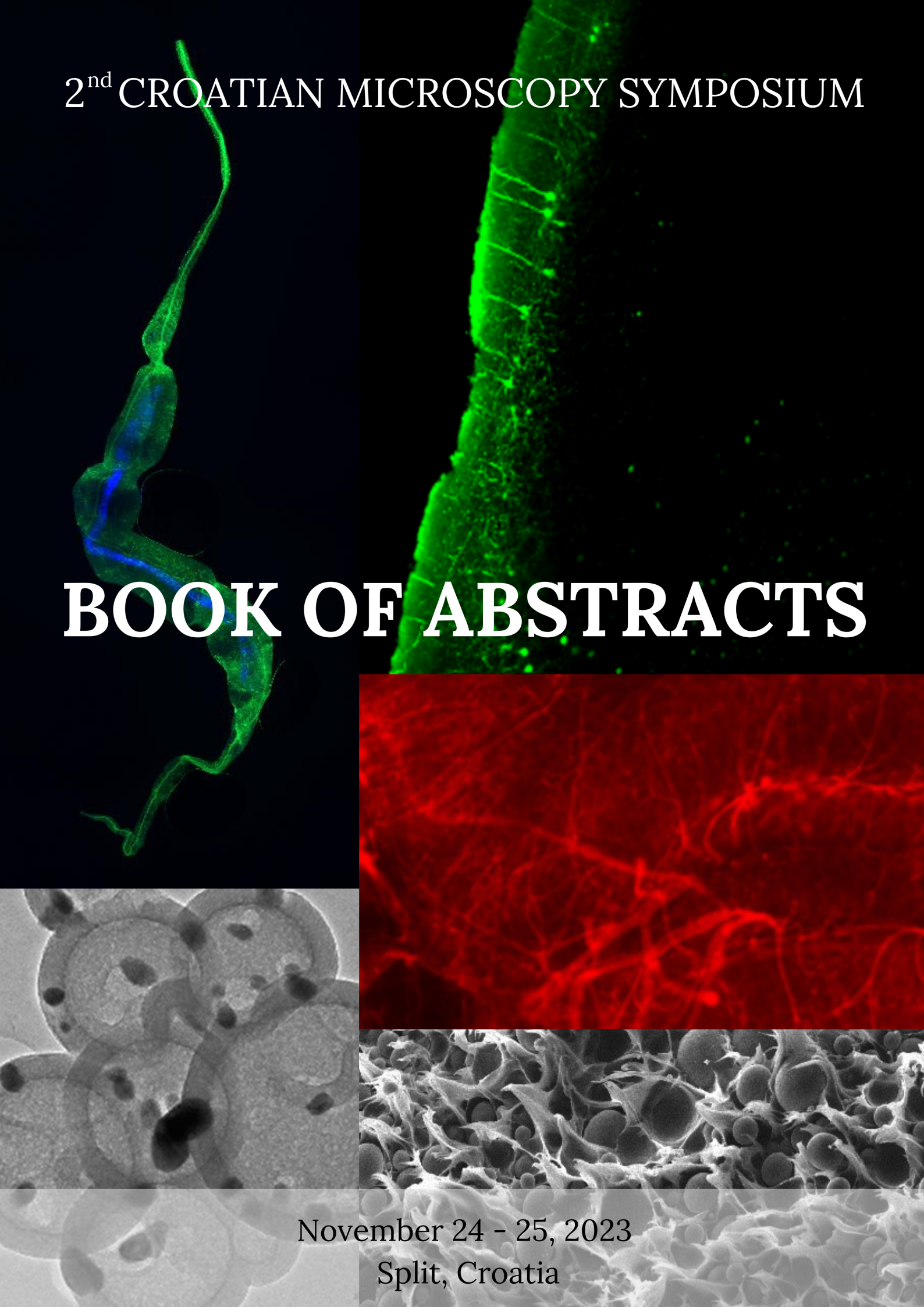


2nd CROATIAN MICROSCOPY SYMPOSIUM

BOOK OF ABSTRACTS



November 24 - 25, 2023
Split, Croatia

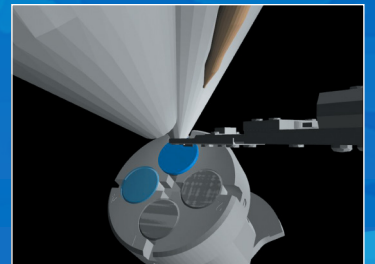
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2nd CROATIAN MICROSCOPY SYMPOSIUM with International Participation:
Book of Abstracts

November 24 – 25, 2023, Split, Croatia

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WELCOME TO THE 2CMS

Ladies and Gentlemen, Dear Colleagues,

It is our great pleasure to welcome you to the 2nd Croatian Microscopy Symposium at the Faculty of Science, University of Split. For the first time in Split, the Faculty of Science, together with Croatian Microscopy Society, is joining up the organization of the Croatian Microscopy Symposium. This symposium is organized in honour of the 40th anniversary of the foundation of the Electron Microscopy Section of the Croatian Society for Natural Sciences, from which the Croatian Microscopy Society was born.

The venue for this symposium is special because the University of Split has got its first transmission electron microscope and its first FEG scanning electron microscope which are both installed at the Faculty of Science. The installation of these devices at the University of Split is of great importance not only for Split and its surroundings, but also for the whole of southern Croatia, as these are the only electron microscopes of this kind in the region. The development of electron microscopy is important not only for the development of science and technology, but also for the development of economy and industry in a given area.

Since microscopy is an interdisciplinary field of science, it is necessary to familiarize the Croatian scientific public and experts with these new devices and their possibilities. During the symposium, its 70 participants will view new microscopes, which will provide them the opportunity to establish new inter-institutional collaborations and collaborations between experts from diverse scientific fields. The symposium will also provide an opportunity to discover the latest research methods in the field of microscopy, especially those available in Croatia. This is of particular importance for young researchers who are just getting acquainted with scientific research, as it enables them to familiarize themselves with the available scientific research equipment and the possibilities that this equipment can provide them in their future work.

The program of the 2nd Croatian Microscopy Symposium includes the presentation of scientific research through two plenary lectures, two invited lectures, three oral presentations and 14 poster presentations in both Life and Material Sciences. During the symposium, the Annual Meeting of the Croatian Microscopy Society will also be held.

In addition to the scientific sessions, the 2nd Croatian Microscopy Symposium also aims to emphasize the importance of informing participants about new advances in the field of microscopy. Therefore, the Symposium will also include presentations of the latest achievements in microscopy through expert lectures held by representatives of equipment manufacturers.

Finally, let us thank you all for your invaluable contributions that make 2nd Croatian Microscopy Symposium a great demonstration of perspectives in microscopy.

On behalf of the Organizing Committee,

Yours sincerely,

Ivana Bočina

President of the 2nd Croatian Microscopy Symposium



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Friday, November 24, 2023

14:30 – 15:00

REGISTRATION AND POSTER SETUP

Lobby

15:00 – 16:15

OPENING CEREMONY

Amphitheatre A0-2

MATERIALS SCIENCE

Plenary lecture

Scanning electron microscopy in science – from physics to dentistry and biology

Ivana Jelovica Badovinac

Golden sponsor lecture

SCAN/JEOL

16:15 – 16:45

VISIT TO THE PMF LABORATORIES

SEM and TEM microscopes (JEOL)

16:45 – 17:15

POSTER SESSION & COFFEE BREAK

17:15 – 19:30

CMS ANNUAL ASSEMBLY

Croatian Microscopy Society - from the beginning to maturity; remembering the first 20 years of our society

Ognjen Milat

Silver sponsor lectures

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19:30

WELCOME DINNER

Saturday, November 25, 2023

9:00–10:20

LIFE SCIENCES

Plenary lecture

Diabetic kidney disease – could polyunsaturated fatty acids (PUFA) help?

Natalija Filipović

Invited lecture

Structural insights into *Anisakis* spp. excretory gland cell

Jerko Hrabar

Oral lecture

Probing the membranolytic activity of novel quaternary ammonium compounds using atomic force microscopy

Doris Crnčević

Nanomorphological and nanomechanical characterization of microalgal reconstructed membrane vesicles

Tea Mišić Radić

10:20– 10:50

POSTER SESSION & COFFEE BREAK

10:50– 12:00

MATERIALS SCIENCE

Invited lecture

Iron, gold, platinum

Marijan Gotić

Oral lecture

Influence of the background salt type on the properties of polyelectrolyte multilayers: AFM study

Tin Klačić

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Closing of the 2CMS

11:55 – 14:00

LUNCH

PMF cafeteria

Departure for Zagreb @ 14:00



HISTORY OF THE CROATIAN MICROSCOPY SOCIETY

Croatian Microscopy Society - from the beginning to maturity; remembering the first 20 years of our society

Ognjen Milat (1)

1) Institute of Physics in Zagreb, Croatia – scientific advisor in retirement

Although the gathering and cooperation of Croatian electron microscopists began with the installation of the first electron microscope at the Ruđer Bošković Institute in Zagreb in 1953, the idea of the Croatian Society for (then Electron) Microscopy dates back to 1983. At the time, the 4th JuSEM conference was held in Kranjska Gora, Slovenia, and it was agreed that the next 5th JuSEM would be organized by Croatian microscopists. Back in Zagreb, Zvonimir Devidé initiated the establishment of the EM society with the general aim of professional and scientific cooperation and socialization of Croatian microscopists, and with the immediate task of organizing the 5th JuSEM. The Society was established on November 20, 1984 as the Section for Electron Microscopy of the Croatian Natural Science Society (SEM HPD). The very successful organization of the 5th JuSEM from May 27 to 30, 1986 at Plitvice Lakes not only strengthened the importance of the Croatian microscopists among the Yugoslav ones, but also secured the highest scientific, professional and organizational position of the Croatian Society at the Yugoslav level. We all participated and happily cooperated, so I remember that as the beginning of the childhood of our society; we held monthly meetings, helped each other, participated in national and international scientific meetings. After the disintegration of the former state, our society strengthened its Croatian identity through intensive cooperation. Thus, with the application for membership in the European Microscopy Association (CESEM) in 1992, then president Nikola Ljubešić, transformed SEM HPD into an independent society: Croatian Society for Electron Microscopy (HDEM/CSEM). It was recognized and admitted to the CESEM and the World IFSEM in 1994. That marked the end of our childhood; the society was accepted into the circle of regional societies and since 1995 was involved as an equal in the organization of the MCEM conference series. The society reached its maturity organizing the 6th MCEM in Pula, June 1 – 5, 2003. It also started to open towards other microscopy techniques, finally changing its name to Croatian Microscopy Society (HMD/CMS), the name we still bear.

