Institute of Scientific Instruments of the ASCR, v. v. i.

Workshop of Interesting Topics of SEM and ESEM

26–31 August 2014

Programme & Abstracts

Editors: Vilém Neděla, Šárka Mašová & Eva Tihlaříková

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Organizer: Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, v.v.i., Královopolská 147, CZ - 612 64 Brno,Czech Republic

Workshop venue: Hotel Templ, Husova 305/50, 692 01 Mikulov

Workshop date: 26–31 August 2014

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Programme of workshop

Tuesday Aug. 26, 2014

	Arriving and transport to Mikulov (Hotel Templ)

Wednesday Aug. 27, 2014

9:00 – 12:00	Guided tour of Mikulov
12:00 – 13:00	Lunch in hotel Templ
15:20 – 15:30	Opening lecture
15:30 – 16:20	**Dr. Müllerová**: MAIN ACTIVITIES OF THE INSTITUTE OF SCIENTIFIC INSTRUMENTS & HISTORY OF ELECTRON MICROSCOPY AT THE INSTITUTE OF SCIENTIFIC INSTRUMENTS
16:20 - 17:10	**Dr. Neděla**: ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY AND ITS APLICATION POSSIBILITIES IN ISI ASCR
18:30	Welcome party and dinner

Thursday Aug. 28, 2014

9:00 – 9:45	**Prof. Shiojiri**: SOME TOPICS FROM OUR RECENT WORKS
9:45 – 10:30	**Dr. Kawasaki**: SCANNING TRANSMISSION ELECTRON MICROSCOPY AND ITS APPLICATION TO THIN FILM CHARACTERIZATION
10:30 – 11:00	Coffee break
11:00 – 11:50	**Prof. Koshikawa**: HIGH BRIGHTNESS AND HIGHLY SPIN-POLARIZED LOW ENERGY ELECTRON MICROSCOPY
12:00 – 13:00	Lunch in hotel Templ

14:00 – 14:20	**Assoc. prof. Jirák**: COOPERATION OF ISI BRNO, ASCR AND DEPARTMENT OF ELECTROTECHNOLOGY, BUT ON DETECTORS FOR ESEM
14:20 – 14:40	**Dr. Oral**: COMPUTATIONS IN CHARGED PARTICLE OPTICS
14:40 – 15:10	Coffee break
15:10 – 16:10	**Prof. Toth**: ESEM AS A TOOL FOR SURFACE CHEMISTRY AT THE NANO-SCALE
16:10 – 16:30	**Dr. Vlašínová**: USE OF ESEM IN SOMATIC EMBRYOGENESIS OF GYMNOSPERMS
18:30	Dinner in the “U Zajíce”, classical music concert

Friday Aug. 29, 2014

9:30 – 9:55	**Prof. Raška**: NUCLEAR ORGANIZATION AND POLYCOMB CHROMATIN DOMAINS
9:55 – 10:25	**Prof. Chongthammakun**: ESTROGEN AND SYNAPTIC PLASTICITY
10:25 – 10:55	Coffee break
10:55 – 11:25	**Dr. Heger**: ICE AND ITS IMPURITIES FROM THE PERSPECTIVES OF PHOTOCHEMISTRY AND ELECTRON MICROSCOPY
11:25 – 11:45	**Dr. Bučko**: PROGRESS IN IMAGING TECHNIQUES FOR CHARACTERIZATION OF POLYELECTROLYTE COMPLEX MICROCAPSULES AS ENCAPSULATION MATRICE FOR BIOCATALYSTS
11:45 – 12:05	**Dr. Schenkmayerová**: PHYSICAL AND BIOCATALYTIC PROPERTIES OF POLYVINYL ALCOHOL LENS-SHAPED PARTICLES VERSUS SPHERICAL POLYELECTROLYTE COMPLEX MICROCAPSULES
12:05 – 13:00	Lunch in hotel Templ
14:00 – 14:20	**Assoc. prof. Maxa**: USE SYSTEMS FOR THE ANALYSIS OF GAS FLOW

14:20 – 14:40	**Hladká**: ANALYSIS OF THE GAS FLOW
14:40 – 15:00	**Ing. Hudec**: THE DIFFERENT PERCENTAGE PERFORMANCE OF NANOPARTICLES AND THEIR EFFECT ON THE PROPERTIES OF EPOXY RESIN
15:00 – 15:30	**Dr. Mašová**: ELECTRON MICROSCOPY AS UNREPLACEABLE METHOD IN PARASITOLOGY: SEM & ESEM UTILIZATION
18:30	Dinner in hotel Templ and cimbal music

Saturday Aug. 30, 2014

Morning	The beauties of Moravia - sightseeing trip: Holly Hill
Lunch	Hotel Templ
Afternoon	Session concerning problems in EM I.
18:30 (dinner)	Barbecue in hotel Templ

Sunday Aug. 31, 2014

Morning	Session concerning problems in EM II.
11:30 – 12:30	Lunch in hotel Templ
Afternoon	Departure to Brno, accommodation

Speaker Abstracts

Abstracts in the following sections are sorted by the order of presentation.

To locate a particular author, please see the index of authors (sorted according author’s surname) in the end of the brochure.

MAIN ACTIVITIES OF THE INSTITUTE OF SCIENTIFIC INSTRUMENTS

Ilona Müllerová - Tomáš Radlička - Filip Mika - Vladislav Krzyžánek - Vilém Neděla - Jaroslav Sobota - Martin Zobač - Vladimír Kolařík - Zenon Starčuk - Aleš Srnka - Pavel Jurák - Pavel Zemánek - Ondřej Číp - Josef Lazar - Libor Mrňa

Institute of Scientific Instruments, AS CR, v.v.i. Královopolská 147, 612 64 Brno, Czech Republic

Abstract Institute of Scientific Instruments (ISI) was established in 1957 to develop diverse instrumental equipment for other institutes of the Academy of Sciences. ISI has long experience in research and development of electron microscopes, nuclear magnetic resonance equipment, coherent optics and related techniques. Nowadays the effort concentrates on scientific research in the field of methodology of physical properties of matter, in particular in the field of electron optics, electron microscopy and spectroscopy, microscopy for biomedicine, environmental electron microscopy, thin layers, electron and laser beam welding, electron beam lithography using Gaussian and shaped electron beam, nuclear magnetic resonance and spectroscopy, cryogenics and superconductivity, measurement and processing of biosignals in medicine, non-invasive cardiology, applications of focused laser beam (optical tweezers, long-range optical delivery of micro- and nano-objects) and lasers for measurement and metrology. ISI works both independently and in cooperation with universities, other research and

professional institutions and with private companies at national and international level.

