSMS za nejlepší PhD disertaci s významným využitím mikroskopických metod:**
cenu získala Zuzana Lhotáková z PřF UK Praha za práci "Study of coniferous needles in relation to environmental factors using approaches of quantitative anatomy and laboratory spectroscopy"

14:00 -15:10 **I. blok přednášek – zvané přednášky** (moderátor: Pavel Hozák)

14:00 -14:35 Alžběta Chorvátová, Mezinárodní laserové centrum, Bratislava: New trends in microscopy: non-invasive screening by time-resolved spectroscopy in living cells and tissues

14:35 -15:10 Richard Přikryl, PřF UK Praha: Využití mikroskopických metod a doplňujících analytických metod při interpretaci historických stavebních materiálů (s příkladem hornina a malt Karlova mostu v Praze)

15:10 - 16:40 **postery a firemní výstava s občerstvením**

16:40 - 17:40 **valné shromáždění ČSMS**

18:00 - 19:00 **řízená ochutnávka vína z produkce vinařství Kovacs**

19:00 - 23:00 **společenský večer s rautem a hudbou skupiny Tonnybluesband**

Čtvrtek 18. února

9:00 -11:00 **II. blok přednášek - firemní prezentace nových přístrojů/technik** (moderátor: Fedor Čiampor)

9:00 -9:15 FEI - Tomáš Vystavěl: FEI Magellan – Extreme High Resolution SEM

9:15 -9:30 LEICA - Andreas Nowak: Industrial sample preparation for TEM and SEM

9:30 -9:45 LEICA - Jan Pala: Optické superrozlišení v konfokální mikroskopii od Leica Mikrosystems

9:45 -10:00 MBSS - Daniel Mikolaj: New developments in MBSS science service and Gatan

10:00 -10:15 OLYMPUS - Tomáš Pop: Novinky v konfokální mikroskopii

10:15 -10:30 TESCAN - Eva Kolíbalová: Vytváření nanostruktur pomocí FIBu

10:30 -10:45 ZEISS - Peter Gnauck - High Resolution Investigation of Biological Cell Tissue using CrossBeam Technology

10:45 -11:00 ZEISS - Joerg Lindenau: The New Detection Quality in Confocal Laser Scanning Microscopy

11:00 -11:30 **přestávka**

11:30 -12:45 **III. blok přednášek - materiálové vědy** (moderátor: Ivo Vávra)

11:30 -11:45 Ivo Vávra: TEM characterization of nanocarbon/polymer composites

11:45 -12:00 Miroslav Kolíbal: Patterned growth of catalytic nanoparticles utilizing focused ion beam

12:00 -12:15 Bojan Dimzoski: Elucidation of morphology changes in immiscible polymer blends using electron microscopy

12:15 -12:30 Peter Švec: Structure and chemistry of rapidly quenched nc systems by advanced image processing

12:30 -12:45 Alena Michalcová: TEM microscopy of rapidly solidified aluminium alloys

13:00 -14:00 **oběd**

14:00 -14:45 **IV. blok přednášek – mikroskopické techniky** (moderátor: Luděk Frank)

14:00 -14:15 Vladislav Krzyžánek: Absolute mass thickness measurement using SEM

14:15 -14:30 Šárka Mikmeková: Mapping of the microscopic strain using scanning low energy electron microscopy

14:30 -14:45 Jan Valenta: Micro-imaging and micro-spectroscopy in the near infrared: technique and practical experience

14:45 -15:45 **V. blok přednášek – biologie a medicína** (moderátor: Lucie Kubínová)

14:45 -15:00 Michal Kozubek: CytoPacq: A web – based toolbox for simulation of 3D cell imaging and quality control of related image analysis

15:00 -15:15 Fedor Čiampor: Ultrastructural and EELS study of carcinoma cell line A549 treated with magnetite nanoparticles

15:15 -15:30 Jaroslav Turánek: Metallochelating nanoliposomes and their application for construction of recombinant vaccines

15:30 -15:45 Pavel Hozák: Advanced methods of multiple target detection of antigens on resin section

15:45 -16:00 **zakončení**

PŘEDNÁŠKY

(uspořádáno podle programu)

1

CURRENT TRENDS IN BIOLOGICAL SPECIMEN PREPARATION

Nebesářová J.[1,2]

[1]Biology Centre, ASCR, Branišovská 31, 37005 České Budějovice
[2]Faculty of Science; Charles University, Viničná 7, 12028 Prague 2

The specimen preparation is a crucial step in a visualisation of biological specimens in electron microscopes. The technical and instrumental development in the last ten years has enabled a large utilization of cryomethods in the procedures of biological specimen preparation. In this lecture advantages and disadvantages of cryomethods are discussed and possible combinations of traditional chemical methods with cryomehods connecting benefits of both approaches, e.g. cryofixation with a high pressure freezing followed by freeze substitution, is presented.

The last part of this contribution is dedicated to the use of extremely ultrathin sections in transmission electron microscopes working with different accelerating voltages and their cutting. They could bring new insight on cell ultrastructure in the combination with newly modified procedures of biological specimen preparation.

Supported by grant projects of Academy of Sciences of Czech Republic No. IQS600220501 and Z60220518.

2

NEW TRENDS IN MICROSCOPY: NON-INVASIVE SCREENING BY TIME-RESOLVED SPECTROSCOPY IN LIVING CELLS AND TISSUES

Chorvatova A.[1], Mateasik A.[1], Chorvat D. Jr.[1]

[1]International Laser Center, Ilkovicova 3, 84104 Bratislava, Slovakia

The spectral characteristics of each fluorophore are unique and can provide their specific identification and separation in complex biological samples. Simultaneously, time-resolved fluorescence decay patterns are additional effective means of fluorophore separation, as spectrally overlapping signals can often be segregated by distinct fluorescence lifetimes. The recent advances in technology, namely combination of fluorescence spectroscopy with time-resolved detection provides a synergic effect with great potential for gathering detailed information on biochemical, functional and structural changes in biomolecular complexes directly in living cells and tissues. This potential is reflected in better diagnostics capabilities to distinguish, for example, between normal and diseased tissues.

We focus on fluorescence measurement in living cardiac myocytes, derived from their naturally occurring autofluorescence (AF), one of the most versatile non-invasive tools for mapping of metabolic state in living tissues. Separation of time-resolved AF spectra to better understand mechanisms underlying cardiac rejection in heart transplanted pediatric patients is presented.

We identify a number of combined approaches, representing various hybrids between time-resolved, spectroscopic and imaging systems that have recently been developed and together form an important family of experimental technologies in biomedical research. Applications of time-resolved micro-spectroscopy of endogenous metabolites in living cells opens new possibilities for advanced non-invasive clinically-relevant biomedical applications.

Authors acknowledge funding from the Laserlab Europe II FP7/2007-2013 under grant agreement n° 228334, VEGA No. 1/0530/09 and APVV-20-056105.

3

VYUŽITÍ MIKROSKOPICKÝCH METOD A DOPLŇUJÍCÍCH ANALYTICKÝCH METOD PŘI INTERPRETACI HISTORICKÝCH STAVEBNÍCH MATERIÁLŮ (S PŘÍKLADEM HORNINA A MALT KARLOVA MOSTU V PRAZE)

Richard Přikryl

Ústav geochemie, mineralogie a nerostných zdrojů, Přírodovědecká fakulta, Univerzita Karlova v Praze, Albertov 6, 128 43 Praha 2

Interpretace materiálového složení, způsobu výstavby a zdrojů surovin původního výplňového zdiva Karlova mostu na základě studia původních materiálů pomocí souboru mikroskopických metod (optická mikroskopie, skenovací elektronová mikroskopie s mikroanalýzou, katodová luminiscence) doplněná o analytické rozbory pomocí infračervené spektroskopie, práškové RTG difrakční analýzy a termální analýzy. Doplněno o laboratorní rozbory fyzikálních a mechanických vlastností, s jejichž pomocí je zdivo interpretováno jako pokračování římské (antické) tradice hydraulických malt pro vysoce namáhané vodní stavby.

4

FEI MAGELLAN - EXTREME HIGH RESOLUTION SEM

Vystavěl T., Chmelík J., Seďa B.

FEI Company, Podnikatelská 6, 612 00 Brno

FEI presents the world's first extreme high-resolution (XHR) SEM. The FEI Magellan system delivers unmatched surface-sensitive imaging performance at sub-nanometer resolution, without compromising the analytical capabilities, sample flexibility or ease of use of a traditional analytical SEM. With sub-nm resolution at voltages from 1 to 30 kV, plus a large tiltable stage for 3-D surface imaging of large or multiple samples, this revolutionary new XHR SEM from FEI lets you see things you've never seen before. New and innovative electron-optical elements together with field-proven industry-leading stage technology deliver breathtaking performance and rock-solid reliability.

The Magellan combines a number of unique technical solutions: patented UC Technology, beam deceleration capabilities and a new solid-state backscatter detector work together to maximize resolution, surface detail and contrast at very low voltages. Constant power lenses optimize beam stability during operation, and electrostatic scanning improves response time. At the same time, the industry-leading, five-axis stage speeds imaging from virtually any angle.

The SEM's analytical-sized chamber features FEI's automated Loadlock for rapid throughput. The chamber's open environment easily accommodates large or multiple samples and a variety of analytical detectors. An optional acoustical enclosure is available to reduce ambient interference, enabling high-resolution, flag-free imaging in a wide variety of lab environments.

5

NEW DEVELOPMENTS IN MBSS SCIENCE SERVICE AND GATAN

D. Mikolaj, M. Baumann

MBSS SCIENCE SERVICE

This work aims at presenting the new developments in the field of sample preparation at micro and/or nano-scale. In a typical scanning electron microscope or focused ion beam imaging, analysis and/or modification of nanostructures are possible. Also the manipulation of small objects inside the SEM or FIB becomes easier through using a nano-manipulation system. On the other hand the recent research and developments in the field of life science, shows need to obtain the structures of molecules at atomic, organelles at electron microscopy, and tissue at light microscopy resolution. Often the structures of cells are so small that we need to use very high resolution, even it's crucial to visualize and reconstruct a sample to use a right method. For an understanding the cellular network is necessary to perform three-dimensional (3D) visualization as a right tool seemed the Gatan 3View^{TM}, which offers the ability to obtain in situ 3D data at remarkably fine depth resolution by operating a high-precision ultramicrotome within a variable-pressure, field emission gun scanning electron microscope (FEGSEM).