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Figure 1. JEOL JSX 1000S – ELEMENT EYE™ - EDXRF Spectrometer



PLENARY LECTURE (Materials Science)

Scanning electron microscopy in science – from physics to dentistry and biology

Ivana Jelovica Badovinac (1)

1) University of Rijeka, Faculty of Physics and Centre for Micro- and Nanosciences and Technologies,
Rijeka, Croatia

The joint laboratories of the current Faculty of Physics and the Centre for Micro- and Nanosciences and Technologies were equipped at the end of 2014 and beginning of 2015 as part of the major project "Development of research infrastructure on the campus of the University of Rijeka" (RISK). At that time Jeol JSM 7800F electron microscope was purchased, and the Laboratory for scanning electron microscopy was established. In this presentation, the role of scanning electron microscopy will be shown, mainly in the research of thin metal oxide films with improved photocatalytic properties for use in water purification, but also the contribution of this technique to the study of various material surfaces, from biological to those used in dentistry. The effects of probiotics on the surfaces of alloys used in orthodontics, the use of biomass ash to improve soil fertility, the visualization and characterization of exosomes – biological nanoparticles and the study of the surface biofilm of the loggerhead sea turtle will also be presented.



PLENARY LECTURE

(Life Sciences)

Diabetic kidney disease – could polyunsaturated fatty acids (PUFA) help?

p

Natalija Filipović (1)

1) University of Split School of Medicine, Split, Croatia

Diabetic kidney disease (DKD) is the one of the most prevalent complications of diabetes mellitus (DM) and a leading cause of chronic kidney disease (CKD). Hyperglycaemia, hyperlipidaemia and hypertension in DM are triggers for a cascade of events, leading to the glomerular hyperfiltration, glomerular hypertension, renal hypertrophy, damage to the tubular system and vasculature, as well as to the immune cell infiltration and extracellular matrix expansion.

A change in a lifestyle, including a diet, presents an essential component in therapy of DM and its complications. In the past several decades, the role of n-6 and n-3 polyunsaturated fatty acids (PUFA) has been studied in numerous clinical studies. Western diet usually contains unfavorable n-6 to n-3 PUFA ratio between 10 and 20:1. Beneficial effects of long chain n-3 PUFA on the CKD have been recorded. In general, n-3 PUFA has been considered anti-inflammatory, while n-6 PUFA have pro-inflammatory effects. However, the effects of PUFA are complex and involve also a regulation of dyslipidemic signals and direct effects in vascular motility.

We used immunohistochemistry, with fluorescence microscopy, and transmission electron microscopy, to determine the influence of PUFA on the consequences of experimentally induced nephropathy in a rat model of streptozotocin-induced DM.

In our studies, involving long and short-term DM models, we found that PUFA supplementation changes the fatty acid composition in different organs, including kidney in diabetic animals. In addition, we found that PUFA supplementation affects different aspects of damage to the rat kidney during the experimental DM. Hence, we believe that supplementation with n-3 PUFA in DKD might have beneficial effects.

Keywords: chronic kidney disease, connexins, diabetic kidney disease, endocytotic vesicles, PUFA

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INVITED LECTURE

(Life Sciences)

Structural insights into *Anisakis* spp. excretory gland cell

Jerko Hrabar (1), J. Tyč (2), F. Kitzberger (2), T. Bílý (2), V. Philimonenko (3), A. Chakroborty (4), Ivona Mladineo (4)

1) Institute of Oceanography and Fisheries, Laboratory of Aquaculture, Split, Croatia

2) Institute of Parasitology, BC CAS, Laboratory of Electron Microscopy, České Budějovice, Czech Republic

3) Institute of Molecular Genetics, BC CAS, Electron Microscopy Core Facility, Prague, Czech Republic

4) Institute of Parasitology, BC CAS, Laboratory of Functional Helminthology, České Budějovice, Czech Republic

Nematodes of the genus *Anisakis* are parasites of marine organisms, being able to cause disease in humans (anisakiasis). A characteristic feature of these worms is the excretory gland cell (EGC), a single large cell essential for worm development, metabolism and pathogenicity. Previous studies of third stage (L3) larvae have revealed an unusually large nucleus with spike-like cytoplasmic projections. Therefore, the aim of this study was to perform electron and light microscopic examinations to determine the spatial organisation of this unusual cell.

For serial block face scanning electron microscopy (SBEM), EGCs were fixed overnight in Karnovsky fixative, stained using the OTO method, embedded in Hard Plus Resin 812 and cut to appropriate size. Images were acquired using Apreo SEM equipped with Volume Scope. Segmentation was done in Microscopy Image Browser and Amira was used for 3D visualisations. For transmission electron microscopy (TEM) and electron tomography (ET), samples were fixed by high-pressure freezing/freeze substitution (HPF/FS) and embedded in Spurr resin. Ultra-thin sections were contrasted according to Reynolds or immunolabelled for lamin and histone H3. For light microscopy, fixed cells were stained with Phalloidin iFluor 488 and DAPI and observed under an Olympus Fluoview confocal microscope. The images were modelled using the IMARIS software.

The nuclear envelope is rich in nuclear pore complexes and wrinkled with large blebs, projecting into the nuclear lumen. The main excretory duct is slightly curved but generally follows the main axis of the cell. Numerous smaller collecting ducts radiate from the main duct into the cytoplasm. Light microscopy revealed an intricate network of actin filaments corresponding to the collecting ducts on the electron microscopy images. In addition, a reticular appearance of chromatin was found with several sites of more condensed chromatin. Such organisation probably plays a role in regulating gene expression through lamin binding of histones.

Keywords: *Anisakis*, excretory gland cell, immunolabelling, scanning electron microscopy, transmission electron microscopy

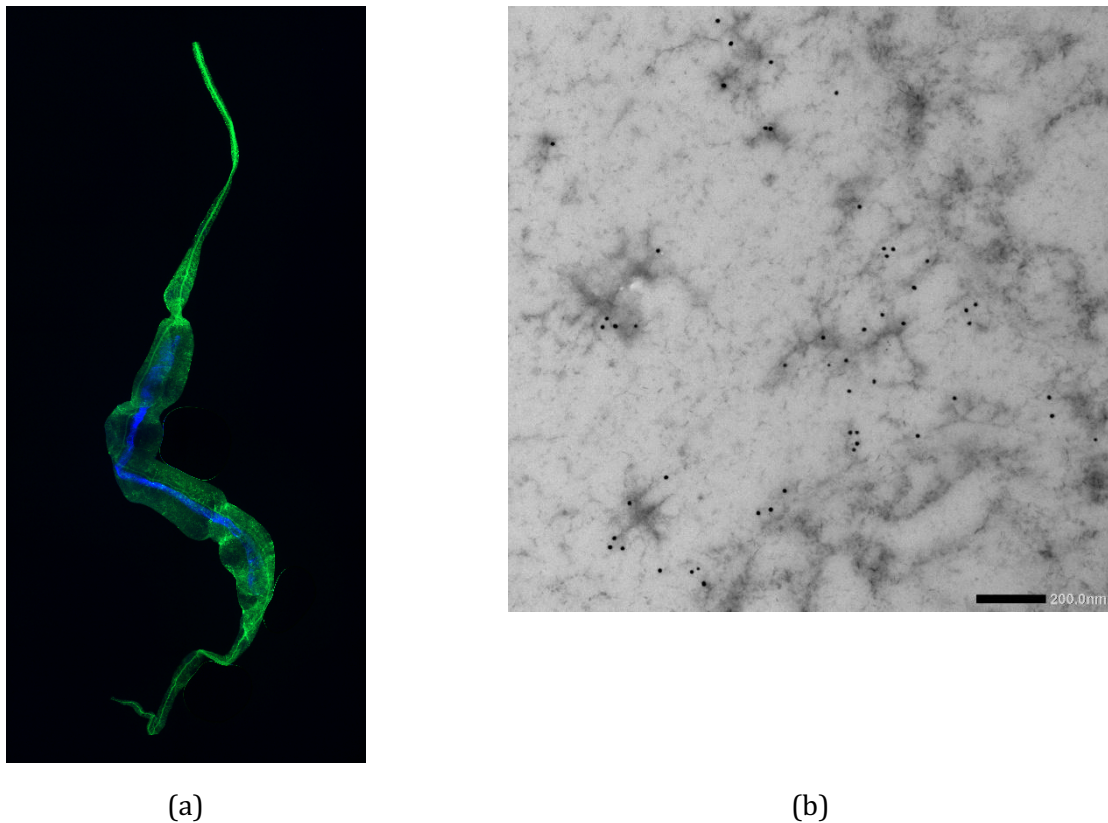


Figure 1: (a) *Anisakis* spp. excretory gland cell with elongated DAPI-labelled nucleus and Phalloidin iFluor488-labelled actin filaments corresponding to excretory canal and collecting canaliculi (4X), (b) Intense histone H3 labelling associated with dark regions in the nucleus (chromatin).