ACKNOWLEDGEMENT

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HISTORY OF ELECTRON MICROSCOPY AT THE INSTITUTE OF SCIENTIFIC INSTRUMENTS

Ilona Müllerová

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Abstract The development of the first transmission electron microscope (EM) at the Institute of Scientific Instruments (ISI) was completed in 1951. In 1954 a functional model of a desktop EM (the Tesla BS 242) was built and it won the Gold Medal at EXPO 1958. Over 1000 of these instruments were produced over a period of 20 years and exported to 20 countries. Unique transmission, emission and scanning EMs were developed and built during the 1960s. At the same time, the issues with high voltage sources, vacuum (and subsequently ultrahigh vacuum) and with the analysis of residual gases were resolved. In 1962, the first electron interference experiments in the world were carried out at ISI. Non-conventional forms of EM were also developed in the 1970s, e.g. interference shadow EM, Lorentz and tunneling EM, emission microscopy, as well as low energy electron diffraction [1]. Since 1973 the finite element method has been exploited for the computation of electrostatic and magnetic lenses. The ultrahigh vacuum scanning EM with cold field emission gun and an Auger spectrometer was fully developed and built at ISI in 1976, and the electron beam writer with a shaped beam and field emission gun in 1985. The development of new scintillation and cathodoluminescent screens began in the 1970s and our

single crystal Yttrium Aluminium Garnet detector significantly improved detection systems all over the world. Low- and very-low-energy scanning EM was introduced to the world in 1990 as a unique technique. Today, it can achieve resolution as low as 4.5 nm at the incident electron energy of 20 eV.

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ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY AND ITS APLICATION POSSIBILITIES IN ISI ASCR

Vilém Neděla

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Abstract The first commercially available environmental scanning electron microscope (ESEM) was introduced in 1988 by Dr. G.D. Danilat and his company Electro Scan. Prof. Autrata and Doc. Jirák of the Institute of Scientific Instruments of the Academy of Science of the Czech Republic, public research institution, and the Brno University of Technology launched a laboratory operation with the first purely Czech ESEM AQUASEM in 1995. The Team of Environmental Electron Microscopy (EEM), headed by Dr. Vilém Neděla, a former student of Professor Autrata, has continued the nearly twenty-year tradition of ESEM progress in the Czech Republic. The team has studied interactions of electrons with high-pressure gas environments, designed, developed and simulated detection systems for SEM and ESEM and performed simulations of gas flows in ESEM. In interdisciplinary cooperation with various partners the team has developed and tested methods of observation of sensitive, native or live specimen studied under conditions of dynamic in-situ experiments using the today already obsolete ESEM AQUASEM II (Figure 1) with directly heated tungsten cathode converted by Dr. Neděla at the Institute of Scientific Instruments of the Academy of Science of the Czech Republic still in his student years. In near future the

institute plans purchase of a new ESEM with high resolution and a unique configuration of accessory analytical and other equipment.

Thus a new laboratory of environmental electron microscopy with state-of-the-art equipment will be established at the ISI ASCR in Brno. The new laboratory will allow for specimen study with electron beam in combination with optional micro handling, dynamic in-situ experiments with specimen temperature variation from -25°C to 1000°C, or local gas and liquid injecting directly onto the sample. The microscope will also be equipped with a state-of-the-art EDS analyser optimised for work in high pressure gas environments with low beam current, so far the most sensitive scintillation photomultiplier detector of reflected electrons, special ionisation detectors of secondary electrons, Raman spectroscopy, software for correlative microscopy and many more special systems developed at the ISI ASCR in Brno.

Figure 1: *Environmental scanning electron microscope AQUASEM II.*

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SOME TOPICS FROM OUR RECENT WORKS

Makoto Shiojiri[1] - Masateru Nose[2] - Masahiro Kawasaki[3]

[1]Kyoto Inst. of Tech.; [2]Univ. of Toyama; [3]JEOL USA Inc.

Abstract We review two topics among our recent investigations; i) photonic crystal structure and coloration of wing scales of butterflies exhibiting selective wavelength iridescence,[1-5] and ii) plant anatomy and biomechanics of canes of common reed (*Phragmites australis*) used for Japanese double reed wind instrument *Hichiriki*,[6] expecting contribution and aiming at expansion to “electron microscopy of susceptible, wet samples and instrumentation”.

The coloration of butterflies that exhibit human visible iridescence from violet to green has been elucidated. Highly tilted multilayers of cuticle on the ridges, which were found in the scales of male *S. charonda* and *E. mulciber* butterflies, produce a limited-view, selective wavelength iridescence (ultraviolet (UV)~green) as a result of multiple interference between the cuticle-air layers. The iridescence from *C. ataxus* originates from multilayers in the groove plates between the ridges and ribs. The interference takes place between the top and bottom surfaces of each layer and incoherently between different layers. Consequently, the male scales with the layers of ~270 nm thick reflect the light of UV~560 nm (green) and the female scales with the layers of ~191 nm thick reflect the light of UV~400 nm (violet). *T. aeacus* does not produce the iridescent sheen whilist *T. magellanus* does. No iridescent sheen is ascribed to microrib layers, which are perpendicular to the scale plane, so that

they cannot reflect any backscattering. The structures of these butterflies would provide us with helpful hints for manipulating the light in photoelectric devices, such as blue or UV LEDs. The observation of wet eyes of butterfly is interesting since the butterfly might have sensitivity to a proper selective wavelength for each species.