One of the most fascinating experiments in recent nanotechnology research is the precise manipulation of nanoparticles, carbon nanotubes (CNT) and other samples at a molecular- or nano-scale. The versatile and easy-to-use nano-manipulation tools, such as the MM3A-EM micromanipulator together with the MGS2-EM microgripper can transform your microscope from an observational instrument into a hands-on tool. By precision control of the probe tips and direct visual feedback of the applied force made working with the CNT's like using chopsticks. We are able to separate the bunch of nanotubes or a single nanotube. For example, another tool such as the FMS-EM, which enhanced our SEM or FIB to perform force measurement, can be useful to provide characterization of the mechanical and tribological properties of materials, such as hardness and Young's modulus.

The use of a nano-manipulation system allows the mechanical manipulation of extremely small objects under microscope control as well as the mechanical and electrical characterization of nano-structures by using special plug-in tools.

6

TEM CHARACTERIZATION OF NANOCARBON/POLYMER COMPOSITES

Vávra I.[1],Križanová Z.[1] , Lobotka P.[1], Jašek O.[2], Biederman H. [3]

[1]Institute of Electrical Engineering, Slovak Academy of Science, Dúbravská cesta 9, 841 04 Bratislava, SLOVAKIA
[2]Department of Physical Electronics, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic
[3]Department of Macromolecular Physics, Charles University, V Holešovičkách 2, Prague 8, Czech Republic

In our presentation we give the results of TEM investigation of carbon nanotubes (SWNT and MWNT) and carbon nanoparticles (expanded graphite, graphene) which are used as a filling material in polymers. Also the structure and electrical properties of prepared nanocomposites (nanocarbon/polymer matrix) will be presented. Polyphyrol, polyaniline and ethylene vinil acetate copolymer have been used as a matrtix. The nanotubes plasmochemicaly coated by polythiophene were investigated as a model composite material.

The goal of our investigations is to prepare nanocomposite which could be used for gas sensing. The origin of chemical sensing in new structural form of carbon will be shortly discussed and some preliminary results will be presented.

The work has been supported by APVV grant agency (project no. 0478-07).

7

PATTERNED GROWTH OF CATALYTIC NANOPARTICLES UTILIZING FOCUSED ION BEAM

Miroslav Kolíbal[1], Jindřich Mach[1], Tomáš Vystavěl[2] and Tomáš Šikola[1]

[1]Institute of Physical Engineering, Brno University of Technology, Technická 2, Brno 61669
[2]FEI Company, Podnikatelská 6, Brno 61200

Nanowire growth by VLS (vapor-liquid-solid) process utilizes mostly gold and aluminium nanoparticles as catalysts. The size of these nanoparticles determines the resulting diameter of nanowires. Since nanowires grow usually in the direction normal to the surface, the position of the catalytic nanoparticle on the substrate determines the position of the nanowire.

A developed technique, called the guided growth, is based on the formation of artificial nucleation sites for nanoparticles growth by focused ion beam milling. Evaporation of a selected material on a patterned surface at high temperatures results in the nanoparticle formation on nucleation sites previously created by ion beam. It is possible to fabricate almost unfaulted arrays of nanoparticles by careful tuning the ion beam parameters.

Finally, we will show our experiments on nanowire growth by germanium evaporation using a gallium catalyst. Due to the simplicity of the process it seems to be possible to perform these experiments inside an electron microscope. This would allow to observe the initial processes of nanowire growth in-situ.

8

ELUCIDATION OF MORPHOLOGY CHANGES IN IMMISCIBLE POLYMER BLENDS USING ELECTRON MICROSCOPY

Dimzoski B., Fortelný I., Šlouf M.

Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovskeho namesti 2, 162 06 Prague 6, Czech Republic

Introduction
Elucidation of the phase structure evolution in molten immiscible polymer blends during isothermal treatment at rest (annealing) is indispensable for tailoring of their end-use performance. Image analysis of SEM micrographs is a powerful tool for understanding of the changes in the morphology of polymer blends.

Experimental
Phase structure development during annealing of PP/EPR blends with different mass fraction of EPR i.e., development of the size of EPR particles with time, was observed using a SEM microscope (Vega TS 5135, Tescan) and evaluated with specialized image analyses program (NIS-Elements, Laboratory imaging).

Results and conclusion
Image analyses consisted of transforming the qualitative information from the acquired SEM micrographs into useful numbers i.e., quantitative structure descriptors such as Equivalent Diameter (ED), which were used for pursuing of changes in the morphology. ED is a size feature describing the diameter of a circle with the same area as the corresponding object on the image. Firstly, ED of EPR particles was determined by time-consuming manual analyses. Secondly, we developed a short script for further automated analyses of SEM images. ED values from the image analyses were used to determine the dependence of EPR particle size on the annealing time for various blendscompositions. Results revealed growth in the EPR particle size with time for all examined compositions of PP/EPR blends. Increase in the mass fraction of EPR led to a higher growth rate of the particle size, more pronounced during the first several minutes. Although the initial particle size has comparable value for blends with different composition, the particle size at the end of the annealing differs more substantially.

Acknowledgement: Financial support of the Grant Agency of AS CR (grant No. IAA200500903) is gratefully acknowledged.

9

STRUCTURE AND CHEMISTRY OF RAPIDLY QUENCHED NC SYSTEMS BY ADVANCED IMAGE PROCESSING

P. Švec[1], R. Portier[2], P. Švec, Sr.[1]

[1]Institute of Physics, Slovak Academy of Sciences, Bratislava, Slovakia
[2]ENSCP, Paris, France

The types of phases, phase structure, local chemical composition and defects in particles of nanocrystalline materials are of primary interest for understanding and interpretation of physical phenomena in these systems as well as of their properties. Three brief examples of application of electron microscopy for these purposes will be presented:
a) application of geometrical phase analysis and phase analysis in reciprocal space for determination of defects in nanograins of rapidly quenched and annealed Ti-based alloy;
b) application of tri-variate analysis to elemental maps of Fe-Ni-based metallic glasses containing nanocrystalline particles in amorphous matrix to determine local processes controlling nanocrystallization and
c) identification of phases at the interface of a rapidly quenched lead-free solder-Cu substrate joint using EDX mapping coupled with tri-variate analysis and spatially localized X-ray diffraction scans.

10

TEM MICROSCOPY OF RAPIDLY SOLIDIFIED ALUMINIUM ALLOYS

Michalcová A.[1,2], Vojtěch D.[1], Novák P.[1], Klementová M.[2]

[1]Institute of Chemical Technology, Prague, Department of Metals and Corrosion Engineering, Technicka 5, 166 28 Prague, Czech Republic
[2]Institute of Inorganic Chemistry of the Academy of Sciences, p.r.i., Husinec-Rez 1001, 250 68 Rez, Czech Republic

Text: Rapidly solidified aluminium-based alloys are promising structural materials. Originally, they are obtained in form of powder or thin ribbons. Hence, the production of material for structural applications includes two steps: 1) preparation of rapidly solidified alloy and 2) compactization by powder metallurgy. From materials point of view, it is interesting to study the alloy in both production steps. Structure observing of consolidated material is comparable with that of common metal materials. Consolidated materials are homogenous and they may exhibit uni axial structure deformation caused e.g. by extrusion. On the other hand, microscopy of rapidly solidified materials is more complicated. Rapidly solidified alloys prepared by inert gas atomisation are produced in form of powder. Microscopy of such alloy is possible after mounting the powder into epoxy. Thin ribbons prepared by melt spinning, can be observed in two ways. The easier way is to prepared longitudinal samples. The three millimetres discs are cut directly from rapidly solidified ribbon, ground and electropolished. Unfortunately, the ribbon thickness is variable and the perforation is not located in sample centre. This can be solved by dimple grinding and ion milling. More interesting task is to observe rapidly solidified ribbon in cross section. However, mounting of ribbons and subsequent preparation of TEM samples is not as easy as described in manuals.

11

ABSOLUTE MASS THICKNESS MEASUREMENTS USING SEM

Krzyžánek V., Reichelt R.

Institute of Medical Physics and Biophysics, University of Münster, Robert-Koch-Str. 31, D-48149 Münster, Germany

Quantitative measurements of thin samples, e.g., mass determination (MD) of macromolecular assemblies, have been performed for many years by the dedicated scanning transmission electron microscopes established at only a few institutions worldwide [1]. However, MD can also be performed by commercial high-resolution field-emission scanning electron microscopes (SEM) extended by a very sensitive annular dark-field (ADF) detector capable of single electron counting and a precise monitoring of the actual electron probe current [2]. Data processing is provided by dedicated software [3]. These extensions enable to simultaneously obtain structural information and data on the mass thickness distribution. For example, this setup allows to determine different types of mass related parameters, such as mass of globular particles, mass per unit length of filaments, and mass per unit area of sheets. Mass measurements can be performed in a large mass range from ~100 kDa to a few GDa. Although this technique was originally developed for proteinaceous specimens only, our extensions go beyond and allow also for quantitative characterization of small organic and inorganic specimens like nanoparticles or nanowires.
To test the overall performance of this technique over a wide thickness range, experimental studies with latex spheres, Epon resin sections, and C-films were performed with 30 keV electrons. The close agreement of experimental and Monte Carlo simulated data proves that the technique is capable of measurements up to the ~7-fold mean free electron path within the specimen.

[1] Adv. Imaging & Electron Phys. 159 (2009), 101-121 & 357-386.
[2] V Krzyzanek et al., Proc. MC 2005, Davos, Switzerland, 49.
[3] V Krzyzanek et al., J. Struct. Biol. 165 (2009), 78-87.
[4] This work is supported by DFG Grant RE 782/11-1.