References:

1. T. Audicana, M. Kennedy, Clin. Microbiol. Rev. 21(2) (2008) 360-379.
2. M. W. Kennedy, W. Harnett (eds.), Parasitic Nematodes: Molecular Biology, Biochemistry and Immunology, CABI Publishing (2013) 432 pp.
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4. Y. Hua et al., Nat. Commun. 6 (2015) 7923.
5. I. Belevich et al., PLoS Biol. 14(1) (2016) e1002340.



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INVITED LECTURE (Materials Science)

Iron, gold, platinum

Marijan Gotić (1)

1) Laboratory for Molecular Physics and Synthesis of New Materials, Division of Materials Physics, Ruder Bošković Institute, Zagreb, Croatia

Studying the precipitation and properties of iron oxides contributes to fundamental knowledge in the fields of materials science and chemistry. This research helps scientists gain a better understanding of the behaviour of iron ions in solution and their interaction with other compounds. Gold nanoparticles (AuNPs) find extensive applications in biomedicine, serving as contrast and radiosensitization agents. Platinum nanoparticles (PtNPs) are highly effective catalysts, for instance, PtNPs can be used for the catalytic reduction of 4-nitrophenol to 4-aminophenol. In general, the properties of nanoparticles are significantly influenced by their size and shape (morphology), highlighting the crucial role of electron microscopy in research.

In this presentation, I will demonstrate the importance of electron microscopy in the study of iron oxide nanoparticles, AuNPs, PtNPs, and other nanoparticles. Figure 1 illustrates the morphology of α -FeOOH (goethite) nanoparticles at various magnifications [1]. Figure 2 showcases AuNPs synthesized via citrate-radiolytical synthesis at room temperature [2]. Figure 3 displays PtNPs dispersed on a SnO₂ substrate [3].

Keywords: Mössbauer spectroscopy, FE-SEM, nanoparticle synthesis, biomedicine, catalysis, gamma-irradiation

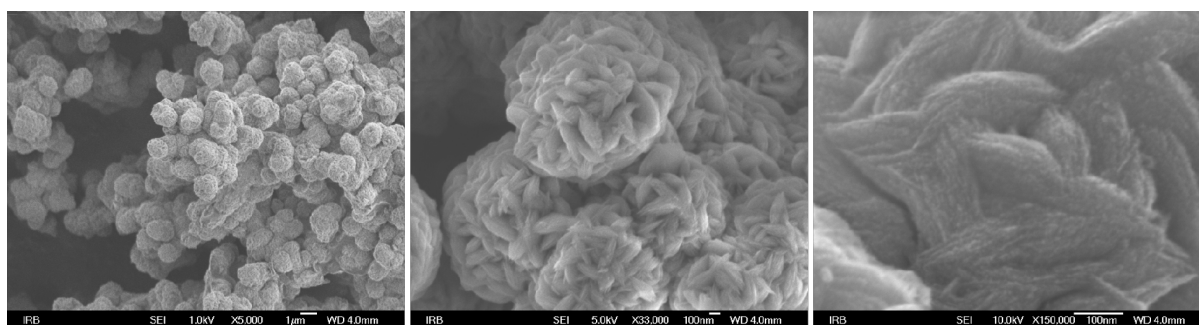


Figure 1: α -FeOOH nanoparticles at different magnifications.

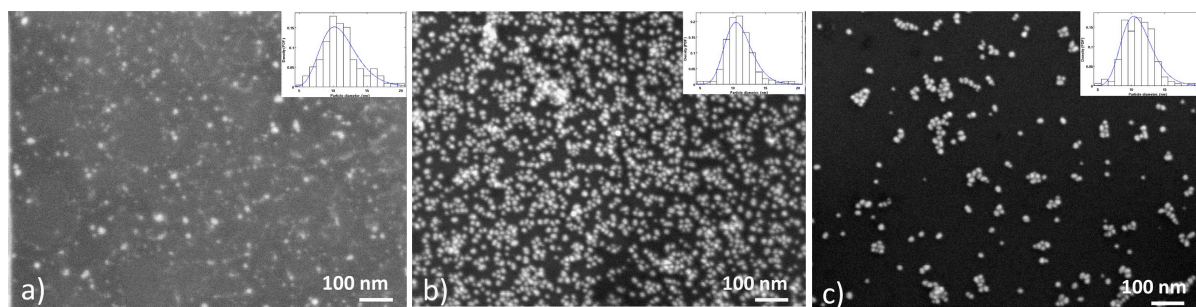


Figure 2: Gold nanoparticles (AuNPs) synthesized via citrate-radiolytical method. Particle diameters were measured using the ImageJ software. The particle size distributions were 11.2 ± 0.25 , 11.0 ± 0.19 and 11.1 ± 0.21 nm for AuNPs radiolytically synthesized at dose of 1 kGy (a), 10 kGy (b) and 30 kGy (c), respectively.

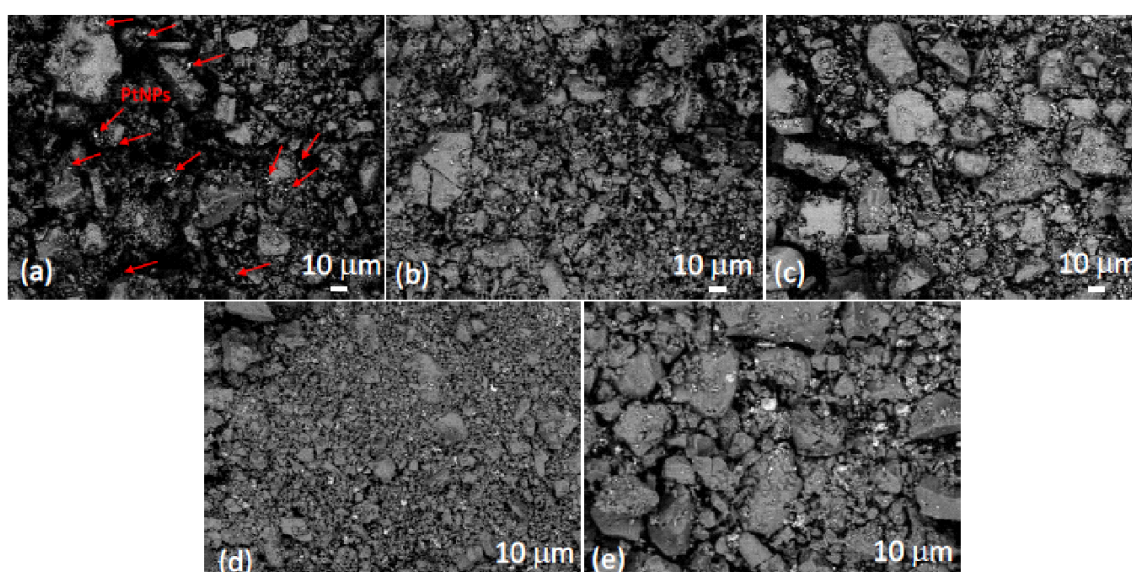


Figure 3: Platinum nanoparticles (white dots) dispersed on the SnO₂ aggregates.

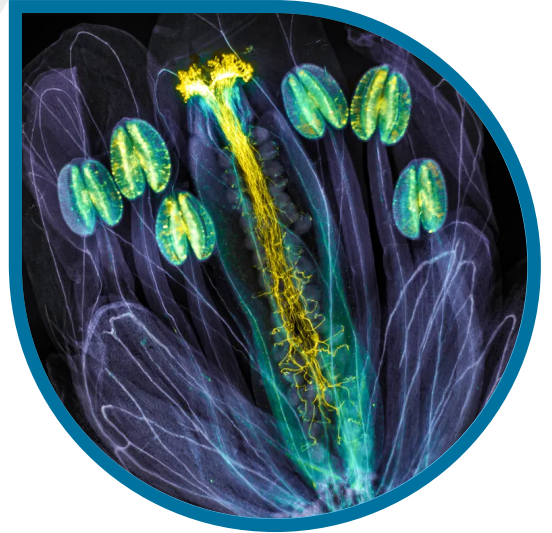
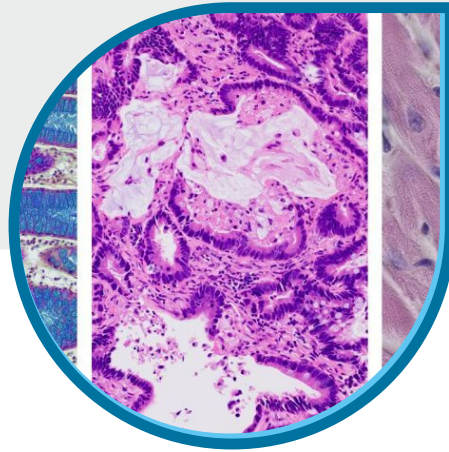
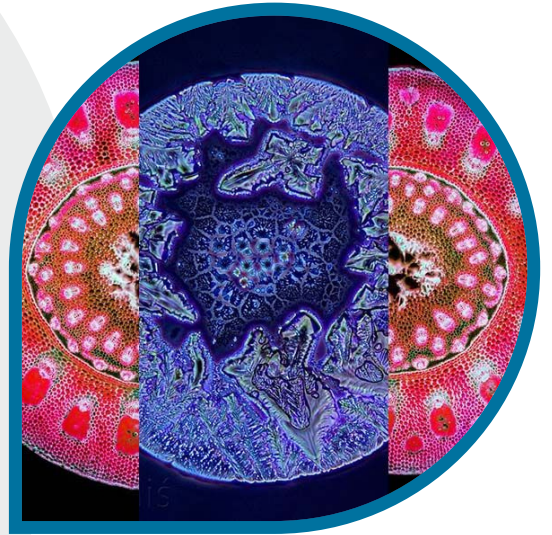
References:

1. M. Gotić, S. Musić, J. Mol. Struct. 834-836 (2007) 445-453.
2. N. Hanžić et al., Radiat. Phys. Chem. 106 (2015) 77-82.
3. I. Đurasović et al., Nanomaterials 13 (2023) 2481.