Hichiriki is a traditional Japanese double-reed wind instrument used in Japanese ancient imperial court music, gagaku, which has been performed since the 7th century. A reed is a thin strip of material which vibrates to produce a sound by setting oscillation in the air column inside the tube of a wind instrument such as clarinet, oboe, bassoon and hichiriki. The best reeds for hichiriki have been made of canes of *Phragmites australis* (*P. australis*) that are harvested from only a limited reed bed at Udono near Kyoto, which is similar to the best reeds for clarinet, oboe or bassoon manufactured from canes of *Arundo donax* grown only in a few areas of the Var in France. The aim of the present investigation is to elucidate why the stems from Udono are the best materials for hichiriki reeds. Plant anatomy was examined for *P. australis* stems grown in different reed beds in Japan as well as morphology, and the local indentation hardness and Young's modulus of tissues on the cross-sections of different hichiriki reeds were measured. It is concluded that the good stems for hichiriki reeds have an outer diameter of about 11 mm, a wall thickness of about 1 mm and comparatively homogeneous structure where harder materials such as epidermis, hypodermis, sclerenchymatous cells and vascular bundle sheaths with hard walls are orderly deployed with softer materials such as parenchyma cells and vascular bundles.

This structure has smaller differences of hardness and Young modulus between the hard and soft materials in the reed, providing the best music performance. The reeds are always wetted for music performance. The observation of the wetted reeds is important for understand the property of reeds as materials for the music wind instruments.

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SCANNING TRANSMISSION ELECTRON MICROSCOPY AND ITS APPLICATION TO THIN FILM CHARACTERIZATION

Masahiro Kawasaki[1] - Masateru Nose[2] - Makoto Shiojiri[3]

[1]JEOL USA Inc.; [2]Univ. of Toyama; [3]Kyoto Instit. of Tech.

Abstract First, we briefly review the history of STEM.[1,2] Early in the 20th century, the light microscope had reached a resolution near the wavelength of the light, which was assigned by Abbe's theory. It was a physical resolution limit due to Rayleigh criterion and therfore it was difficult to observe the clear structures of substances in sub-microns. In contrast, developments of modern physics provided the greatest discovery of electrons and led to elementary particle physics. On the basis of the phenomena that the electron beam is refracted by the electric and the magnetic fields, similarly to the light by the glass lens, the geometrical electron optics was evolved. Encouraged by Abbe's theory, Knoll and Ruska, German electrical engineering physicists, succeeded in constructing a transmission electron microscope (TEM) in 1931 using the electron beam with a very short wavelength. Ruska won the Novel Prize in Physics in 1986 for his work in electron optics, including the design of the first electron microscope. However, it was not well known that von Ardenne, a German applied physicist, invented a scanning transmission electron microscope (STEM) besides a scanning electron microscope (SEM). His handwritten sketches in 1937 depicted the essential features of current SEM, STEM and electron microprobe. STEM which has no imaging lens is free

from color aberration of the lens so that it allows to observe thicker specimens than TEM would allow. He added inovational features such as stereo imaging in SEM (1940), micobeam diffraction in STEM (1942), and furthermore a 200 kV high-voltage EM (1944), a high-temperature EM (1941), a reaction chamber EM (1942), EM of living matter (1941), electron vacuum micocinematography (1943), a condenser-objective lens with side-entry specimen exchange (1944), *etc.* Futher developments of STEM were surprisingly accelerated in 1966~70 by Crewe, who was a British born American physicist, with a field emission electron gun for imaging with an atomic resolution.

Next, we show the principle and practice of STEM, in particular, high-angle annular dark-field (HAADF) STEM[3,4], comparing with conventional transmission electron microscopy.

Lastly, our recent STEM investigations on Au/TiO_2 thin films deposited on the glass substrate[5] and multilayered Cr(Al)N/SiO*x* nanocomposite coatings[6,7] are reviewed as examples of characterization of thin films.

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HIGH BRIGHTNESS AND HIGHLY SPIN-POLARIZED LOW ENERGY ELECTRON MICROSCOPY

Takanori Koshikawa [1] - Masahiko Suzuki[1] - Kazue Kudo[2] - Kazuki Kojima[3] - Tsuneo Yasue[1] - Noriko Akutsu[1] - Agerico Dino[3] - Hideaki Kasai[3] - Ernst Bauer[4] - Tsutomu Nakanishi[5] - Xiuguang Jin[6] - Yoshikazu Tkeda[7]

[1]Osaka Electro-Communication University, Osaka, Japan; [2]Ochanomizu University, Tokyo, Japan; [3]Osaka University, Osaka, Japan; [4]Arizona State University, Tempe, USA; [5]School of Science, Nagoya University, Nagoya, Japan; [6]KEK, Tsukuba, Japan; [7]Aichi Synchrotron Light center, Aichi, Japan

Abstract We have already developed a novel very high brightness and highly spin-polarized low energy electron microscope (SPLEEM) [1-3]. Our developed SPLEEM can make us the dynamic observation of the magnetic domain images possible. However the size of the spin-polarized electron gun is large and we have developed a new compact spin-polarized electron gun with a new idea. In principle two devices are necessary to operate 3-dimensional spin direction; one is a spin manipulator which changes the out-of-plain spin direction and another one is a spin rotator which can change the in-plain spin direction. We have proposed a multi-pole Wien filter which enables 3-dimensional spin operation with one device [4].

Current induced domain wall motion is a key phenomenon to realize novel spintronics devices such as a race-track memory (IBM) and a domain wall motion magneto-resistive random access memory (NEC). It has been indicated that domain walls in nanowires with

perpendicular magnetic anisotropy can move with lower current density than those with in-plane magnetic anisotropy. Multilayer [$CoNi_X$] multi-layer is known to exhibit perpendicular magnetic anisotropy and is expected as a material for the devices with low operation current. We investigated magnetic property during growth of the [$CoNi_x$]y multi-layers with our high brightness and highly spin-polarized LEEM [1-3]. We will also reproduce the magnetic domain pattern formation of the surface of Co/Ni multilayers by numerical simulations based on the Landau-Lifshitz-Gilbert (LLG) equation, which describes the dynamics of local magnetization. Fig. 1 shows experimental and simulation results of magnetic domain images of multilayers of pairs of [$CoNi_2$] on W(110) [5,6]. The numerical simulations well reproduce the magnetic domain patterns observed in the experiments.