12

MAPPING OF THE MICROSCOPIC STRAIN USING SCANNING LOW ENERGY ELECTRON MICROSCOPY

Mikmeková Š.[1], Hovorka M.[1], Müllerová I.[1], Frank L.[1], Man O.[2], Pantělejev L.[2], Kouřil M.[2]

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[2]Institute of Materials Engineering, FME BUT, Technická 2, 612 96 Brno, CR

Detection of a strain in the microscopic scale is very important under multiple circumstances.

The cathode lens mode in the scanning electron microscope enables us to detect slow but not only slow electrons backscattered under large angles from the optical axis. These electrons carry mainly crystallographic contrast based on the channelling contrast, mostly in the Mott scattering angular range.

Local strain can be effectively imaged using the scanning low energy electron microscopy (SLEEM). SLEEM is very sensitive to perfection of the crystal lattice and to arrangement of atoms within the interaction volume. Examples of the SLEEM images of ultrafine-grained copper (UFG) in as-pressed state and after annealing are shown in Fig. 1.

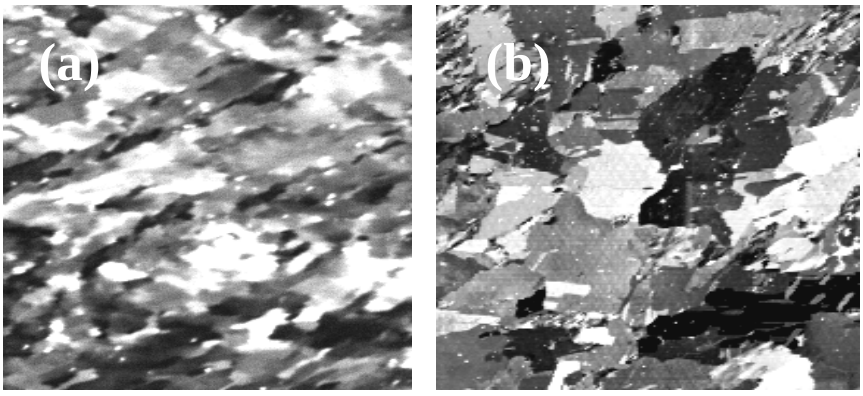

Figure 1: UHV SLEEM images of ultrafine-grained Cu obtained at 10 eV (a) as-pressed state, (b) after annealing.

13

MICRO-IMAGING AND MICRO-SPECTROSCOPY IN THE NEAR INFRARED: TECHNIQUE AND PRACTICAL EXPERIENCE

Jan Valenta

Charles University in Prague, Faculty of Mathematics & Physics, Department of Chemical Physics & Optics, Ke Karlovu 3, 121 16 Prague 2

Micro-spectroscopy in the visible spectral region is becoming a widespread and commercially available technique which is capable of detecting luminescence spectra with micron resolution at very low signal level (e.g. fluorescence of single organic molecules or photoluminescence of single semiconductor nanocrystals). Extension of these techniques to the near infrared (NIR) spectral region (here we aim to the region 800 - 1700 nm) is mainly limited by the poor properties of the NIR imaging (2D) detectors While in the visible range perfect Si-based CCD cameras (with high quantum efficiency, dynamic range and low dark noise) are available, the NIR cameras are mostly based on InGaAs detectors with substantially worse parameters. In this contribution we describe the design and construction of VIS/NIR micro-spectroscopy set-up at FMP CU in Prague. The apparatus is based on the electron-bombardment CCD camera with the InGaAs photocathode and a silicon CCD chip. We will discuss also the choice of objective lenses, filters and other components for the NIR region. Finally, a few practical applications will be shown.

14

CytoPacq: A web-based toolbox for simulation of 3D cell imaging and quality control of related image analysis

Kozubek M.[1], Svoboda D.[1], Stejskal S.[1]

[1]Centre for Biomedical Image Analysis (CBIA), Faculty of Informatics, Masaryk University, Brno

Fluorescence microscopy still faces the problem of the quality of cell image analysis results. Degradations caused by cell preparation, optics and electronics considerably affect most 2D and 3D cell image data acquired using optical microscopy. That is why image processing algorithms applied to these data typically offer imprecise and unreliable results. As the ground truth for given image data is not available, the outputs of different image analysis methods can be neither verified nor compared to each other.

In order to overcome these difficulties, we have created a toolbox [1] that can generate 3D digital phantoms of specific cellular components along with their corresponding images degraded by specific optics and electronics. The user can then apply image analysis methods to such simulated image data. The analysis results (such as segmentation or measurement results) can be compared with ground truth derived from input object digital phantoms (or measurements on them). In this way, image analysis methods can be compared to each other and their quality (based on the difference from ground truth) can be computed.

The present version of the simulation toolbox can generate cell nuclei in 3D using deformation of simple shapes and adding texture to the cell interior. Further, it can simulate optical degradations using convolution with supplied point spread function as well as electronic artifacts such as impulse hot pixel noise, additive readout-noise or Poisson photon-shot noise.

The work was supported by the Ministry of Education of the Czech Republic (Grants No. 2B06052 and LC535).

[1] Svoboda, Kozubek, Stejskal, „Generation of Digital Phantoms of Cell Nuclei and Simulation of Image Formation in 3D Image Cytometry," Cytometry Part A, 75A/6, pp. 494-509, 2009.

15

ULTRASTRUCTURAL AND EELS STUDY OF CARCINOMA CELL LINE A549 TREATED WITH MAGNETITE NANOPARTICLES

Čiampor F[1]., Vávra I[2]., Križanová Z[2]., Mésarošová M[3]., Gábelová A[3].

Virologický ústav SAV[1], Bratislava, SR
Elektrotechnický ústav SAV[2], Bratislava, SR
Ústav experimentálnej onkológie SAV[3], Bratislava, SR

The past decade has seen a remarkable increase in the use of electron microscopy as a research tool in nanomedicine. Techniques utilized in the ultrastructural domain have become extremely complex.
Magnetite nanoparticles (MNPs) are the object of rapidly-moving developmental efforts aimed at the improvement of diagnosis and treatment of cancer.
Magnetite particles used in this study were prepared by M.Timko group in the Institute of Experimental Physics, SAS, Košice. Ultrathin sections were studied by electron microscopes JEOL 1200 EX at 100 kV and JEOL 2200 FS at 200 kV with EELS analyses.
A549 carcinoma cell line was investigated after long-term (24h) exposure to nanospheric superparamagnetic magnetite particles coated with two surfactants – sodium oleate and polyethylene glycol (PEG Mw=1000) and with /or without fetal bovine serum (FBS) in the growth medium.
Macropinocytosis is a triggered process used by cells to internalise large amounts of fluids. MNPs are internalised into the vacuoles with the cytosol. EELS carbon map and iron map have shown, that MNPs are internalised together with surfactant and that they are not transported into the cell nuclei.
Internalisation is supported by the presence of FBS in the growth medium. MNPs do not penetrate the cell plasma membrane in the absence of FBS.
Cytotoxicity of individual MNPs and surfactants was evaluated using MTT, trypan blue exclusion test and LDH, and the genotoxic activity of MNPs was investigated by alkaline single cell gel electrophoresis.

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16

METALLOCHELATING NANOLIPOSOMES AND THEIR APPLICATION FOR CONSTRUCTION OF RECOMBINANT VACCINES

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Liposomes represent almost ideal carrier system for the preparation of targeted drug delivery systems and synthetic vaccines due to their biodegradability and versatility as regards the incorporation of various molecules having different physical-chemical properties. The molecules and antigens can be either sterically entrapped into the liposomes (the internal aqueous space), or embedded into the lipid membrane (e.g. membrane-associated proteins/antigens) by hydrophobic interactions. Further, they can be attached to either the external or the internal membrane by electrostatic, covalent or metallo-chelating interactions. It is possible to encapsulate simultaneously various adjuvans into the liposomes (e.g. MPL A, CpG oligonucleotides, MDP and its analogues, etc.), Preparation of metallochelating proteoliposomes, the study of their structure by DLS, GPC and TEM, AF and confocal microscopy together with examples from *in vitro* and *in vivo* studies will be presented and discussed.

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17

ADVANCED METHODS OF MULTIPLE TARGET DETECTION OF ANTIGENS ON RESIN SECTIONS

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Various means for obtaining high-yield immunogold labelling together with fine ultrastructure details and spatial statistics evaluation of immunogold labelling will be presented and discussed with a special attention to achieving multiple immunolabellings. First, various resins (Lowicryl, LR White, LR Gold, Unicryl) used for embedding were compared for their preservation of samples morphology and for their sensitivity to reveal antigens by antibodies-coupled nanoparticles on their surface. Their surfaces after cutting with a diamond knife were also assessed using atomic force microscopy, and results were statistically analyzed. Second, we introduce the use of LR White resin in combination with high-pressure freezing and freeze-substitution. We conclude that the LR White resin in combination with HPF/FS can be successfully used for fine ultrastructural immunocytochemistry allowing one to avoid the toxic Lovicryls. Third, various conditions were tested that are supposed to improve sample quality during the freeze-substitution step (water or glutaraldehyde additions). The results will be discussed in the direction of recommending an optimal approach depending on the biological sample. Fourth, further progress in multimodal detection of antigens in electron microscopy will be described.

This work was supported by the Academy of Sciences of the Czech Republic (reg. no. KAN200520704), grants LC545 and 2B06063 of the MŠMT ČR, Grant Agency of the Czech Republic (reg. no. P205/10/0348); and by the institutional grants no. AV0Z50520514, AV0Z60220518, and AVOZ40500505..

POSTERY

(uspořádáno podle jména prezentujícího autora)

1

DETECTION OF MANNOSYLATED PROTEINS IN SALIVARY GLANDS OF PARTIALLY FED FEMALES OF TICKS *IXODES RICINUS*

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The aim of our study concerns the characterization and localization of mannosylated glycans in the salivary glands (SG) of the tick *I. ricinus.* SG and saliva act as a main route for pathogens to infect the vertebrate host through arthropod vectors. Structure and properties of glycoproteins in ticks are poorly understood but it is believed that carbohydrate/protein interactions participate in host/vector/parasite relationship.