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ORAL LECTURE (Life Sciences)

Probing the membranolytic activity of novel quaternary ammonium compounds using atomic force microscopy

Doris Crnčević (1), Lucija Krce (2), Renata Odžak (1), Matilda Šprung (1)

1) University of Split, Faculty of Science, Department of Chemistry, Split, Croatia

2) University of Split, Faculty of Science, Department of Physics, Split, Croatia

Bacterial resistance is one of the greatest threats to human health, therefore the discovery of new antimicrobial agents to combat this emerging problem is of great importance. Among the potent candidates with a broad spectrum of biological activity are quaternary ammonium compounds (QACs). Nowadays, efforts are being made to synthesize QACs with improved antibacterial properties while being less susceptible to activation of bacterial resistance mechanisms. The nature of their antibacterial mode of action implies a membrane-targeted approach leading to cell lysis [1]. The aim of our study was to synthesize new QACs derived from naturally occurring precursor structure [2]. Atomic force microscopy (AFM) served as a sensitive technique to elucidate the changes in bacterial cell morphology and membrane damage caused by treatment with the selected most potent QACs. The smooth cell surface of the untreated control cells was disrupted after incubation with desired compound, while the height of the cells decreased. Treated cells were further stained with a mixture of SYTO9 and propidium iodide (PI) fluorescent dyes. Optical fluorescence imaging additionally confirmed membrane damage for the majority of bacterial populations and pointed out novel QACs as promising small molecules to combat emerging pathogens.

Keywords: antibacterial agents, atomic force microscopy, membranolytic activity, quaternary ammonium compounds

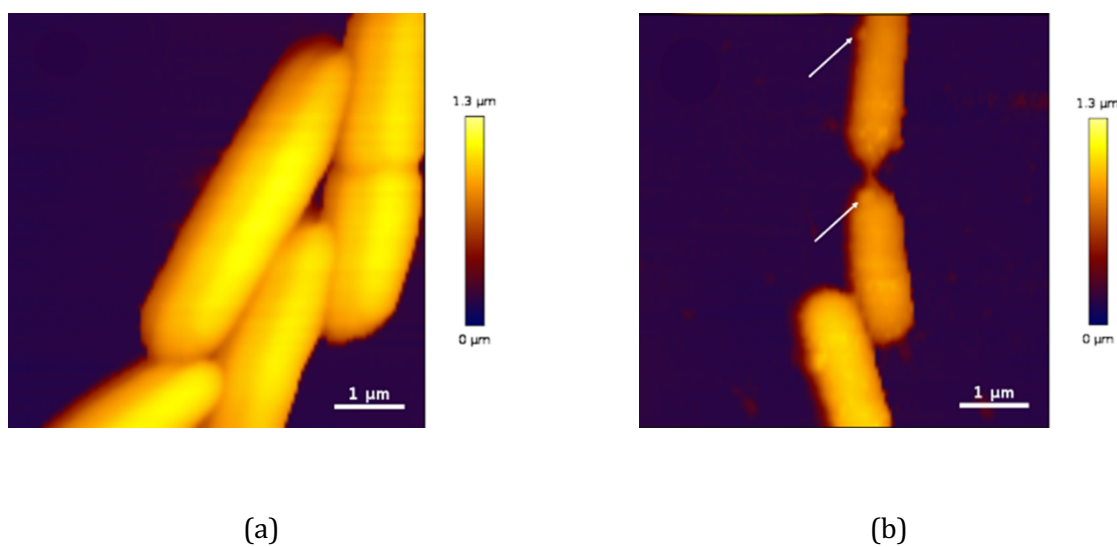


Figure 1: Atomic force microscopy height images of (a) untreated *Escherichia coli* DH5 α cells and (b) the same bacterial cells upon the treatment with the selected newly synthesized compound.

References:

1. P. Gilbert and L. E. Moore, *J. Appl. Microbiol.* 99 (2005) 703-715.
2. D. Crnčević et al., *Pharmaceuticals* 15 (2022) 775-796.

ORAL LECTURE

(Life Sciences)

Nanomorphological and nanomechanical characterization of microalgal reconstructed membrane vesicles

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The green microalga *Dunaliella tertiolecta*, known as a naked cell, surrounded by a glycocalyx surface coat, has the potential to be used for the preparation of reconstructed membrane vesicles [1, 2]. Microalgal cells burst after hypoosmotic shock, and the remaining membrane fragments self-assemble into micrometer-sized membrane vesicles. The aim of this study is to prepare nanometer-sized reconstructed membrane vesicles and characterize them in terms of their nanomorphological and nanomechanical properties using atomic force microscopy. Nanometer-sized vesicles were prepared by hydration of lyophilized material followed by ultrasonic treatment and extrusion through a 200 nm filter pore. The nanometer-sized vesicles showed spherical shape with an average diameter of 160 nm. The elastic properties of vesicles were quantified using the apparent Young's modulus (E). The reconstructed nano-sized vesicles could serve as a basis for the development of marine bioinspired drug delivery systems.

Keywords: atomic force microscopy (AFM), drug delivery system, *Dunaliella tertiolecta*, reconstructed nano-sized vesicles

Acknowledgement - This work was supported by the project "Research network V4-Croatia for the development of novel drug carriers from algae" (No. 22220115) funded through the International Visegrad Fund.

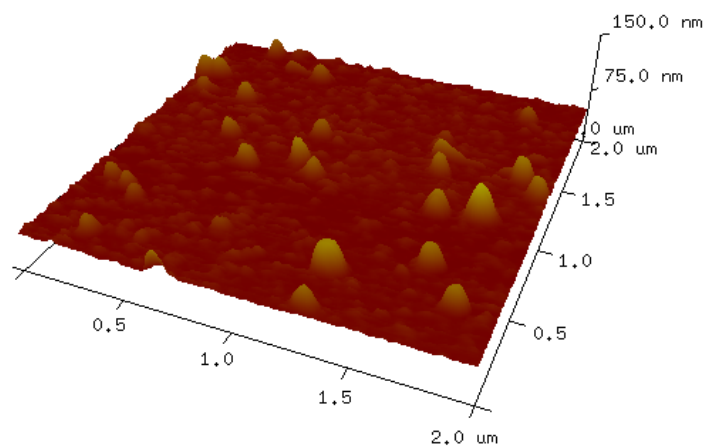


Figure 1: AFM 3D topographical image of reconstructed nano-sized vesicles of microalga *D. tertiolecta*.

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ORAL LECTURE (Materials Science)

Influence of the background salt type on the properties of polyelectrolyte multilayers: AFM study

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Polyelectrolytes are macromolecules (*e.g.*, polymers) with dissociating functional groups that can carry positive or negative charge in solution. By alternating adsorption of oppositely charged polyelectrolytes on a substrate surface, polyelectrolyte multilayers (PEMs) are formed. Nowadays, these ultrathin films are applied in various fields [1]. The wide application potential of polyelectrolyte multilayers lies in the fact that their structure and properties can be fine-tuned by varying preparation parameters [2]. One such parameter is a type of background salt present in a polyelectrolyte solution [3]. In this study, PEMs made of poly (allylamine hydrochloride) (PAH) and poly (acrylic acid) (PAA) have been fabricated on silicon wafers using a layer-by-layer method [4]. The atomic force microscope (AFM) was used to investigate the influence of the supporting sodium salt (NaF, NaCl, and NaClO₄) on the build-up and properties of PAH/PAA multilayer. It was found that the type of background salt has a significant effect on the surface morphology (Figure 1), film thickness, and surface roughness of the PAH/PAA multilayer. For the same number of adsorbed polyelectrolyte layers, PEMs prepared from NaClO₄ solution were thicker and had higher surface roughness than PEMs prepared from NaCl and especially NaF solution.

Keywords: atomic force microscopy, polyelectrolyte nanofilm, specific ion effect

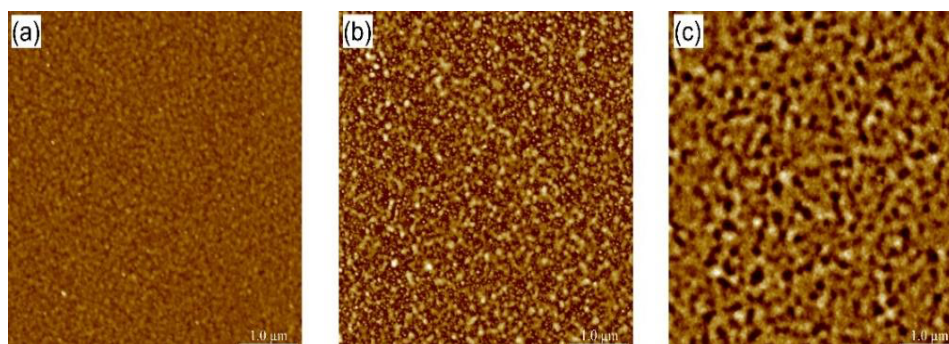


Figure 1: AFM image of (PAH/PAA)₅ multilayer prepared at pH = 7 from polyelectrolyte solutions ($c = 0.01$ mol/L) containing 0.1 mol/L of (a) NaF, (b) NaCl, and (c) NaClO₄. In all images, the z-scale is 40 nm, while x- and y-scales are 5 μ m.