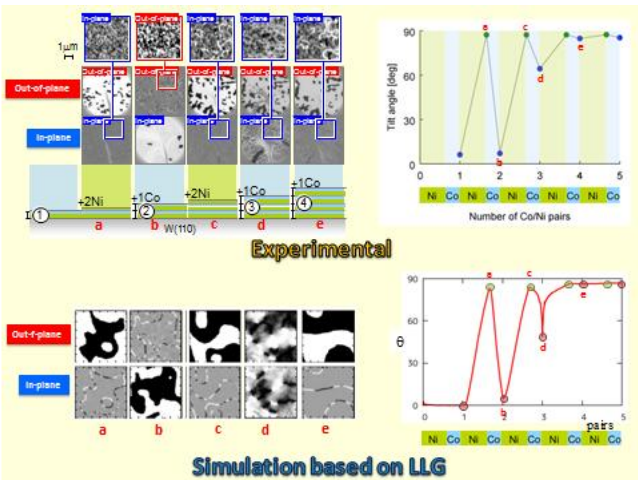

Fig.1 Magnetic domains of Co/Ni multi-layers

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COMPUTATIONS IN CHARGED PARTICLE OPTICS

Martin Oral - Tomáš Radlička

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Abstract The design of modern electron microscopes could not be possible without appropriate software tools. With the sub-nanometer resolution in SEM, and the sub-ångström resolution in TEM, one can see that the simulations involved in designing the instruments need to be tremendously accurate. A simulation starts with the computation of the electric and magnetic fields generated by various optical elements. That is followed by determinig the paraxial properties, aberrations and accurate particle trajectories (ray tracing). The distributions of the fields are mostly detemined with the Finite Element Method (FEM), the Boundary Element Method (BEM) or the Finite Difference Method (FDM). As the field data are at the input of all the subsequent calculations, they need to be very accurate, especially in the region close to the optical axis. Current expertise includes a set of rules that need to be applied in generating a FEM or BEM mesh. Advanced field interpolation techniques are necessary for accurate aberration analysis and particle tracing with high-order integration methods. Specialized software has been developopped for the use in charged particle optics which aids the user in getting meaningful and accurate results. For instance, the EOD (Electron Optical Design) is a comprehensive package for particle optical sumulations. Field data produced by SIMION and Comsol need a specialized post-processing before their use in accurate ray tracing. Presented will be different methods of

computing the optical aberrations, intensity distribution and probe size (resolution) on basic as well as more advanced examples (electron and ion optical columns, deflection systems, ToF spectrometers etc.) that were solved in the EOD and using custom programs.

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ESEM AS A TOOL FOR SURFACE CHEMISTRY AT THE NANO-SCALE

Milos Toth

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Abstract Environmental scanning electron microscopy (ESEM) is a key technology in an emerging suite of techniques for charged particle beam driven growth [1-2], etching [3-4] and functionalization [5] of solids using gas-phase [2-5] and liquid [6] precursors. Here I will outline recent applications of ESEM with an emphasis on electron beam induced deposition of high purity materials [2], damage-free etching of optically active nanostructures [4], and fluorination of optoelectronic materials [5]. I will review advances in hardware, experimental methods and computational modeling techniques which have enabled the use of ESEM for nanofabrication, and for studies of chemical reactions at surfaces. I will also discuss the related technique of environmental photo yield spectroscopy (EPYS) [5], and show how it can be used for real-time analysis of surface reactions, and for fundamental studies of the gas cascade amplification process employed by most gaseous secondary electron detectors.

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USE OF ESEM IN SOMATIC EMBRYOGENESIS OF GYMNOSPERMS

Helena Vlašínová[1] - Vilém Neděla[2] - Roman Businský[3] - Jiří Hřib[1] - Ladislav Havel[1]

[1]Mendel university in Brno; [2]Institute of Science Instruments, Academy of Science of the Czech Republic, [3]Silva Tarouca Research Institute for Landscape and Ornamental Gardening

Abstract Somatic embryogenesis is a wonderful method for the micropropagation of woody plants *in vitro*. This method was tested in bog pine (*Pinus uncinata* DC. *subsp. uliginosa* (Neumann) Businsky), one of the most endangered tree species in the Czech Republic. The bottleneck of this technique is the development of embryos with a functioning apical meristem, able to give well-growing plant. For the identification of meristem development damages the non-commercial environmental scanning electron microscope (ESEM) was used. In compare to classical light imaging and classical EM imaging, ESEM has been found as a very useful tool for quick recognising and detailed studies of apical meristem disruptions and also for the detailed study of the previous early somatic embryo development. This study could bring a new light to this process uderstanding.

ACKNOWLEDGEMENT

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NUCLEAR ORGANIZATION AND POLYCOMB CHROMATIN DOMAINS

Ivan Raška[1] - Jana Šmigová[1] - Pavel Jůda[1] - Eva Bártová[2]