Here we demonstrate the presence of Man(α-1,3)Man in N-linked oligosaccharides of a high-mannose type, which are typical for invertebrates. Mannose-specific lectin isolated from *Galanthus nivalis* (GNA) was used for the detection of Man(α-1,3)Man in SG from partially fed females of the tick *I. ricinus*. Affinity isolated glycoproteins were characterized using western blotting and affinity/lectin labeling. Several proteins ranging from 40 to 170 kDa were distinguished and 120 kDa large GNA-specific protein was isolated. Enzymatic deglycosylation implied the presence of N-glycosidic bond and Man(α-1,3)Man structure.

Mannose localization in SG was determined on ultrathin sections by GNA-affinity labeling for TEM and florescence microscopy. Mannose specific labeling was present in granules of *b* and *c* cells of acinus type II and in *f* cells of acinus type III. This may suggest the secretion of mannose containing glycoprotein by the tick itself. Other labeling was observed on surfaces of the granular cells of acinus type III. No reaction was noted on cuticular structures of SG.

The project was supported by grants of the Grant agency of the AS of the CR (KJB600960906, Z60220518) and Ministry of Education, Youth and Sports of the CR (6007665801, LC 06009).

2

CONFOCAL LASER SCANNING MICROSCOPY AND TWO PHOTON EXCITATION MICROSCOPY AS TOOLS TO STUDY TESTATE AMOEBAE

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We applied CLSM and Two-Photon Excitation (TPE) microscopy techniques to visualize testate amoebae (TA), their test, morphology and physiology. TA are usually examined using SEM and E-SEM. It is not possible to acquire images of living TA and physiology of the amoeba inside the test by SEM or E-SEM. The goal of the study was to examine the potential of CLSM and TPE for imaging of TA.

We tried 17 fluorescent dyes to label different structures of TA. CLSM enabled us to acquire images of TA from depths up to 40 μm, whereas TPE was able to penetrate to 60 μm. Stereological methods were employed to estimate the volume of the biomass of TA visualized by CLSM.

Ecologists often face challenges with the identification of TA or with the estimation of volumes of cells. 3D reconstructions obtained from the CLSM data proved to be helpful. The amount and quality of data regarding the morphology, biometry, distribution, and ecological preferences varies among TA species. Especially, the cytoplasm, the types of pseudopodia and nuclei for the majority of these species have not been observed and this causes difficulties in their systematic identification. But CLSM and TPE methods give us images of these structures. CLSM and TPE may contribute to improving knowledge on TA morphological characteristics with implications to taxonomical and ecophysiological research.

As far as we know, this is the first time the CLSM, TPE, 3D reconstruction and stereological methods have been used for studying inner structures of TA.

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3

ELASTIC ALIGNMENT OF MICROSCOPIC IMAGES USING PARALLEL IMPLEMENTATION ON A GRAPHICS CARD

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Elastic registration is a task of finding the matching of two images, using geometric and elastic transformations, so that objects in images have the same size, position and orientation. We apply elastic registration in the framework of volume reconstruction, where an object acquired by CLSM from parallel physical sections is composed and mutual positions of the sections including deformations caused by their cutting have to be found. Our aim was to find a parallelizable algorithm that can be implemented on a graphics card using NVidia CUDA programming environment.

The correspondence between two images to be registered can be found by minimization of a functional penalizing the dissimilarity of corresponding image elements together with roughness of the correspondence function. The functional consists of two parts where the first part is the *discrete total variation* as a measure of roughness and the second one represents *L1 norm* as a measure of dissimilarity of images.

The proposed functional is well-suited to be solved by optimization of *(max,+)-labelling problems*. A parallelizable version of these optimizations represents an equivalent transformation of a *(max,+)-labelling problem*. Then the functional can be computed in a parallel way using horizontal and vertical lines of images only.

The proposed elastic registration algorithm was implemented both running on CPU using Matlab and C language and running on a graphics card using Matlab and NVidia CUDA programming environment. We found that CUDA-based implementation of the algorithm is approx. six times faster than CPU-based implementation, depending on the size of images to be registered.

CUDA-based implementation is reasonably fast, requires seconds to minutes of calculations, and, thus, can be used for practical tasks dealing with alignment of microscopic images.

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4

DIVERSE EFFECTS OF AL^{3+} IN ACIDIC MEDIUM AND ACIDITY ALONE ON TISSUE AND CELL STRUCTURE IN *LOTUS CORNICULATUS* ROOTS

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Aluminium toxicity is a serious stress factor influencing plants. The toxic form of Al^{3+} is present only in acidic soils. Thus testing aluminium effects on plants must be carried out exposing plant root systems to media with pH below 5. Great amount of data on structural and physiological responses of plants to Al toxicity is available. The aim of this work was to compare structural responses of roots exposed (for 24 h) to aluminium (2.0 mM $AlCl_3$, pH 4.0), or acidity alone (pH 4.0 without Al^{3+}) using light (Olympus BX51) and transmission electron (Tesla BS500) microscopes.

Root cells were more vacuolated in both stress conditions. Disturbed arrangement of cell files in the cortical tissue, and irregular wall thickenings reminding of local deposition of callose present frequently in the root cells occurred only in Al-treated roots. Interestingly, the structural integrity was better preserved in the cells with callose deposits. Peculiar modification of nuclei with dark central part reminding of nucleolus, surrounded by a transparent ring and a continuous envelope as well as small membranous fragments but no organelle compartments in the cytoplasm occurred only under the low pH but not with Al.

The structural responses suggest that cell wall deposits induced by Al^{3+} might have protective role and can ameliorate a parallel effects of H^+ on acid-labile bonds in pectic polysaccharide network of cells walls and, on proton toxicity in general.

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5

CHARACTERIZATION OF BIOLOGICAL OBJECTS PREPARED FOR EM STUDY USING MICRORADIOGRAPHY

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An opportunity to study internal state of biological samples prepared for electron microscopy non-destructively would be advantageous in some special cases. For example, we study hymenopteran parasitoids living inside of larvae and pupae of horse chestnut leafminer, *Cameraria ohridella* (*Insecta)*. It is almost impossible to recognize parasited developmental stages of horse chestnut leafminer. Our experimental setup provides digital high resolution X-ray in-vivo micro-radiography (maximum is about 1 μm). This setup was built-up especially for X-ray imaging as well as for the observation of real-time in-vivo processes in living organisms. The single photon counting pixel device Medipix2 was used as an image area. Hamamatsu micro-focus X-ray tube or FeinFocus micro-focus X-ray tube served as X-ray sources. The implementation of detectors Medipix2 opens new possibilities to perform non-invasive observation of living objects.

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6

THE CHARACTERISATION OF METAL NANO PARTICLES WITH ELECTRON MICROSCOPY

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The Transmission Electron Microscope (TEM) is often used as a method to assay the size of nanoparticles.
In this experiment we examine the possibilities of using a Field Emission Scanning Electron Microscope (FESEM) equipped with an Autrata improved Yttrium aluminium garnet **(**YAG) detector of back-scattered electrons for the size measurement of nanoparticles.
The diameters of different metal nanoparticles obtained from the FESEM consistently exhibits smaller values in comparison with data from the TEM. The reduction in size was influenced by a lot of parameters, e.g. the size of nanoparticle, element used for a nanoparticle production, accelerating volatage, and other working conditions in the FESEM.
The concluding data from this experiment indicates that the FESEM using for the detection of nanoparticles backscattered electrons is not a favourable tool for the size determination and characterisation.

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7

STUDY OF INESTINAL MUCOSA IN HIGH PRESSURE CONDITIONS OF VARIABLE PRESSURE SEM

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Nature state of low conductive wet biological samples cannot be studied in high vacuum environment of conventional scanning electron microscope. Samples have to be fixed, dehydrated, dried and coated before they can be observed. High pressure of water vapour in a specimen chamber of variable pressure SEM enables observation moist of even liquid biological samples without preparation, dehydration nor conductive coating.
Chemical fixation causes the precipitation, denaturing and cross-linking of cell, but it's necessary for later observation biological organisms and materials. This work deals with study of the chemically fixed but fully hydrated intestine villi in high pressure conditions of variable pressure SEM.

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[2]Neděla, V: Micros. Res. Tech. 70 (2007), p. 95 – 100.
[3]This work was supported by the Academy of Sciences of the Czech Republic, Grant No. P 102/10/1410

8

ELECTRON MICROSCOPY OF METAL NANOPARTICLES DEPOSITED ONTO THE SURFACE OF InP

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We report on the SEM characterization of metal nanoparticles deposited onto the surface of n-type InP wafers and epitaxial layers. The main motivation is to prepare high quality metal/InP interfaces, which are indispensable in high speed electronic and optoelectronic devices, radiation detectors, or gas sensors. Pd, Ag, and Au nanoparticles were deposited by electrophoresis from reverse micelle colloid solutions containing particular metal nanoparticles of 10 nm in diameter. We discuss the influence of (i) the final substrate surface treatment, (ii) the properties of the deposited colloid solution, (iii) the elecrophoretic deposition conditions (time, electrode polarity, applied voltage), and (iv) the post-deposition treatment of the layers (chemical treatment in peroxide and annealing at elevated temperatures) on the morphology of the deposited layers of nanoparticles observed in JEOL JSM 7500F scanning electron microscope. We also present some preliminary data from AFM measurements.

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9

DIVERSITY OF PLANKTIC DIATOMS OF TWO EUTROPHIC WATER BASINS IN WESTERN SLOVAKIA

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Diversity of planktic diatoms (Bacillariophyceae) of two small eutrophic water basins, at Devínske jazero in Bratislava and Modra, Western Slovakia, is presented. Our taxonomical and morphological studies were focused to the dominant species, as well as to the biologically interesting taxa.