Acknowledgements: This research was supported by the Croatian Science Foundation under the bilateral Slovenian-Croatian APPLPEMS project (IPS-2020-01-6126).



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**POSTER PRESENTATIONS
(Life Sciences)**

P1: Morphometric analysis of *Trypanosoma* flagellates (Kinetoplastea: Trypanosomatida) from the blood smears of northern pike (*Esox lucius*) from the Mrežnica river, Croatia – a case report

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Trypanosoma remaki Laveran and Mesnil, 1901 from the genus *Trypanosoma* Gruby, 1843 is a common and widely distributed parasitic flagellate of pikes. Based on variation in size, two varieties are recognised, *parva* and *magna*. The life cycle involves stages in both the blood and tissues of the fish and the digestive tract of the invertebrate vector. The freshwater leeches, *Hemiclepis marginata* and *Piscicola geometra* are the only known natural vectors for *T. remaki*. This case report presents morphological and morphometric analysis of *Trypanosoma* sp. from the blood smears of a naturally infected northern pike. During spring and autumn of 2021, as part of a fish health and ecotoxicological study conducted under the Croatian Science Foundation project „Metal-binding biomolecules and health disturbances of freshwater organisms exposed to industrial wastes“ (IP-2019-04-2636), a total of 62 pikes were captured by electrofishing from two locations along Mrežnica River in Croatia. Blood samples were collected from 37 pikes (10/spring; 27/autumn). Smears were stained using May-Grünwald-Giemsa staining protocol, analysed for differential blood count, and examined for the presence of blood parasites by light microscopy using an Olympus BX41. Morphometric analysis of *Trypanosoma* sp. was performed by Cell B software on digital images captured by Olympus DP12 digital camera. The overall prevalence of infection was 5.4 %, with higher value in autumn (7.4 %) than in spring (0 %), and the intensity of infection was 1.75 trypomastigote per slide. The measurements of the specimens studied correspond mainly to those provided in the literature for *T. remaki*. However, these data must be considered cautiously due to the small number of specimens and the lack of molecular analysis. This case report provides morphological characteristics and measurements of *Trypanosoma* sp. from naturally infected northern pike. This is the first report of *Trypanosoma* sp. in northern pike from Croatian waters.

Keywords: blood smear, morphometric analysis, pikes, *Trypanosoma* sp.

P2: A combination of electron microscopy methods for a better view

Mia Bužančić (1), Jasna Arapov (1), Sanda Skejić (1), Tina Bonačić (1), Tina Tomašević (1), Ana Bakrač (1), Maja Straka (1), Živana Ninčević Gladan (1)

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This study presents the application of STEM (scanning transmission electron microscopy) in phytoplankton taxonomy. STEM combines some of the advantages of the SEM and TEM methods for easier observation of ultrafine structural features of phytoplankton frustules. In this study, STEM is used for imaging and morphological determination of the marine diatom genus *Pseudo-nitzschia* at nanoscale resolution. Several species of the genus *Pseudo-nitzschia* from the central and southern Adriatic Sea are described using the Tescan FE-SEM /STEM MIRA3 microscope. The STEM micrographs show *P. calliantha*, *P. delicatissima*, *P. galaxiae*, *P. hasleana*, *P. manni*, *P. multistriata*, *P. pseudodelicatissima*, *P. subfraudulenta*. The STEM method has greatly improved and facilitated the morphological characterization of *Pseudo-nitzschia* species.

Keywords: STEM, morphology, phytoplankton taxonomy, *Pseudo-nitzschia* spp.



P3: Tissue clearing procedures for preparing transparent brain sections and visualization of fluorescent structures with various techniques including light sheet and confocal microscopy

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Structures in large samples are easy to visualize using light sheet fluorescence microscopy (LSFM). The variety of tissue clearing procedures, which are necessary for imaging thick samples, are used for achieving sample transparency. The main aim of this research was to verify if the clearing procedures can be beneficial even if “classical” easily available fluorescent microscopes were used for sample visualisation. Mouse brains were isolated from Thy1-YFP-16 mice, which produce yellow fluorescent protein in neurons. Blood vessels staining was achieved by using LEL Texas Red (Invitrogen) dye which was injected in the left heart ventricle prior perfusion. Subsequently, mice were perfused with 1× PBS and 4 % formalin solution and cleared. Different clearing methods for making transparent brain samples can be applied [1]. For whole brain tissue clearing, three methods were used: ECi, PEGASOS and FluoClearBABB. Previously naturally transparent parts of mouse embryos were also imaged using fluorescence microscopy by our group [2,3]. Cleared mouse brain samples were cut on approximately 1 mm thick slices using cutting mold, mounted on the glass slides in the drop of the final clearing solution, covered by coverslips, and imaged using inverted fluorescence microscope (The EVOS®, ThermoFisher Scientific) and confocal microscope (Olympus FV3000). Neurons in mouse hemisphere were labeled with primary conjugated antibody (NeuN) and cleared using MACS Clearing Kit (Miltenyi Biotec). For visualization of antibody labeled neurons in cleared mouse brain hemisphere LSFM was used (Ultramicroscope II). Using all three clearing methods satisfying transparency of whole mouse brain was achieved. PEGASOS and FluoClearBABB method were preferred for their YFP signal preservation. Primary conjugated antibody showed strong signal in neurons and good penetration into deeper parts of mouse brain (Figure 1). Even without using LSFM, it was possible to visualize neurons (Figure 2) and fluorescently labelled blood vessels in thick samples (Figure 3).

Keywords: antibodies, brain, fluorescence, mouse, PEGASOS

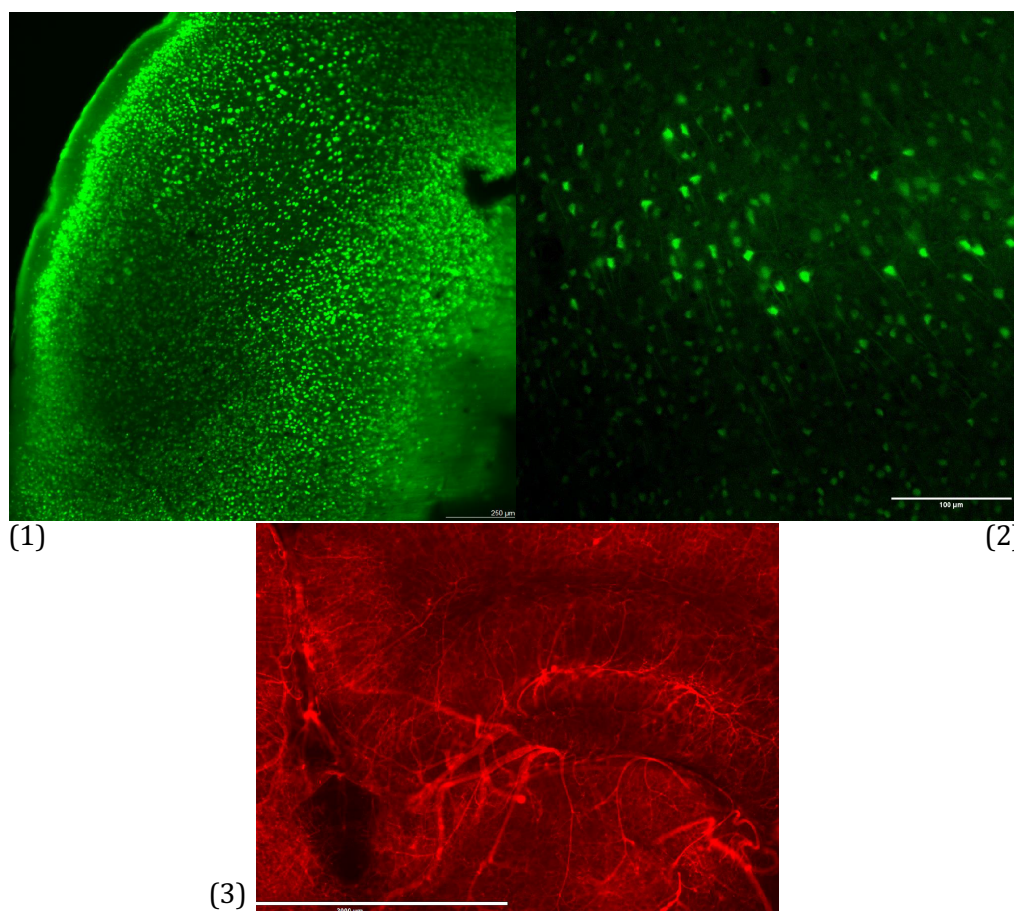


Figure 1: Neurons in cleared mouse hemisphere labeled with NeuN antibody (NeuN Antibody Vio® R667, Miltenyi Biotec B.V. & Co. KG, Bielefeld, Germany) and imaged using Ultramicroscope II LaVision Biotec, Bielefeld, Germany). Brain hemispheres were cleared using MACS Clearing Kit (Miltenyi Biotec B.V. & Co. KG, Bielefeld, Germany).

Figure 2: YFP-positive neurons in cleared mouse brain using PEGASOS clearing method under confocal microscope Olympus FV3000. The image was taken on approximately 1 mm thick cleared brain slice.

Figure 3: Blood vessels in mouse brain labeled with LEL Texas Red using inverted fluorescence microscope (approximately 1 mm sample slice (sample sliced with Alto Acrylic 1 mm Mouse Brain Coronal 40 75gm, CellPoint Scientific) cleared using ECi method; blood vessels labelled with *Lycopersicon Esculentum* Lectin Texas Red (Invitrogen)).