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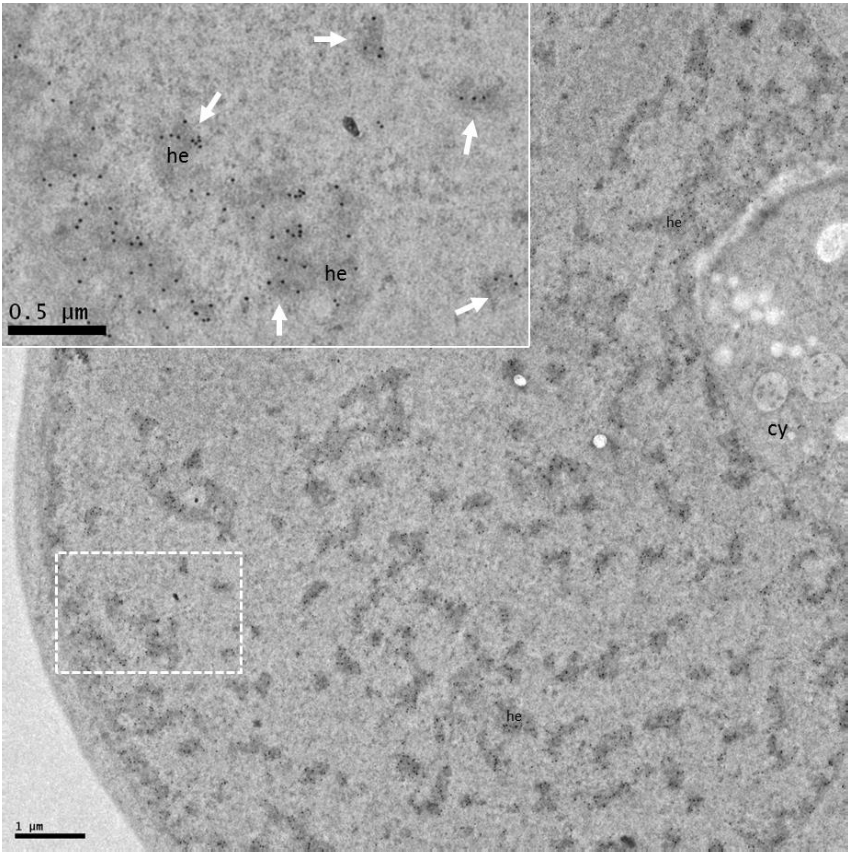
Abstract Nuclear interior is compartmentalized and consists of chromatin and interchromatin compartments. Interchromatin compartment encompasses a variety of nuclear bodies that contain little or no DNA and are identifiable by electron microscopy as distinct nuclear foci/domains. A Polycomb (PcG) body, that has been for the first time identified in human cells by immunofluorescence microscopy, represents a nuclear focus characterized by accumulations of Polycomb repressive complex 1 (PRC1) proteins. Surprisingly, our correlative light-electron microscopy results with implemented on-section immunogold PRC1 protein labeling showed that the gold label was specifically enriched all over nuclear heterochromatin fascicles (Figure 1). This and other results support the concept that the PcG body in human cells is not a nuclear body of the interchromatin compartment, but a chromatin domain.

Figure 1. Immunogold labeling of polycomb BMI-1 protein in U2-OS cell line. The immunogold label (15 nm gold particles) is specifically enriched within the electron-dense heterochromatin structures throughout the nucleus.The cell was processed by high-pressure freezing followed by freeze substitution that allowed the preservation of the cellular fine structure and antigenicity. The image represents a 70 nm thick resin section through the unstained Lowicryl-embedded cell. He - heterochromatin, cy-cytoplasm. In the inset, white arrows point to labeled heterochromatin.



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ESTROGEN AND SYNAPTIC PLASTICITY

Sukumal Chongthammakun[1] - Siriporn Chamniansawat[2]

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Abstract Evidence suggests that both exogenous and endogenous estrogen influence memory function and neuroprotection through estrogen receptors (ER), which are highly expressed in cerebral cortex and hippocampus. Exogenous or gonadal estrogen is mainly synthesized in the gonad and reaches its target organ via blood circulation. In addition to gonad, endogenous estrogen production and secretion in hippocampus was demonstrated to affect the neuronal functions. Regarding the synaptic plasticity, exogenous estrogen significantly upregulates the dendritic spine scaffolding protein, postsynaptic density-95 (PSD-95), as well as expression of the presynaptic vesicle protein, synaptophysin (SYP). These expressions follow the rising of mRNA and protein expression of the neuronal activity-regulated cytoskeleton associated protein (Arc), a protein which is known to be induced by synaptic plasticity following memory consolidation. Estrogen-treated neurons revealed a progressive decrease in membrane and cytosolic ERβ along with the increased nuclear ERβ, in time-dependent manner, suggesting estrogen-dependent nuclear translocation of ERβ. The increased PSD-95 and SYP mRNA expression indicates the classical genomic estrogenic action on synaptic plasticity. Therefore, the estrogen-enhanced synaptic plasticity is ERβ-dependent and involves both non-genomic and genomic estrogenic actions. Not only the exogenous but the endogenous estrogen was demonstrated to influence the hippocampal neuronal function. The hippocampal estrogen

also enhances the ERβ expression in basal ERα-PI-3K dependent mechanism. The priming action of local hippocampal estrogen on ERβ expression is suggested to be essential for exogenous estrogen-enhanced hippocampal synaptic plasticity.

ACKNOWLEDGEMENT

This work was partially supported by European Commission and Ministry of Education, Youth and Sports of the Czech Republic (project No. CZ.1.07/2.3.00/20.0103).

ICE AND ITS IMPURITIES FROM THE PERSPECTIVES OF PHOTOCHEMISTRY AND ELECTRON MICROSCOPY

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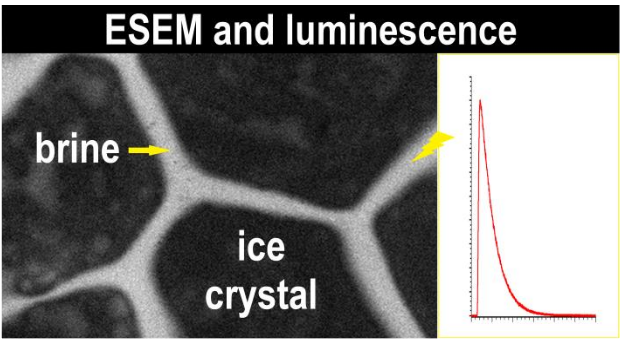
Abstract Ice as a solid phase of water is an interesting but still not well understood medium [1, 2]. Our photochemical group utilized chemical and spectroscopic tools to acquire information relevant to the environmental and applied science. In the introduction I would like to stress some important facts on ice and frozen aqueous solutions stemmed from our work. [3-7] Still opened question, that we are trying to answer by the ESEM, is where and at what forms are the impurities located after the freezing. [8] The recently published results from the pilot collaboration with Dr. Neděla's group will be detailed.