Generally, in eutrophic water basins in Slovakia, cyanobacteria and green algae are predominating in summer and autumn periods. While in the investigated fishpond Devínske jazero in Bratislava diatoms dominated during the whole year (namely centric types *Cyclotella meneghiniana, Cyclostephanos invisitatus, Stephanodiscus hantzschii* f. *tenuis*), in the water basin at Modra, centric diatoms (*Stephanodiscus binderanus, Cyclostephanos invisitatus*) were present only in cold first spring weeks, otherwise most important dominants were chlorococcal algae (*Golenkiniopsis longispina* Korshikov) and cyanophytes from the genera *Aphanocapsa* and *Microcystis*. As the fishpond Devínske jazero is situated near the Morava River, the present diatom assemblages were influenced by this river.

Due to the ecological demands and distribution of diatoms in Europe, representatives of the genus *Thalassiosira* (*Th. pseudonana, Th. duostra*), *Cyclotella* (*C. atomus), Discostella* (*D. woltereckii*) and *Cyclostephanos delicatus* appear to be interesting for the Flora of Slovakia.

This study was supported by APVV, project No. 0566-07.

10

MORPHOLOGICAL STUDY OF ADULT PARAZITE *EUDIPLOZOON NIPPONICUM* BY DIFFERENT MICROSCOPIC TECHNIQUES

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The use of a combination of different morphological approaches (i.e. confocal, electron and light microscopy) has allowed us to map the body organization of the adult parasite *Eudiplozoon nipponicum* (Monogenea, Diplozoidae) from the gills of carp and to describe structures noticeable only when using specific microscopic method.

Based upon applying fluorescein-conjugated phalloidin as a specific probe for filamentous actin to whole-mount preparations of *E. nipponicum*, the study reports on organization of the parasite's major muscular structures. The body wall musculature is highly organized, with lattice like outer circular, intermediate longitudinal and inner diagonal somatic fibres.

Buccal suckers, glandulo-muscular organs and pharynx are the dominant structures of the parasite's forebody.

The hindbody bears the prominent attachment apparatus. The hindbody of an adult is equipped with two haptors, each with four pairs of clamps organized in two rows and controlled by extrinsic muscle bundles.
The doubled reproductive tract, consisting of female and male organs, almost fills the adult's entire body. Walls of the reproductive organs have well-developed, densely assorted circular and longitudinal muscle fibres while diagonal fibres are absent.

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11

EXPERIMENTAL APPARATUS FOR AUTOEMISSION CATHODE

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An experimental apparatus for cathode activation, debugging and testing was designed and made up. The high-vacuum bakeable apparatus is pumped by one ion pump, ultimate pressure is better than 10^{-7} Pa. Dry fore-vacuum pumping system consisting of turbomolecular pump and diaphragm pump is used.

The function part of the apparatus consists of cathode, Wehnelt cylinder, anode, anode aperture and metallized scintillator. The distance between the anode and the Wehnelt cylinder can be set in a range of 0÷10 mm. Power supply for cathode has an independently regulated power supplies for the cathode high voltage (HV), the Wehnelt cylinder bias and the cathode heating. The measurements of a cathode emission current is included in HV power supply and measurement of a beam current from electrons bombarding scintillator is possible using external picoammeter. Apparatus is equipped with two sight glasses for visual process monitoring. The first one is positioned on the optical axis of the apparatus behind the scintillator and allows the observation of the cathode emission pattern. A camera can be attached to this glass window. The second one is positioned radially and it allows the observation of the space between the Wehnelt cylinder and the anode and also of the part of cathode standing out of the Wehnelt cylinder. Relative measurements of the distance between the anode and the Wehnelt cylinder can be made using joined telescope.

The purpose of the apparatus is to prepare autoemission cathodes in conditions geometrically nearly equivalent to the conditions of the final device where the cathode will be operated.

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[2] This work was supported by GA ASCR, grant no. IAA100650803 and MPO TIP project no. FRTI1/576.

12

TOWARDS UNDERSTANDING A ROLE OF NUCLEAR MYOSIN I IN TRANSCRIPTION

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Nuclear myosin I (NM1) is a 120 kDa molecular motor localized in the cell nucleus. It consists of a single head or motor domain which binds actin and has ATPase activity, neck domain which provides a site for calmodulin binding and regulation of motor activity by calcium, and tail domain which binds cargo through phosphatidylinositol-4,5-biphosphate (PIP_2). NM1 was shown to be involved in chromatin remodeling, repositioning of transcriptionally activated regions in the nucleoplasm and also in transcription with RNA polymerase I. Here we tested whether NM1 also takes part in transcription with RNA polymerase II and III and try to resolve the mechanism of NM1 involvement in transcription by mutational analysis of NM1. We observe a significant decrease in transcription elongation after NM1 immunodepletion by all three RNA polymerases in two different in vitro transcription systems. Furthermore, NM1 co-purifies with the promoter region during the transcription reactions. The NM1 immunodepleted transcription systems allows us to test whether add-back of wild-type NM1 or mutants with disabled actin binding, motor function, and PIP_2 binding would restore normal transcription. For this we are currently preparing NM1 and its mutants in the insect cells.

13

SCREENING OF SYNTHETIC NUCLEOTIDE-ANALOGS AND PEPTIDES AS INHIBITORS OF *CANDIDA ALBICANS* PROLIFERATION AND MORPHOTYPE CHANGES

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Candida albicans is a commensal fungus which can turn from saprophyte – yeast growth to pathogenic – hyphae or pseudohyphae forms associated with Candida invasiveness. Therefore, the inhibition of morphotype changes present desirable goal of antifungal therapy.

The panel of synthetic nucleotide-analogs and peptides was used for identification of the *C. albicans* inhibitors based on their candidastatic activity and inhibition of blastoconidia to hyphae transition.

The *C. albicans* blastoconidia were grown in the presence of tested chemicals in RPMI 1640 medium at 37°C for 24, 48, and 72h. The inhibitory effect of tested chemicals was determined by counting the cell number using Bürker chamber and death-live cell FACS analysis using eosin Y staining.

The peptides denoted LL8 and LL8/12 exhibited strongest inhibitory activity. Furthermore, both peptides inhibited the blastoconidia to hyphae transition after 48h of incubation and affected Candida cytokinesis as evident from phase contrast and fluorescence microscopy (DAPI and Rylux).

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14

CVD PRODUCTS of $Ge_2(CH_3)_6$ - $(C_2H_5)SiH_3$

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Samples were prepared by conventional pyrolysis of hexamethyldigermane $Ge_2(CH_3)_6$ and ethylsilane $(C_2H_5)SiH_3$ using Low Pressure Chemical Vapor Deposition (LPCVD). The pyrolytic apparatus worked in a flow-through mode at total vapor pressure of 180Pa, from which there was 110Pa of $Ge_2(CH_3)_6$ and 70Pa of $(C_2H_5)SiH_3$. Deposits were collected on copper substrate at 500°C for 70 minutes.

The deposits contain flowerlike aggregates of micrometer-size GeSi platelets and GeSi nanowires. The platelets exhibit different shapes from hexagonal to elongated serrated leaves. They are up to several micrometers in size but have thickness of only about 40nm. The platelets grow perpendicular to <111> direction. However, the electron diffraction patterns show interesting modulations probably caused by Si-Ge ordering. Nevertheless, the electron diffraction/ordering might also be affected by the presence of oxygen which was detected by EDS analysis.

The nanowires are only about 10nm thick. They are single crystals which grow in <111> direction and contain no defects. Some nanowires are wrapped in amorphous jacket. In addition, some nanowires are decorated with isometric Ge nanocrystals about 5nm in diameter.

SEM, TEM, HRTEM, EDS, ED, NBD, PED results will be presented.

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15

ANALYSIS OF A COMBINED ELECTROSTATIC AND MAGNETIC OBJECTIVE LENS

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Cathode lens (CL) is an electron optical element commonly used in the photo-emission electron microscopy (PEEM), low energy electron microscopy (LEEM) and last but not least in the scanning electron microscope (SEM) [1]. The electrostatic field of the cathode lens decelerates primary electrons down to arbitrarily low energy. Lenc and Müllerová [2,3] derived approximate analytical expressions for the axial aberration coefficients for the CL alone and for CL in combination with a magnetic focusing lens. The sequential arrangement of electrostatic and magnetic fields was considered.

In order to analyse the optical properties of the CL in combination with a focusing magnetic lens (sequential fields) and a focusing immersion-magnetic lens (overlapped fields) we employed the EOD software [4].

The spherical and chromatic aberration coefficients and the spot size were calculated for the landing energy varying from the primary beam energy to only 1 eV. The 3D distribution of the electrostatic and magnetic fields in the specimen chamber principally influences trajectories of signal electrons.

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16

PHYSICAL AND BIOLOGICAL CHARACTERISATION OF LIPOSOMAL ANTICANCER DRUGS

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Liposomes represent advanced nanodelivery systems for wide range of drugs. Paclitaxel (PTX) is approved for the treatment of ovarian and breast cancer. In general, the developed liposomal PTX formulations are troubled by low PTX encapsulation capacity and PTX crystallisation. We developed and characterised stable lyophilised liposomal PTX preparation. PTX crystallisation and morphology of the liposomes was monitored during storage by DLS, phase contrast microscopy and TEM. α-Tocopheryl succinate (α-TOS) is a semi-synthetic analogue of α-tocopherol with selective toxicity to the cancer cells. The hydrophobic character and low aqueous solubility of α-TOS predetermine liposomes as suitable delivery systems. We developed and characterised a stable lyophilised liposome based α-TOS formulation. Stabilised liposomal α-TOS formulation was prepared by lyophilisation in the presence of sucrose. The size distribution, ζ-potential and morphology of the liposomes were preserved during storage in the lyophilised form as assessed by DLS, Doppler velocimetry and TEM. The anticancer effect of α-TOS and other analogues was tested *in vitro* and *in vivo* on various cancer cell lines and mouse models.

Acknowledgement
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PLANAR-SECTION TEM SPECIMENS OF MULTILAYER STRUCTURES

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The structural analysis of epitaxial III-V multilayers need detailed analysis of each interface by electron diffraction. For the characterization of all multilayer interfaces we used the technology of planar section TEM specimen [1]. By this method we can perform the detailed structural analysis of all multilayer interfaces in one TEM specimen.