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P4: Extracellular polymeric substances (EPS) are an important factor in alleviating toxicity of copper oxide nanoparticles and ions in *Chlorella vulgaris*

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Copper nanoparticles have unique properties but are of concern because of their potentially harmful effects on aquatic ecosystems. In order to investigate the effects of CuO and Cu₂O nanoparticles and copper ions on the freshwater alga *Chlorella vulgaris*, cells were treated with concentrations that allow 75 % cell survival after 72 hours in liquid Bold's Basal medium. Ultrastructural and morphological changes and the localization of copper in cells and in the extracellular layer composed of polymeric substances (EPS) were examined using a transmission electron microscope. The elemental composition of the observed particles was confirmed with an EDX detector, while the EPS layer was quantified using an organic carbon analyser. The EPS layer of algal cells was also visualized by light microscopy in conjugation with the fluorescently labelled lectin ConA, while its quantification was performed on a microplate reader. The results obtained by TEM analysis showed that CuO nanoparticles bind to the surface of the cell wall, while Cu₂O nanoparticles bind, among other things, to thylakoid membranes (Figure 1). Moreover, all treatments resulted in disruption of membrane and cell wall integrity, with CuSO₄ having the most deleterious effect. Furthermore, a significant accumulation of copper nanoparticles on the EPS cell layer was observed after all applied treatments. Light microscopy showed that treatments with CuO, Cu₂O and CuSO₄ resulted in increased EPS synthesis compared to the control, which was also demonstrated using a microplate reader, while further quantification using an organic carbon analyser showed that only the CuSO₄ treatment significantly contributed to the synthesis of the EPS layer. These findings show that copper oxide nanoparticles and ions can easily bind to EPS which then alleviates their harmful effects on algae by reducing copper internalization into cells.

Keywords: *Chlorella vulgaris*, copper oxide, lectins, nanoparticles, TEM

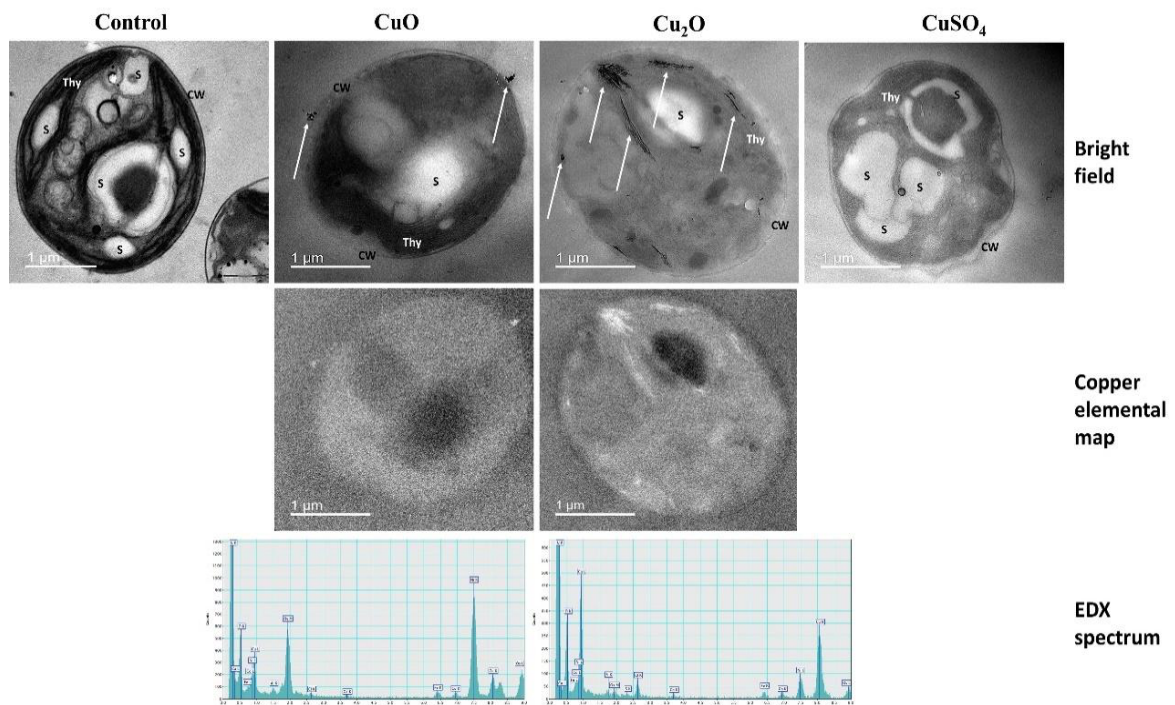


Figure 1: *C. vulgaris* cells 72 h after exposure to 14.45 mg L⁻¹ CuO, 11.89 mg L⁻¹ Cu₂O nanoparticles and 8.36 mg L⁻¹ CuSO₄ obtained by TEM, showing nanoparticles (arrows) on or in the algae cells (bar = 1 μm). Thy-thylakoids, S-starch, CW-cell wall.

P5: Light sheet fluorescence microscopy reveals the spatial arrangements of neurons and blood vessels in the mouse brain

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Microscopy has made it possible to visualize structures of interest that are not visible to the bare eye in various fields of science, including neuroscience. The preparation of tissue samples and whole organs for visualization by classical microscopic methods can be complicated, but also time-consuming due to cutting the tissue into thin enough sections so that the structures can be visible. Using fluorescence microscopy, it is possible to image naturally transparent parts of organisms such as parts of mouse embryo [1,2]. Novel light sheet fluorescence microscopy (LSFM) enables the visualization of large samples and even entire organs and organisms. Quality tissue clearing is imperative in this type of microscopy [3]. The aim of this research was to visualize both neurons and blood vessels as fluorescently labeled structures in the whole mouse brain. Two-month-old mice (Thy1-YFP-16 strain), which naturally express yellow fluorescent protein in neurons, were used for brain isolation. For blood vessels visualization, *Lycopersicon esculentum* Lectin Texas Red (Invitrogen) was administered in the left heart ventricle of living mouse before perfusion. For whole brain tissue clearing and visualization of blood vessels and neurons these methods were used: ECi (optical clearing using ethyl-cinnamate) and iDISCO+ (modified immunolabeling-enabled three-dimensional imaging of solvent-cleared organs). After anesthesia, mice were perfused with 1× PBS and 4 % formalin solution and brains were isolated and cleared by the methods mentioned above. In iDISCO protocol, for visualization of neurons Anti-GFP (green fluorescent protein) antibody - ChIP Grade (Abcam, Cambridge, United Kingdom) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (Abcam, Cambridge, United Kingdom) were used. Samples were imaged with Ultramicroscope II, LaVision Biotec, Bielefeld, Germany. Clearing protocols enabled good visualization of blood vessels (Figures 1a, 1b), while iDISCO+ which uses antibody labeling proved to be better for visualization of neurons (Figure 1c). Of all used protocols, the ECi method is recommended because it lasts only one day, while the other methods are more time-consuming and require more toxic chemicals, however the visualization the neurons are currently not possible using the ECi protocol. The visualization of both neurons and blood vessels would allow monitoring the events and morphological recovery in mouse brain after ischemic stroke.

Keywords: brain, clearing methods, ECi, iDISCO+, mouse

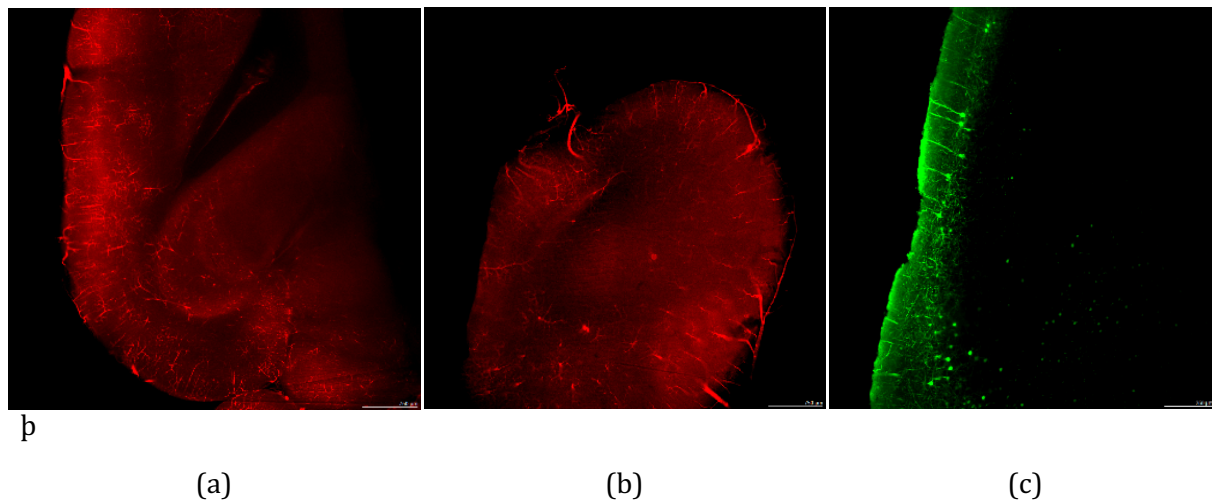


Figure 1: (a) Blood vessels in mouse brain cleared using ECI method and labelled with *Lycopersicon esculentum* Lectin Texas Red (Invitrogen) imaged on light sheet microscope (Ultramicroscope II, LaVision Biotec, Bielefeld, Germany, bar 750 μm); (b) Blood vessels in mouse brain cleared using iDISCO+ method and labelled with *Lycopersicon esculentum* Lectin Texas Red (Invitrogen) imaged on light sheet microscope (Ultramicroscope II, LaVision Biotec, Bielefeld, Germany), bar 750 μm ; (c) Neurons in mouse brain cleared using iDISCO+ method and labelled with Anti-GFP (green fluorescent protein) antibody - CHIP Grade (Abcam, Cambridge, United Kingdom) and Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (Abcam, Cambridge, United Kingdom) imaged on light sheet microscope (Ultramicroscope II, LaVision Biotec, Bielefeld, Germany), bar 250 μm .