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PROGRESS IN IMAGING TECHNIQUES FOR CHARACTERIZATION OF POLYELECTROLYTE COMPLEX MICROCAPSULES AS ENCAPSULATION MATRICE FOR BIOCATALYSTS

Marek Bučko[1] - Peter Gemeiner[1] - Alica Vikartovská[1] - Andrea Schenkmayerová[1] - Anikó Bertóková[1] - Igor Lacík[2] - Dušan Chorvát Jr.[3] - Vilém Neděla[4]

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Abstract Polyelectrolyte complex microcapsules showed to be an univerzal encapsulation matrice for wide range of biocatalysts including viable, native and recombinant bacterial cells, non-viable bacterial cells as well as enzymes. Developed continuous encapsulation protocol based on the polyelectrolyte complexation of oppositely charged polymers enabled production of uniform microcapsules with a controlled shape, size, membrane thickness, permeability and mechanical resistance. The characterisation and control of the microcapsule properties including the inner structure and surface properties was inevitable to determine their proper function and predictability in important applications such as the stabilisation and reuse of encapsulated biocatalysts for more efficient production of valuable compounds. Recent utilisation of unique environmental scanning electron microscope enabled an innovative observation and characterization of capsule morphology in

native and fully hydrated state. Achieved results and comparison with previously used conventional microscopic techniques will be presented.

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PHYSICAL AND BIOCATALYTIC PROPERTIES OF POLYVINYL ALCOHOL LENS-SHAPED PARTICLES VERSUS SPHERICAL POLYELECTROLYTE COMPLEX MICROCAPSULES

<u>Andrea Schenkmayerová</u>[1] - Marek Bučko[1] - Peter Gemeiner[1] - Dušana Treľová[2] - Igor Lacík[2] - Dušan Chorvát Jr.[3] - Pavel Ačai[4] - Milan Polakovič[4] - Lukáš Lipták[5] - Martin Rebroš[5] - Michal Rosenberg[5] - Vladimír Štefuca[6] - Vilém Neděla[7] - Eva Tihlaříková[7]

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Direct comparison of key physical and chemical-engineering properties of two representative matrices for multipurpose immobilisations was performed for the first time. Polyvinyl alcohol lens-shaped particles LentiKats® and polyelectrolyte complex microcapsules were characterised by advanced techniques with respect to the size distribution of the particles, their inner morphology as revealed by fluorescent probe staining, mechanical resistance, size-exclusion properties, determination of effective diffusion coefficient and environmental scanning electron microscope imaging. Recombinant cells *E. coli* overexpressing enzyme cyclopentanone monooxygenase were immobilised in polyelectrolyte complex microcapsules and LentiKats® for comparison of their operational stability using model Baeyer-Villiger oxidation of (±)-cis-bicyclo[3.2.0]hept-2-en-6-one to regioisomeric lactones as important chiral synthons for potential pharmaceuticals.

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USE SYSTEMS FOR THE ANALYSIS OF GAS FLOW

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Abstract Environmental scanning electron microscope is explaining withdrawing of two differentially pumped chambers in a drawing area [1] [2]. To solve this problem is used finite volume method.

This method includes several steps:

• With using of mesh the area is dividing into discrete volume.

• Discretization and balancing of the unknown quantities in each finite volume

• Numerical solution of discretized equations

The finite volume method divides a continuous function into the discrete functions. The calculation is passed in iterations. Boundary conditions effect to the surrounding cells and the calculation is passed in the entire area of each cycle named iteration, in which the equations are solved according to the given task.

The mesh could be structured only from hexahedron cells or it could be unstructured with using other types of cells like a Prisma, a Tetrahedron or Pyramide (Fig. 1).

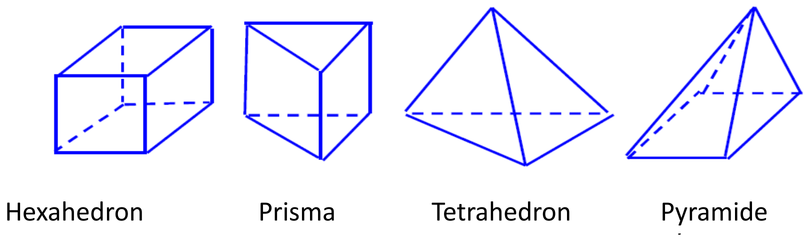


Fig. 1 Elements.

The calculation could be solved in different way of the interpolation [3] (Fig. 2).

First-order upwind

It is assumed that the value of φ on the face is equal to a value in the centroid staying on the left (upwind).

Second order upwind

It specifies the value of φ on the face from the values in the two centroids staying on the left (upwind).

Central differencing

We determine the value of φ on the face using linear interpolation between the values in the centre of the adjoining cells.

QUICK

A quadratic curve is approximated by the two nodes situated in the upstream and one node which is located downstream.

In our case, we are using the SolidWorks system for Creating 3D solids and then systems for mathematical and physical analysis:

• SolidWorks Flow Simulation
• Ansys CFX
• Ansys Fluent

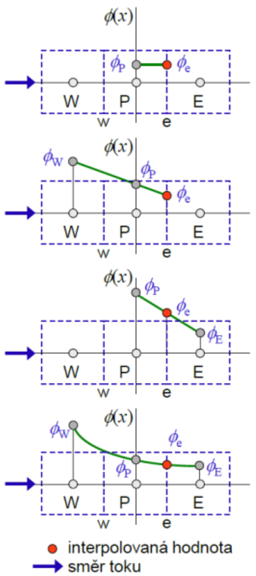
Each system has its own advantages.

In the SolidWorks Flow Simulation system we are putting every useful step for creations and the calculation of the task is very simple. For example, it is using only structured mash, which makes it very quickly, but it cannot use any other type of interpolation than First-order upwind.

Ansys CFX is allowed to use the unstructured mash and interpolation of Second order upwind. Generally, the system is useful for the calculation of the turbo machinery.

The Ansys Fluent system is allowed to use the unstructured mash and all of these types of the interpolation. Its another advantage is ability to make 2D calculations with the help of the 2D calculating mash, which speeds up the calculating time.

In practice, it is good to combine the use of all of these systems according to the type of solving task for effective task solving.



First-order upwind

Second order upwind

Central differencing

QUICK

Fig. 2 Interpolation.

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[2] MAXA, J.; NEDĚLA, V.; JIRÁK, J.; VYROUBAL, P.; HLADKÁ, K. Analysis of Gas Flow in a Secondary Electron Scintillation Detector for ESEM with a new system of Pressure limiting Apertures. *Advances in Military Technology.* 2012. 7(2). p. 39 - 44. ISSN 1802-2308.