The specimen was thinned from the substrate side by mechanical grinding and polishing. Finally ion-milling by Ar^+ ions (4 keV) was used to open hole. At the hole edges we can perform the TEM structural investigation of multilayer on its surface. After this the TEM specimen is carefully ion-milled from the surface side. In the process of milling from surface side the specimen is clamped eccentrically. (The milling from the surface was controlled by optical microscopy.) As a consequence the multilayer is thinned unevenly along the perimeter of the hole. It means that in each perimeter spot we observe another planar section of the original multilayer.

The method is demonstrated on the TEM analysis of epitaxial multilayer InAs/InMnAs/GaAs.

[1] Vavra I. et al, phys.stat. sol. (a) **150**, 371 (1995).

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FIB TECHNIQUE APPLICATIONS IN MATERIAL RESEARCH

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Commercial availability of instruments equipped with focused ion beam (FIB) allows development of novel techniques in materials science. In this contribution, two applications of FIB are presented.

We present the use of the microcompression technique to measure unique mechanical properties of thin films. This method combines the preparation of micro-sized specimens of a given geometry (pillars in our case) using a focused ion beam followed by compressive test by a nanoindeter. An Al–Cu thin film was studied. The cylindrical pillars with diameter of about 1.3 □m and their height was determined by the thickness of the film 2 □m were prepared and examined. The optimized and reproducible process of the pillars preparation by FIB milling was found. The preparation process parameters were optimized considering both the specimen geometry and the preparation time. Successfully executed compressive tests confirmed suitability of the specimens for microcompressive testing.

In the second case, the FIB technique together with other advanced microscopic techniques were applied to study early microstructural changes leading to crack initiation in fatigued polycrystals. True shape of the surface relief of the slip bands (extrusions, intrusions) and the path of initiated fatigue cracks were assessed in three dimensions by serial FIB cross-sectioning (FIB-SEM tomography). The potential of FIB technique in fatigue crack initiation studies in polycrystals is highlighted.

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NEW METHOD FOR MEASUREMENT OF CHLOROPLAST DENSITY IN MESOPHYLL OF NORWAY SPRUCE NEEDLES

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Chloroplasts, as organelles of photosynthesis in plants, belong to the most important components of plant cells. Chloroplast density in mesophyll of needles represents one of the quantitative anatomical characteristics, which could be influenced by external conditions. Particles in 3D can be counted in an unbiased way by the optical disector method, however, according to our knowledge, this method has never been used for estimating chloroplast number. In this study we applied the disector method to counting chloroplasts using 3D images of needles acquired by confocal microscopy.
Needles collected from Norway spruce trees were stored frozen before processing. Several cross-sections were cut off by a hand microtome in a systematic uniform randomly chosen positions along the needle and their images were captured by a Leica SP2 AOBS confocal microscope using 10x objective. Then systematically sampled series of optical sections were acquired with higher resolution using 63x objective and 2x zoom. The series were then used for counting chloroplasts by the optical disector. The chloroplast density was calculated by the ratio of chloroplast number and mesophyll volume, which was estimated by a point counting method.
The optical disector method proved to be suitable for unbiased counting chloroplasts in needles. We are going to use this method for studies of effect of elevated CO_2 concentration on the anatomical structure of sun and shade needles.
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WETSTEM: TRANSMISSION ELETRON MICROSCOPY OF SMALL PARTICLES IN WATER

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Introduction

Evaporation of the water from water dispersed or swollen samples may result in collapse these systems and aggregation of the particles as observed in a conventional transmission electron microscopy (TEM). That is why FEI company developed a detector combining scanning transmission electron microscopy (STEM) and environmental scanning electron microscopy (ESEM), which is called STEM-in-ESEM or wet-STEM [1]. Here we report the first results obtained in the wet-STEM mode.

Experimental

Wet-STEM detector (won in FEI world contest by one of the co-authors (MS)) was installed in a FESEM microscope (Quanta 200 FEG, FEI, Czech Republic). Three types of samples were observed in wet state (+2 °C, ≈ 710 Pa, humidity ≈ 100 %): water-dispersed polymer microspheres (average diameter ≈ 1 μm), solution of polymer micelles (≈ 80 nm) and colloidal solution of gold nanoparticles (≈ 30 nm).

Results and Conclusion

All type of particles (polymer microspheres, micelles, colloidal metal nanoparticles) was successfully visualized in the wet-STEM mode. In comparison with a standard TEM microscopy of dried specimens, wet-STEM exhibited the following features: (i) contrast was higher, (ii) resolution was lower, (iii) sample tilt was impossible and (iv) particle agglomeration was similar.

Acknowledgement: KAN200520704, GACR P205/10/0348 and P208/10/0353, MSMT 2B06053, FEI Company.

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EGGS OF BASAL TAPEWORMS (CESTODA)

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Tapeworms (Cestoda) bear a number of morphological and biological characteristics unique among animals, including extremely high reproductive capacity and morphology of eggs. The morphology of tapeworm eggs has proven to be a key characteristic in their diagnostics, but it has also extraordinary phylogenetic importance. The principal objective of the recently approved project by the Grant Agency of the Czech Republic is to obtain new data on egg morphology of different orders of cestodes considered to be most primitive (basal) and that possess polylecithal eggs using “modern” techniques of electron microscopy.

Transmission electron microscopy (TEM) has proven to be crucial for the advancement of modern cell biology. However, better understanding of the composition and functions of biological models is not possible without obtaining images of intact assemblages *in situ*. Cryofixation by high pressure freezing will be applied to selected model species. In some cases, life cycles will be studied experimentally to obtain ontogenetic stages (eggs and early larval stages) suitable for a study using “modern” techniques of electron microscopy.

Application of modern methods of electron microscopy (cryomethods, tomography) combined with studies on life cycles will represent a key innovation, which should help us better understand the evolutionary history and diversity of cestodes.

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NOVEL PREPARATION AND CHARACTERISATION OF MICROBUBBLES

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Microbubbles have an increasing role in ultrasound diagnosis and delivery of the therapeutic agents including genetic material (DNA/RNA constructs), proteins and chemoterapeutic agents. Especially delivery of genetic material for gene therapy purposes is greatly enhanced by ultrasound in the presence of microbubbles, which have become a necessary tool for the treatment and diagnostics in medicine.

Lipid microbubbles (MB) were prepared from liposomes composed of 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (with addition of 1,2-Dioleyl-*sn*-Glycero-3-phosphoethanolamine-N-Carboxyfluorescein) at final concentration 10 mg/ml.

The created microbubbles' size and concentration were characterized using optical (light) and fluorescence microscopy (*Nikon Eclipse TE200),* confocal microscopy (with addition of recombinant green fluorescent protein – rGFP) *(True Confocal Scanner, Leica TCS SP2)* and transmission electron microscopy *(EM Philips 208 S, MORGAGNI software, FEI, CZ)*. Afterwards, the microbubble size was measured using Static Light Scattering *(Horiba LA-300 Laser Diffraction Particle Size Distribution Analyzer)* and Flow cytometry *(FACScan cell analyzer, Beckton-Dickinson, Franklin Lakes, NJ).*

All methods showed to be relevant and significant ways of microbubble characterization.

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INNOVATIVE RESEARCH OF MODERN TYPES MAGNETIC LENS AND CHAM-BERS OF ELECTRON MICROSCOPES - MAGNETIC PROPERTIES OF LENS STEEL AND SIMULATION OF LAYOUT MAGNETIC FIELD

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The paper deals the basic objectives and results of the project which is specialized on the innovation research of the magnetic lens and chamber from electron microscopes. The first part of the project solution contains the analyses of magnetic properties of the steel which is designed for construction of chamber and lens. The other part of project is aimed at the model simulation of the electromagnetic field layout on type electron microscopes chamber. This project and paper were created by financial support of state budget through the Ministry of Industry and Trade MPO-CR, project n. FR-TI1/334.
The measurement of magnetic properties of steel lens is made on professional and unique measurement systems which could be used to define both the DC quasistationary and AC dynamic BH hysteresis and magnetization characteristics for various frequencies and intensities of actuating magnetic field. Mentioned measuring systems are Remacoph a Pemagraph.

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ENDODERMAL SUBERIN LAMELLAE – BARRIERS CONTROLLING TRANSPORT OF IONS THROUGH THE APOPLASMIC SPACE – DEVELOP INDEPENDENTLY IN EACH ROOT OF THE SAME INDIVIDUAL DEPENDENT ON THE ENVIRONMENTAL CONDITIONS

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Deposition of suberin in the form of suberin lamellae on the inner surface of endodermal cell walls of the roots plays a key role in the control of material transport through the apoplasmic space.
The aim of this study was to find out if the development of the endodermal suberin lamellae in different adventitious roots of the same plant is between the roots coordinated or it is independent.
Adventitious roots of the same *Allium cepa* plant were splited to the two pots containing either control medium, or cadmium medium in the both of them, or control medium in the first pot and cadmium medium in the second pot. The development of suberin lamellae in the endodermal cells were detected on cross sections of the adventitious roots by staining with Fluorol yellow 088 and observation under fluorescence.
Cadmium treatment significantly decreased the distance of suberin lamellae development from the root apex compared to the control medium. Endodermal suberin lamellae develop independent in each root of the same *Allium cepa* plant. Their development is dependent on the place specific environmental conditions.

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COMPARISON OF ESEM AND SEM IN MORPHOLOGY STUDIES OF PARASITIC VERTEBRATE NEMATODE

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Ascaridoid nematode species *Brevimulticaecum heterotis* obtained by parasitological dissection from the African bonytongue, *Heterotis niloticus* (Osteoglossidae) collected in Senegal has been chosen for studies by scanning electron microscope to redescribe its external morphology. Scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) were used for the study.
For SEM, specimens were fixed with hot formalin. They were dehydrated, critical-point dried, mounted on stubs and coated with gold. For ESEM, fixed specimens were hydrated with water; desiccation was not applied. Quanta™ 3D FEG and Quanta™ 250 FEG in SEM and ESEM mode were used for specimen examination.
Combination of both techniques brings new level to nematode investigation. While SEM remains unsubstitutable method for study of nematode morphology due to its ease of use, ESEM is an unexceptionable instrument when there is only one unique specimen that cannot be destroyed or must remain available for molecular studies.