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P6: Exploring plant morphology as a contribution to the assessment of the phytochemical potential of *Ailanthus altissima* (Mill.) Swingle

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Invasive alien plants are rich in specialized metabolites that play a crucial role in their adaptations to new environments. However, the mode of action of these metabolites beyond their invasive mechanisms is still not clarified. *Ailanthus altissima* (Mill.) Swingle, commonly known as the Tree of Heaven, is a highly invasive, widespread plant species whose potential to provide phytopharmaceutical ecosystem services is investigated in the project NATURALLY (HRZZ IP-2020-02-6899). Extrafloral nectaries (ENs) are a major component of the *A. altissima* secretory system, but the knowledge of their morphology and role in the tree's physiology is still limited. One of the hypotheses is that the role of ENs is to excrete metabolic waste. This research aims to examine the morphology of ENs, compare findings to previous research, and discuss their possible role and function. With this purpose, ENs on leaves have been monitored through different phases of leaf development from June to August in 2015 and 2022. The nectaries' morphology was investigated using light and scanning electron microscopy (SEM) techniques. Our study revealed the lack of previously reported pores or ducts on the upper surface of the glands, leading us to propose a mechanism for nectar secretion involving the tearing of epidermal tissue. Our findings align with one of the first systematical investigations of *A. altissima* extrafloral nectaries conducted in Croatia a century ago, which had been forgotten until recently. The study will contribute to new insights into the mechanisms of plant response to environmental conditions, extending beyond their potential to provide phytochemical ecosystem services.

Keywords: excretion, extrafloral nectaries, invasive alien plant species, light microscopy, SEM, tree of heaven

Acknowledgements: This research was supported by the Croatian Science Foundation under the project "NATURE as an ALLY: Alien Invasive Plants as Phytopharmaceuticals—NATURALLY" (IP-2020-02-6899). The work of Mirela Uzelac Božac was supported by the Croatian Science Foundation "Young Researchers' Career Development Project—Training New Doctoral Students" (DOK-2021-02-3094).

P7: *In vitro* genotoxic activity of leaf and flower extracts of alien invasive species *Ailanthus altissima* (Mill.) Swingle

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Invasive alien plants are rich in specialized metabolites (Sandilyan and Klooster, 2016) and have the potential to serve as a valuable source of bioactive phytochemicals and potent antioxidant systems with applications in the pharmaceutical industry. An essential condition for their utilization is their non-toxic nature. This study aims to assess the genotoxic activity of leaf and flower extracts of *Ailanthus altissima* (Mill.) Swingle from Istria (Croatia) by the comet (single cell gel electrophoresis) assay. Plant extracts were prepared using finely minced air-dried leaves and inflorescences collected and pooled from 15 locations in Istria and solved in 2 % dimethyl sulfoxide (DMSO) in Endothelial Basal Medium-2 (EBM-2, Lonza, San Diego, CA). The ability to induce *in vitro* genotoxic effect in human liver-derived endothelial cells (HLEC) was assessed for three extract concentrations: 1.0 mg/ml, 0.5 mg/ml and 0.1 mg/ml. Comet assay was conducted according to the method of Cvjetko et al. (2018), stained with GelRed Nucleic Acid Gel Stain, visualized with Zeiss Axioscope 5 fluorescence microscope and analyzed using the software KOMET5 (Kinetic Imaging Ltd., Liverpool, UK). The tail DNA percentage (% tDNA) was used as the primary measure of DNA damage. The treatments with leaf extracts in concentrations 1.0 and 0.5 mg/ml resulted in a significant increase of % tDNA compared to the control (Figure 1). The treatment with flower extracts showed a genotoxic effect in HLEC only in the highest tested concentration of 1.0 mg/ml. These findings suggest that *A. altissima* extracts in concentrations below 0.1 mg/ml for the leaves and below 0.5 mg/ml for the flowers do not cause substantial DNA damage in human hepatocytes, indicating a promising avenue for further research regarding the potential use of these extracts in pharmaceutical applications.

Keywords: Comet assay, DNA damage, human liver endothelial cells, invasive alien plant extracts

Acknowledgements: This research was supported by the Croatian Science Foundation under the project “NATURE as an ALLY: Alien Invasive Plants as Phytopharmaceuticals—NATURALLY” (IP-2020-02-6899). The work of Mirela Uzelac Božac was supported by the Croatian Science Foundation “Young Researchers' Career Development Project–Training New Doctoral Students” (DOK-2021-02-3094)

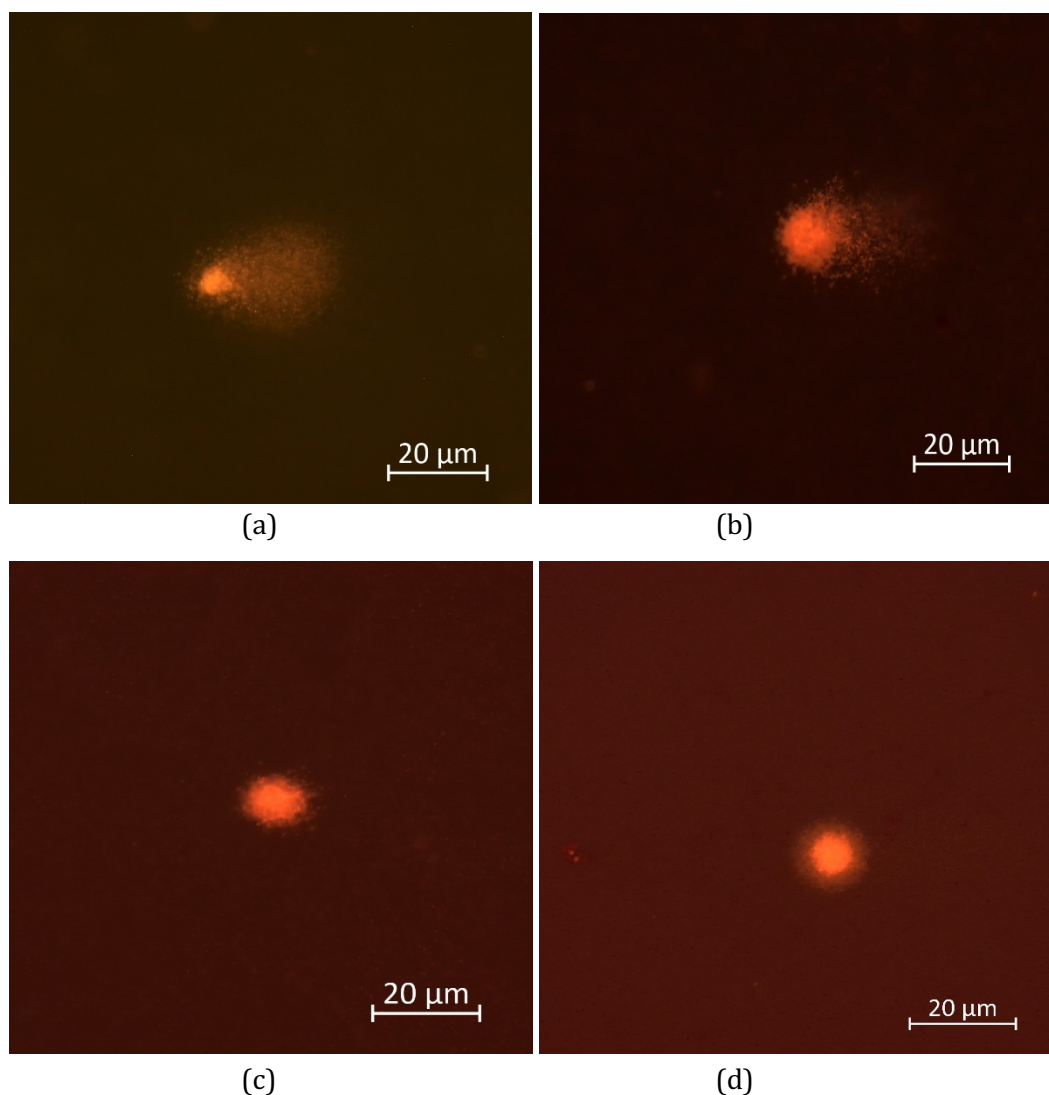


Figure 1: DNA damage of human liver-derived endothelial cells (HLEC) recorded by Comet assay after 24 h treatment with *Ailanthus altissima* leaf extracts in the concentrations of 1.0 mg/ml (a), 0.5 mg/ml (b), and 0.1 mg/ml (c). Only the concentrations 1.0 and 0.5 mg/ml caused significant damage compared to the control (d). Images were taken with 40x magnification using the ZEISS Axioscope 5 fluorescence microscope.