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ANALYSIS OF THE GAS FLOW

K. Hladká[1] - J.Maxa[1] - V. Neděla[2] - P. Vyroubal[1] - S. Vaculík[1] - P. Hlavatá[1]

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Abstract In order to optimize the shape of the Differentially pumped chamber in the area of the Primary Electron Beam passage for the current concept of the location of the foci of the Primary Electron Beam, it was necessary to adapt to the size and shape of the limitations of the existing structure. On (Fig. 1a) is the total cross sectional view of the Differentially pumped chamber [1] [2].

Three variants were examined:

Current concept with an easily machined shape of the passage of the primary beam (Fig. 1 b).
Semi-closed concept with a conical shape (Fig. 1 c).
Cone concept with a wider cone (Fig. 1d).

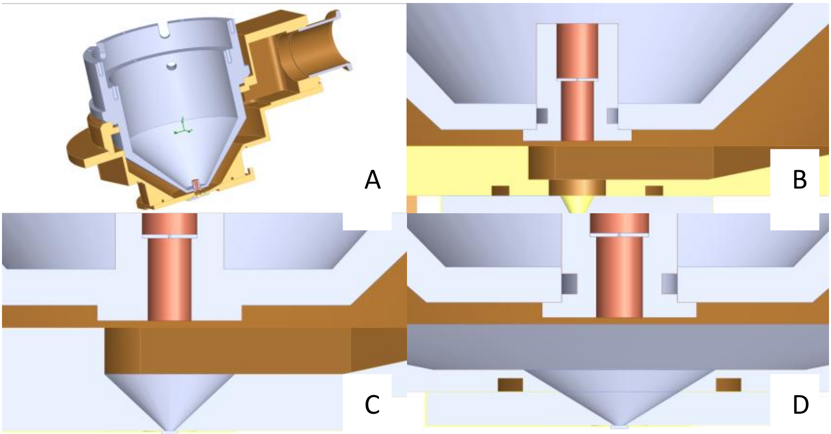

Fig. 1 Differentially pumped chamber.

Using the finite volume method to simulate withdrawing gas from the Differentially pumped chamber to achieve the boundary condition; which is the specimen chamber pressure of 1000 Pa, one of two variants of drawing with the vacuum pump were used: Lavat - pumping speed 25 m3/h and Pfeiffer- pumping speed of 62 m3 /h.

The results show that the course of the static pressure in the space of the primary electron beam path has the lowest Cone concept where the pumped gas stream is directed to the primary beam axis. The reason is that in this area there is a significant supersonic gas flow, causing the formation of an area of lower pressure in this space.

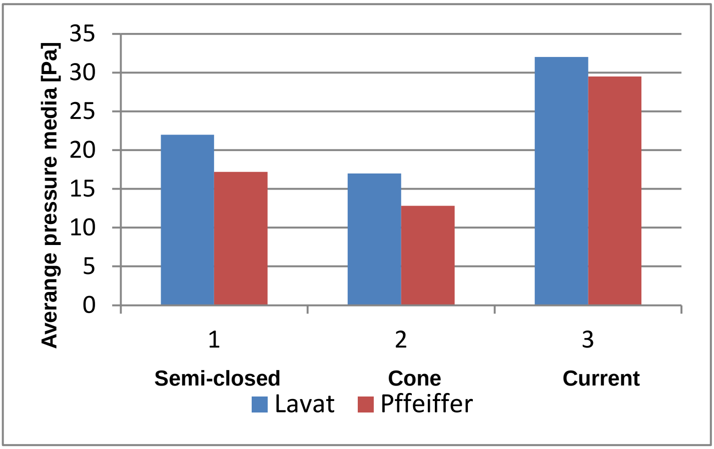

Fig. 2 Results.

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THE DIFFERENT PERCENTAGE PERFORMANCE OF NANOPARTICLES AND THEIR EFFECT ON THE PROPERTIES OF EPOXY RESIN

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[1]Institute of Scientific Instruments of the ASCR, v. v. i.in Brno; [2]Brno University of Technology Faculty of Electrical Engineering and Communication

Abstract The study deals with impact of different percentage filling of nanoparticles on the electrical properties of epoxy resin, which has very good mechanical and electrical properties. The sample is the blended mixture which is evacuated, subjected to ultrasound and then cured. It is expected that the formation of lumps should be minimised due to the influence of microwaves. Nanoparticles should be equally distributed in epoxide volume for this case. Unfortunately, this assumption was not proven. The mixture contains an epoxy resin CY228, hardener HY918, softener DY045 and accelerator DY062. Nanoparticles of alumina (Al_2O_3), sulfur dioxide (SiO_2), titanium dioxide (TiO_2) and tungsten oxide (WO_3) from Sigma Aldrich Company were used as a filler. There were made samples for each filler with 0.25, 0.5, 1, 2 weight percent for our experiment and were determined values of the dissipation factor tgδ, permittivity ε_r and resistivity ρ_v by measuring.

We are able to prepare samples with better electrical properties. Unfortunately, despite the advanced procedure of samples production, our main problem is the inhomogeneity

of distribution of nanoparticles in the sample manifested by the formation of lumps. Scanning electron microscope REM Jeol JSM 6700F was used to detect lumps of nanoparticles.

The lowest permittivity was encountered in the samples with the 2% filling of Al_2O_3 and SiO_2, in the case of TiO_2 it was 1%. In the samples containing Al_2O_3 a SiO_2 the impact of the nanoparticles on the intrinsic resistivity is evident in the full temperature range. The highest intrinsic resistivity is in the sample with the 0.5% content of SiO_2, apart from the sample with 0.5% of Al_2O_3 which has a lower resistivity than pure epoxide. The most pronounced improvement in electrical properties of the resulting nanocomposite was achieved by adding Al_2O_3 and SiO_2. The influence of TiO_2 was less obvious, and adding the nanoparticles of WO_3 caused no change in any of the measured parameters.