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PREPARATION OF MONODISPERSED NICKEL-CHELATING NANOLIPOSOMES FOR IMMOBILISATION OF HIS-TAGGED PROTEINS: STUDY OF THEIR STRUCTURE BY TEM, AF MICROSCOPY AND DLS

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We designed and constructed flow-through ultrafiltration cell for reliable preparation of liposomes from lipid micelles by detergent removal method. Well defined lipid micelles were prepared from EPC by application of sodium cholate and ethanol, so that we avoided laborious and a non-economic method based on re-solubilisation of liposomes. The influence of ionic strength, lipid concentration and flow rate (fast and slow detergent removal) on final liposome structure and size distribution were examined. By standardised procedure we were able to prepare monodisperse liposomes (PI <0.1). The size can be tuned within the size range of 30 – 90 nm. The mean size depends on the ionic strength of the buffer used. Metallo-chelating liposomes were used for binding of various recombinant antigens e.g. rHSP90, HIV-1 rgp120, rGFP, rOSPC and recombinant antigen from circovirus 1. Preparation of metallochelating proteoliposomes, the study of their structure by DLS, GPC, TEM, AF and confocal microscopy together with examples from *in vitro* and *in vivo* studies will be presented and discussed.

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AMORPHOUS BILAYER RIBBONS STUDIED BY TRANSMISSION ELECTRON MICROSCOPY AND PHYSICAL PROPERTIES MEASUREMENTS

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Amorphous bilayers with layers of Fe-Si-B and Co-Si-B have been prepared by planar flow casting from a single crucible with two nozzles close to each other and with a partition between them forming two separate vessels. Such an arrangement has allowed us to obtain ribbons with two homogeneous layers, one on top of the other, along the whole ribbon length with high quality surface and with contact interlayer having submicron thickness. The character of the interlayer has been investigated by Cross-sectional TEM, XRD, SEM/EDX and resistometry in the as-quenched state and after annealing below and after crystallization. From the results it seems evident that the process of connection of the two layers takes place below the crystallization temperature by mutual interdiffusion of component atoms, thus giving rise to mechanically solid connection between the two layers localized to a narrow well-defined interface.

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ACQUIARIUM: FREE SOFTWARE FOR IMAGE ACQUISITION AND IMAGE ANALYSIS IN CYTOMETRY

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Acquiarium (http://cbia.fi.muni.cz/acquiarium/) [1] is free software for carrying out the common pipeline of many spatial cell studies using fluorescence microscopy. It can be used for image acquisition and/or image analysis. The image acquisition part is optimized for fast image capture using spinning disk microscopes, which are suitable for 2D or 3D imaging of both fixed and living cells. The image analysis part is focused on comfortable work with a collection of many 3D images. It has a modular design and is extensible via plug-ins. Acquiarium can execute a batch of plug-ins on selected images, which makes extensive analyses possible.
Acquiarium is intended for the following main applications:
• **Quantification of objects**, especially dot counting in 2D, 3D, as well as time lapse images and counting the number of larger domains in cells, e.g., the number of nucleoli or protein sites.
• **Spatial arrangement studies**. For example, colocalization studies, or radial distribution of hybridization dots in nuclei.
• **Measurement of geometrical and shape parameters** of cellular structures. For example, volume, surface area, etc.
The Acquiarium software contains general purpose algorithms suitable for various image cytometry applications.
Grant sponsor: Ministry of Education, Youth and Sports of the Czech Republic (project No. 2B06052).

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COMPENSATION OF LATERAL ILLUMINATION INHOMOGENEITIES IN CONFOCAL MICROSCOPY IMAGES USING MORPHOLOGY FILTERS

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Due to multiple distortion effects, images acquired by confocal laser scanning microscopy (CLSM) of even homogeneous specimen regions exhibit irregular intensity variations, e.g. darkening of image edges and lightening of the centre, which complicates image postprocessing such as image stitching or registration of images of successive sections for three-dimensional (3D) reconstruction.
Previously, we developed a fast method for lateral inhomogeneity compensation based on estimating a spatially variable gain which models the adverse effects of uneven illumination, and multiplying acquired CLSM images by the inverse of the estimated gain. Gain estimation was based on a mathematical morphology operator called the upper Lipschitz cover. The method worked well for images with pronounced biological structures without too much noise, but did not prove equally efficient for noisy images with relatively flat brightness profile. In these cases, due to image noise, the spatially variable gain estimate returned values which did not correspond to actual dye distribution.
In this study, we improved the Lipschitz cover-based method by preprocessing raw images using another mathematical morphology operator, the median filter. The modified method yields much better results than its predecessor, both for noiseless and noise-distorted images. The proposed approach is fast and thus well suited for routine preprocessing of large stacks of CLSM images: As an example, brightness compensation of a stack of sixty 512x512x8bit grayscale images of a cross section of a rat brain takes less than 12 seconds on a 3GHz Intel Core Duo CPU. The method is fully automatic and does not need any special calibration images.
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STUDY OF INTRINSIC STRESS IN CN_x FILMS PREPARED BY MAGNETRON SPUTTERING DEVICE USING ELECTRON MICROSCOPY

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Preparation and analysis of amorphous carbon nitride thin films (CN_x) deposited by RF magnetron sputtering device on silicon wafers (100) is reported. The films have been prepared from graphite target (purity: 99.9999 %) in a pure nitrogen atmosphere. The substrate bias voltage was systematically varied from 0 V to 55 V. This change is one of the parameters for reduction of intrinsic stress (σ) in this type of films.

The elimination of residual stress is necessary for preparation films with a good adhesion to substrate. The intrinsic stress was calculated from the curvature of monocrystalic substrate with a use of goniometer. The influence of residual stress on the quality of CN_x films has been studied in details by scanning low energy electron microscopy (SLEEM) and transmission electron microscopy (TEM). While compressive stress (coming from ex situ contamination) leads to wrinkling and film delamination, tensile stress (originating from growth) can cause the fracture of thin films.

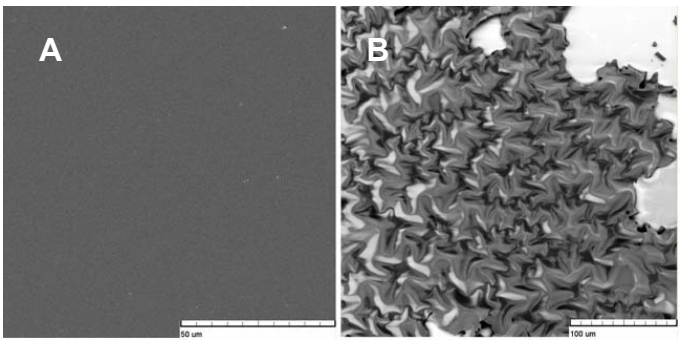

Figure 1: SLEEM images of : (A) CNx film, σ = 459 MPa obtained at 3 keV, (B) CNx film, σ = 88 GPa obtained at 1 keV.

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FORMATION OF Sn/Ag/C NANOALLOY THROUGH LASER ABLATION OF Ag TARGET AND SIMULTANEOUS DECOMPOSITION OF TMT IN THE GAS PHASE

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Increasing environmental and health concerns over the toxicity of lead-based solders have provided an inevitable driving force for the development of lead free solders. The Sn-Ag alloys are widely considered as a promising alternative due to higher strength and superior resistance to creep and thermal fatigue.

We have examined a novel approach to gas-phase deposition of nanosized Sn-Ag alloys embedded in a carbonaceous phase based on IR laser ablation of Ag in a plume (dielectric breakdown) of tetramethyltin (TMT). In this approach, Ag nanoparticles are propelled from the target and mixed with Sn clusters produced in the gas phase by the decomposition of TMT. The resulting Sn/Ag structures are during their formation subject to heating and subsequent cooling within some ms. The decomposing TMT, serving as a carbon source, may provide additional stabilization of these metastable structures.

We obtained thin Sn/Ag/C films that were characterized by scanning electron microscopy (SEM), Raman spectroscopy; micro X-ray diffraction (μ-XRD) and high-resolution transmission electron microscopy (HRTEM) and we draw some conclusions on the structure of these materials. We revealed that obtained materials are consist blend of amorphous and crystalline phase correspond to amorphous Sn-Ag-C alloy and crystalline nanobodies of β-Sn covered by crystalline shell.

This work was supported by Ministry of Education, Youth and Sports of the Czech Republic (Grant no. LC523).

The use of a nano-manipulation system allows the mechanical manipulation of extremely small objects under microscope control as well as the mechanical and electrical characterization of nano-structures by using special plug-in tools.

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MOLECULAR CYTOGENETICS OF FISH TAPEWORNS

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Molecular cytogenetics involves combination of molecular biology and cytogenetics. In general, it utilizes a series of techniques referred to as fluorescence *in situ* hybridization, or FISH, in which labeled DNA probes are commonly used to localize specific DNA sequences or identify chromosome region and/or individual chromosomes. This approach has already been applied in fish tapeworm species (Cestoda, Platyhelminthes). Here we present examples of several differential light-microscopy and fluorescent methods including FISH with small subunit of ribosomal genes (rDNA) in chromosome complements of four caryophyllidean non-segmented tapeworms, namely diploid *Caryophyllaeus laticeps*, *Caryophyllaeides fennica* and *Khawia saurogobii*, and triploid *Atractolytocestus huronensis*. In all the species, rDNA-FISH successfully localized nucleolar organizer regions (NORs) including inactive clusters of rDNA. The data improved alignments of the chromosome pairs and, being phylogenetically informative, have great potential to be applied in evolutionary studies of this group of parasites.

This work was supported by the projects of the Grant Agency of the Slovak Republic (VEGA 2/0014/10 and VEGA/0148/09), by the National Science Foundation, USA (PBI award Nos. 0818696 and 0818823) and the Grant Agency of the Czech Republic (no. 524/08/0885) and from research projects of the Institute of Parasitology (Z60220578, LC 522).

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SEM IMAGING OF STRUCTURES FABRICATED BY SHADOW NANOSPHERE LITHOGRAPHY

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Self-assembly of the hexagonal closed-packed monolayer of latex spheres, is a basis of the nanosphere lithography. This technique is used for creation of masks for deposition or sedimentation of various materials. It is known that nanosphere lithography can be used to make honeycomb lattices of triangularly shaped islands on various substrates. Using spheres with different diameters, one can change the spacing and size of periodically arranged islands.

The method of nanosphere lithography will be utilized, in combination with other methods as sputtering and deposition, electrolytic methods and sedimentation. The goal will be the preparation of metallic structures, e.g. Pd, Ni, Cu, Ag.

By varying the position of the substrate with respect to the evaporation source during the sample preparation, we make morphologies such as dots, triangles and rings, that are not accessible by the standard nanosphere lithography.

Planar structures of Pd, Ag, and ZnO were prepared and studied. Final structures were analyzed by AFM and SEM.

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DIRECT IMAGING OF THE LOCAL DENSITY OF STATES ABOVE VACUUM LEVEL BY VERY LOW ENERGY ELECTRON REFLECTIVITY

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The local density of states is an important characteristic of solids, in particular the crystalline ones. One way of probing the local density of states is to measure the reflectivity of very low energy electrons (units to tens of eV) from the sample surface. The reflectivity is inversely proportional to the local density of electronic states coupled to the impinging electron wave [1].

A scanning electron microscope equipped by the cathode lens [2] can effectively map the reflectivity of very low energy electrons with a high lateral resolution. The cathode lens is a zero working distance electrostatic lens with the specimen serving as the cathode at a negative potential, thus allowing one to decrease the energy of the incident electrons arbitrarily.

Since differently oriented crystal faces have specific densities of states and therefore specific energy dependence of the very slow electron reflectivity is expected, a series of demonstration experiments has been carried out on single crystal aluminum (111) and (100) specimens. The reflectivity was compared to theoretical calculations [3,4] and influences of surface cleanliness and other conditions were reviewed. [5]

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OXYGEN ORDERING IN $La_{1-x}Sr_xCoO_{3-y}$ CONDUCTIVE OXIDE

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$La_{1-x}Sr_xCoO_{3-y}$ (LSCO) is a non-stoichiometric oxide drawing attention due to its magnetoresistivity, conductivity and perovskite-like structure. It is a mixed conductor with strong dependence of conductivity on oxygen content. Its electrical behavior is well described, but there is still a discussion concerning phase structure and phase stability.
We deposited epitaxial LSCO thin films on single crystal $SrTiO_3$ by MOCVD. With help of annealing in reducing atmosphere we changed content of oxygen in the oxide and observed changes by TEM. We observed three types of electron diffraction patterns in dependence of different sample treatment. Their presence confirmed oxygen ordering in pseudo-cubic lattice with double and triple period in one lattice direction. The triple period superstructure is rarely mentioned in literature, we suppose it is due to its instability. In our thin films it is stabilized by strain induced by epitaxial growth on single crystal substrate.

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MORPHOLOGY AND CRYSTALLINE STRUCTURE OF SYNTHETIC AND COMERCIAL TiO_2-BASED NANOFIBRES

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Introduction
Titanium oxide nanotubes (Ti-NT), synthesized in our laboratory, represent a new morphology of TiO_2. Their morphology and crystalline structure is different from all common modifications of TiO_2, i.e. rutile, anatase and brookite. Here we demonstrate that they are also different from commercially available TiO_2-nanofibers from Elmarco company.

Experimental
Our synthesis of TiO_2-based nanotubes was based on the hydrothermal treatment of TiO_2 powder and concentrated NaOH solution as described elsewhere [1]. Commercial TiO2-nanofibers were obtained from Elmarco company, Liberec, Czech Republic. TEM microscope (Tecnai G2 Spirit Twin, 120kV) was employed in studying of morphology (BF), purity (EDX) and crystal structure (SAED, DF) of both products.

Results and conclusion
Our laboratory-synthesized TiO_2 nanotubes were uniform, with diameter about 10 nm and non-anatase crystal structure. The diameter of commercial TiO_2 nanofibers were more than 10 times thicker and the crystalline structure corresponded to pure anatase modification. TEM analyses proved that both products are different.

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ION BEAM PATTERNING OF MICROELECTRONIC COMPONENTS

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New possibilities for the patterning of microelectronic devices are open by application of focused ion beam (FIB). In our contribution we present first results of metallization patterning by FIB. Various metallization systems and photonic structures were patterned in equipment Quanta 3D 200i.

The re-deposition of sputtered material in whole trench profile and radiation damage of semiconducting substrate involved by ion etching were studied by cross-sectional TEM. No linear defects introduced into semiconducting substrate by ion etching were observed. In multilayered metallization (Au(100 nm/Ti(40 nm) on SiO_2/Si)) the effect of big difference of sputtering coefficients was expressed in the side profile of etched trench.

Degradation of superconducting properties of high temperature superconducting films was observed after high current density ion beam etching.

Surprising resistance to the ion beam etching was observed in natural photonic material.

To suppress the non desirable effects during FIB patterning the proper parameters of used ion beam must be found.

The FIB equipment (Quanta 3D 200i) was purchased within the project of the structural funds of the European Union entitled: “Centre of excellence for new technologies in electrical engineering”, ITMS code 26240120011.

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STRUCTURE AND PROPERTIES OF LEAD-FREE SOLDERS AND SOLDER JOINTS

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Special types of lead-free solders based on Sn-Sb and Sn-Sb-Cu for soldering at elevated temperatures were prepared by rapid quenching in form of ribbons. Wetting of Cu substrate has been studied by sessile drop method at temperatures between 280 and 380 ^{o}C. The structure of the solder-Cu substrate interface was studied on cross-sectioned samples from wetting experimements by conventional X-ray diffraction, spatially resolved X-ray diffraction, SEM and EDX. In order to obtain a more accurate (semiquantitative) distribution of constituent elements in crystalline phases the elemental maps from EDX were analyzed also using tri-variate element distribution analysis. The method has allowed to confirm the phase structure of the solder at the interface with Cu substrate indicated from X-ray diffraction analyses. Of particular interest is the observation about enhanced content of Sb in the vicinity of the joint interface which may be responsible for increased strength of the joints.

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CHANGES OF CONNEXIN 43 STATUS IN WB344 CELLS TREATED WITH PCBS

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Polychlorinated biphenyls (PCBs), persistent enviromental contaminants, can be divided into two groups according to their structure and the main modes of action. The coplanar dioxin-like PCBs activate AhR, the latter, noncoplanar nondioxin-like act through CAR and other mechanisms. Nondioxin-like 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) has been shown to cause toxic effects linked to neurotoxicity, immunotoxicity, endocrine disruption or tumor promotion.. In this study, performed in liver epithelial WB-F344 cells, we investigated the impact of PCB 153 on gap junctional intercellular communication (GJIC) by fluorescence microscopy and Western blotting using antibodies against connexin 43 (Cx43), a transmembrane protein forming gap junctional channels in rat liver progenitor cells, and by PCR. PCB 153 induced a sustained inhibition of gap junctional intercellular communication (GJIC), decreased the size and number of gap junction plaques in plasma membrane and reduced the protein but not the mRNA level of Cx43. It seemed that multiple post-translation modes of action contributed to downregulation of Cx43 protein, among others, delayed GJ assembly, enhanced internalization and degradation. The observed changes of cell-to-cell communication might be related to toxic effects of non-dioxin-like PCBs in liver cells, including tumor promotion.

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COVALENT CONJUGATION OF PALLADIUM NANOPARTICLES TO SREPTAVIDIN

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The conjugation of colloidal palladium nanoparticles to streptavidin were performed with a modified protocol used for conjugating streptavidin to carboxylated quantum dots. First, palladium nanoparticles were incubated under various conditions with dihydrolipoic acid (DHLA) to cover nanoparticles with a surface layer bearing hydrophilic deprotonated carboxyl groups. The carboxyls were then covalently coupled to streptavidin using the coupling agent 1-ethyl-3-(3-dimethylaminopropyl) carboiimide (EDC). After the conjugation reaction, aggregates and unbound proteins were removed. The concentrations of streptavidin conjugates were measured using a fluorescence-based assay. The conjugates were proved using SDS-PAGE under reducing conditions. Electrophoretically separated proteins were silver stained. Conjugates were used to detect antigens labeled with ConA-biotin or GNA-biotin. Negative controls were carried out by omitting biotinylated lectins. The specificity of the binding reaction between both commercial streptavidin-gold and the streptavidin-palladium conjugates was consistent. Labeled ultrathin sections were observed using TEM, operating at 80 kV, without adding contrasting agents. The protocol described here may be used for conjugating other proteins of interest.
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AUTOMATED SPINNING DISK CONFOCAL MICROSCOPY IN 3D LIVE CELL IMAGING

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Fluorescence microscopy has become the leading technology to study structure and dynamics of cellular components and processes. The studies can be performed in two-dimensional (2D) but also in three-dimensional (3D) spatial coordinate system as well as in time and spectral dimensions. Several years ago the microscopy of living cells was introduced based on discovery of fluorescent protein GFP. Fluorescent proteins allow us to study protein dynamics, localization, and interactions in living cells. In our laboratory, we have been developing special systems for automated cell image acquisition and analysis using fluorescence microscopy working up to five dimensions (x, y, z, t, lambda), whose hardware and software was optimized for studies on living cells. The presentation will focus on the latest developments in our technology. Besides microscopy hardware and software, also examples of possible applications will be presented. For the first time, we discovered interaction of apoptotic proteins AIF and endonuclease G, expressed using one DNA plasmid, in living human cells during apoptotic cell death. We were also able to analyze the movement speed and directionality of movement of telomeres in 3D and 2D in living human cells with unsurpassed time and spatial resolution.

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