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ELECTRON MICROSCOPY AS UNREPLACEABLE METHOD IN PARASITOLOGY: SEM & ESEM UTILIZATION

<u>Šárka Mašová</u>[1,2] - Ivona Foitová [2] - Eva Tihlaříková[1] - Vilém Neděla[1]

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Abstract Systematic parasitology studies diversity and its origin. It includes mainly collection, naming, classification and describing of different species of parasites as well as a reconstruction of their evolutionary history. SEM is very important method in study and describing new species or redescribing insufficiently described species of parasites, because it empower opportunities for more detailed studies of their outer morphology. This method was applied in our research mainly for study of several species of parasitic roundworm (Nematoda) and one species of spiny headed worm (Acanthocephala). Our second topic concerning with SEM in parasitology is orangutan self-medication by plants with mechanical influence. However this method condemns parasite samples for destroying and do not allow other using of it [1].

In case of specimens deficiency, environmental scanning electron microscopy (ESEM) is helpful. It would speed up a preparation of sample and is not invasive so valuable specimens can be used for other types of

taxonomical studies. Non-invasivity of this method is shown in [2]. Two species of already fixed roundworms (*Multicaecum heterotis*, *Contracaecum osculatum*) and one species of spiny headed worm (*Corynosoma pseudohamanni*, Fig. 1) were documented also by ESEM. The samples were examined on a non-commercial ESEM AQUASEM II [3], they were placed to the Peltier specimen holder with silicon surface, into a drop of water (approx. 5 μl). Observation conditions were: beam accelerating voltage 20 kV, probe current 50 pA, sample temperature 2 °C, water vapour pressure 680 Pa.

ESEM seems to be good alternative to classical SEM, in a case of lack of samples or need to use samples for molecular study or depositing them as type material in museum.

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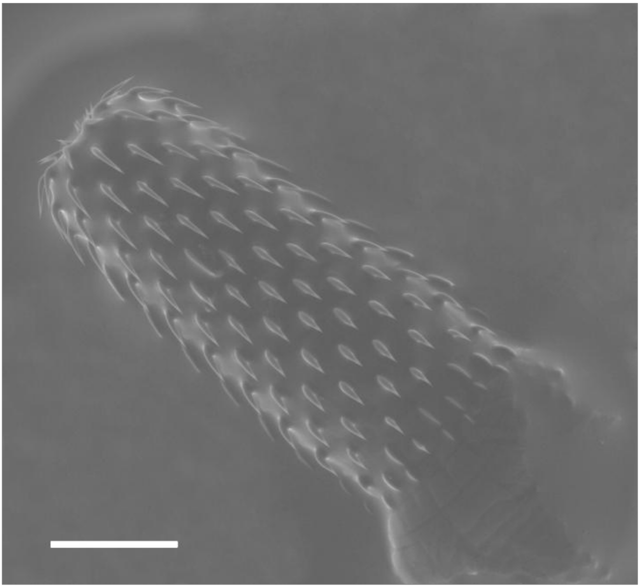

Fig. 1. ESEM observation of proboscis with hooks of cystacanth of *Corynosoma pseudohamanni* (Acanthocephala). Due to small field of view a superposition was needed. (Ionization detector, HV: 20 kV, LowVac 680 Pa, scale bar 200 µm.)

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STUDY OF SCOTS PINE (*PINUS SYLVESTRIS* L.) NATIVE EMBRYOGENIC TISSUE BY ESEM

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Abstract Environmental scanning electron microscopy enables the investigation of uncoated pine early embryogenic tissue samples *in situ*. The samples were examined under low vacuum conditions (air pressure 550 Pa) at a temperature of around -18°C by the AQUASEM II non-commercial environmental scanning electron microscope. The native extracellular matrix surface network was imaged by the environmental scanning electron microscope (Fig.1). The backscattered electron detector disclosed brightness loci in the cells of early embryogenic culture. Scots pine embryogenic tissue contained long suspensor cell aggregates, aggregates of bottle shape cells and early somatic embryos composed from embryonal heads with suspensor cells. Suspensor cells were with a smooth surface. The cell surface of the bottle shape cells, as well as the embryonal heads, was covered with mucilaginous matrix - ECM. Results of our experiments with Pinaceae) suggest adhesion of early embryogenic tissue of Scots pine (*Pinus sylvestris*) is more substantial than adhesion of early embryogenic tissue of firs (Neděla et al. 2012). The

continuity of extracellular matrix surface network with structural integrity of plant organism is discussed.

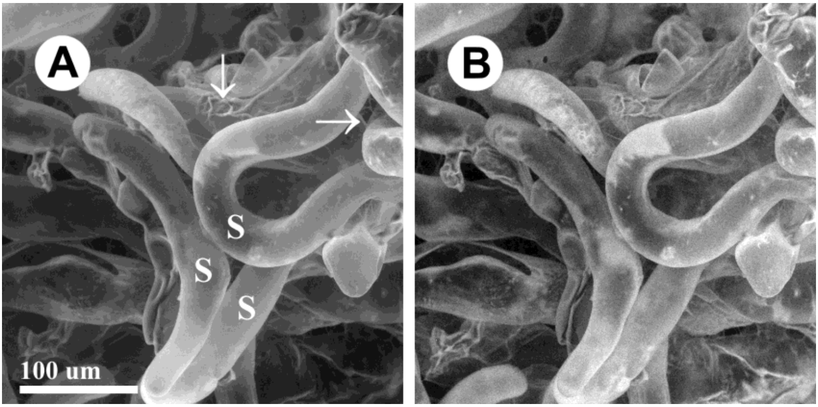

Fig. 1. ESEM observations of early embryogenic tissue (*Pinus sylvestris* L.). (A- using ionization detector, B- using the BSE YAG detector (accelerating voltage 20 kV, probe current 70 pA, ionization detector 270 V, pressure of air in the specimen chamber 550 Pa), the presence of the ECMSN is indicated by full arrows, S-long suspensor cells). Bar= 100 um.

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Index of authors' names

Workshop of Interesting Topics of SEM and ESEM
26–31 August 2014
Programme & Abstracts

Vilém Neděla, Šárka Mašová & Eva Tihlaříková (Eds.)

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