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**POSTER PRESENTATIONS
(Materials Science)**

P8: TEM analysis of Ni-based electrocatalyst

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Water electrolysis for hydrogen production is governed by the oxygen evolution reaction (OER). The most efficient catalysts for the OER so far are Ir and Pd-based catalysts. Because of their high prices new catalysts are readily investigated [1]. Among the potential candidates are also Ni-P/C-based catalysts that contain Ni-P nanoparticles supported in a carbon matrix. The aim of present work was to investigate the morphology, crystallinity and chemical composition of synthesized Ni-P/C catalysts using TEM/EDS. Ni-P/C-based catalysts were synthesized by hydrothermal process in which Ni salts were mixed with phosphate/polydopamine (P/PDA) medium with increasing concentration of phosphorous (P/PDA ratio 0, 0.15, 0.3, 0.6, 0.9) followed by a pyrolytic treatment in an inert atmosphere. The TEM samples were prepared by grinding the powder samples and dispersing them in ethanol. The solution was applied to a lacey carbon mesh on a copper grid and coated with 2 nanometres of carbon using PECS (Model 682) precision etching and coating system. The TEM/EDS analyses were carried out at 200 kV in a JEOL JEM-2100 TEM. Ni-P/C samples with zero or low ratio of P/PDA medium (0.0, 0.15, 0.3) exhibit amorphous carbonaceous phase with embedded, randomly shaped, Ni-P nanoparticles ranging up to 200 nm in size (Fig. 1a). However, when ratio of the P/PDA medium increases to 0.6 the morphology of supporting carbonaceous phase changes to well defined hollow spheres with the shell thickness of app. 70 to 80 nm (Fig. 1b). EDS analysis confirmed concentration variations in Ni-P nanoparticles and carbonaceous matrix/spheres depending on the initial P/PDA ratio. The transformation mechanism from amorphous carbonaceous matrix to well defined spheres, although not yet fully understood, nevertheless implies that these Ni-P/C-based nanostructures may be interesting catalysts for the OER catalysts, which is additionally supported by already measured electrochemical properties.

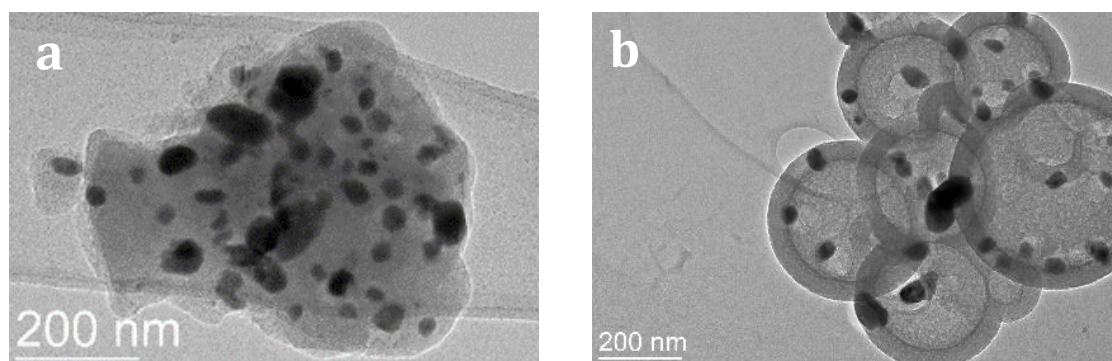


Figure 1: (a) TEM image of a Ni-P/C-based electrocatalyst with a phosphorous/polydopamine ratio of 0.15. (b) TEM image of a Ni-P/C-based electrocatalyst with a phosphorous/polydopamine ratio of 0.6.

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P9: Establishment of a microscopy laboratory at the Department of Physics, University of Sarajevo-Faculty of Science

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From June 2022 to May 2023, a major reconstruction project at the Department of Physics, University of Sarajevo-Faculty of Science took place. During that time, a scanning electron microscope JEOL JSM IT 200 LA and an atomic force microscope Nanosurf CoreAFM were purchased, installed, and put into operation. The main motivation behind this project was to support and strengthen research and teaching capabilities, mainly in solid state physics, but also in chemistry, life science, and material science in Bosnia and Herzegovina. Due to a lack of equipment, these kinds of analyses could not be performed in Bosnia and Herzegovina. Various researchers from Universities across the country had to use the services of different laboratories abroad. According to preliminary interest, we conclude that the number of potential users of microscopy techniques in our Department will be high. Thus far, measurements of metals, semiconductors, thin films, and various biological samples have proved to be successful, regardless of lack of previous experience.

Keywords: scanning electron microscopy, atomic force microscopy

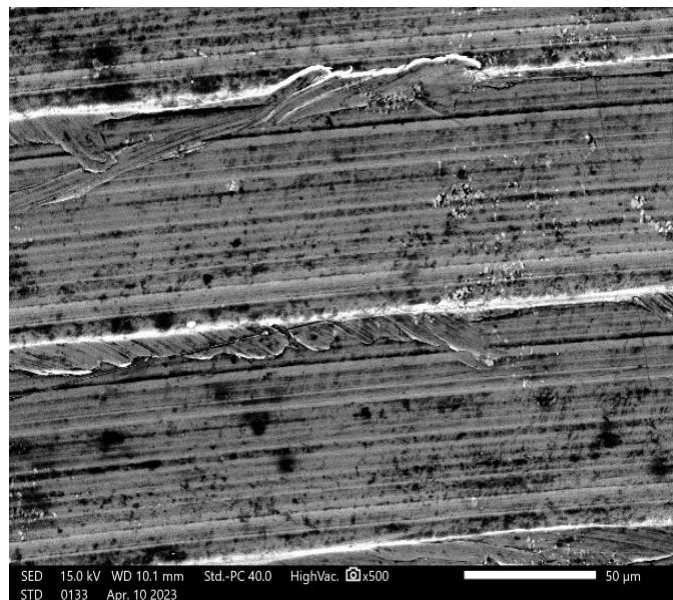


Figure 1: SEM image of CuZrAlY metallic glass.

P10: Surface reduction of tungsten oxide thin film by low energy hydrogen ion bombardment

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Tungsten trioxide, WO_3 , is widely known for its technological importance in the fields of electrochromic sensing and catalyst devices. The incorporation of hydrogen in WO_3 can significantly influence the electrical, optical and structural properties of the material. In this work, the changes in the chemical states of tungsten and the electronic structure of WO_3 during H_2^+ bombardment were monitored in situ by X-ray photoelectron spectroscopy (XPS). WO_3 thin films were bombarded with H_2^+ ions of three different energies (1, 2 and 5 keV), and hydrogen implanted films were afterwards characterized by scanning electron microscopy, X-ray diffraction and secondary ion mass spectrometry. XPS analysis shows that, under H_2^+ irradiation, lower and intermediate oxides, as well as metallic W, are formed in the surface layers of WO_3 . At the beginning of H_2^+ bombardment, oxidation states of W in WO_3 film were partially reduced to W^{5+} . With the increasing bombardment time, further reductions to W^{4+} , W^{2+} , and finally to W^0 , were observed. After 180 min of H_2^+ bombardment the concentration of different tungsten oxides saturated. The morphological evolution of WO_3 films bombarded with different energies of H_2^+ ions was observed *ex situ* by scanning electron microscopy. The observed changes in the crystal morphology (Fig. 1) are confirmed with the X-ray diffraction results. The as prepared sample exhibits the monoclinic WO_3 phase, which under hydrogen bombardment transforms into the tetragonal phase. The present work demonstrates that the surface reduction of WO_3 under H_2^+ bombardment substantially depends on the energy of impinging ions. Moreover, by increasing the ion energy, the bulk of WO_3 sample transforms from the monoclinic to the tetragonal phase, indicating the formation of hydrogen tungsten bronzes – non-stoichiometric materials in which hydrogen atoms are incorporated into the structure of WO_3 .

Keywords: hydrogen bombardment, tungsten trioxide, surface modification, X-ray photoelectron spectroscopy

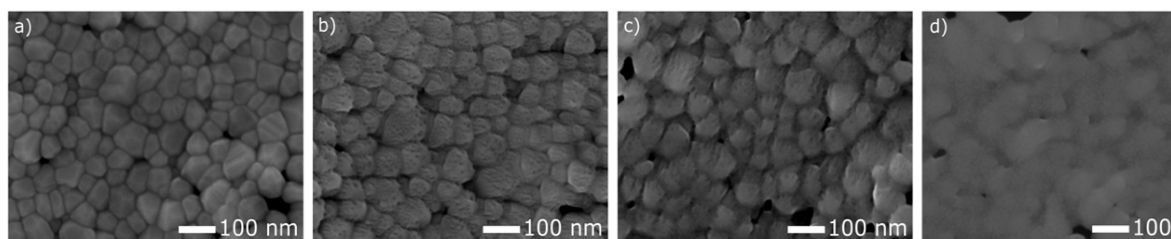


Figure 1: SEM surface morphologies of WO_3 thin films: a) as grown, b) H_2^+ irradiated at 1 keV 180 min, c) H_2^+ irradiated at 2 keV 180 min, d) H_2^+ irradiated at 5 keV 180 min.

P11: A decade of scanning electron microscopy at Faculty of Chemical Engineering and Technology

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The first scanning electron microscope at the Faculty of Chemical Engineering and Technology (FCET) was installed in March 2013. Since then, it has taken over 36 000 images, helped educate over 30 users, most of them early career researchers, and was used for numerous national and international research projects. The bibliography of the instrument numbers almost 100 papers and one of its micrographs was featured on the title page of the Journal of Applied Polymer Science. The SEM is also widely used in partnerships with industry and for commercial analysis of samples.

The instrument is extensively used in teaching, primarily through yearly workshops for Ph.D. students enrolled at the FCET doctoral study programme and through a graduate-level course Materials Engineering Laboratory. Students working on their bachelor and graduate theses in the field of materials science also commonly use the SEM for the analysis of their samples. Demonstration exercises are held for graduate students of the Department of Biology, Faculty of Science, and the capabilities of the instrument are demonstrated to the public during FCET Day of Open Doors and the Festival of Science.

The instrument was upgraded over the years, first by the addition of a STEM detector in 2019, financed by a grant from the Ministry of Science and Education, and then in 2022 with a new EDS detector with mapping software, financed by the European Regional Development Fund.

Keywords: commercial use, education, energy-dispersive X-ray spectroscopy (EDS), scanning transmission electron microscopy (STEM) detector, scientific research

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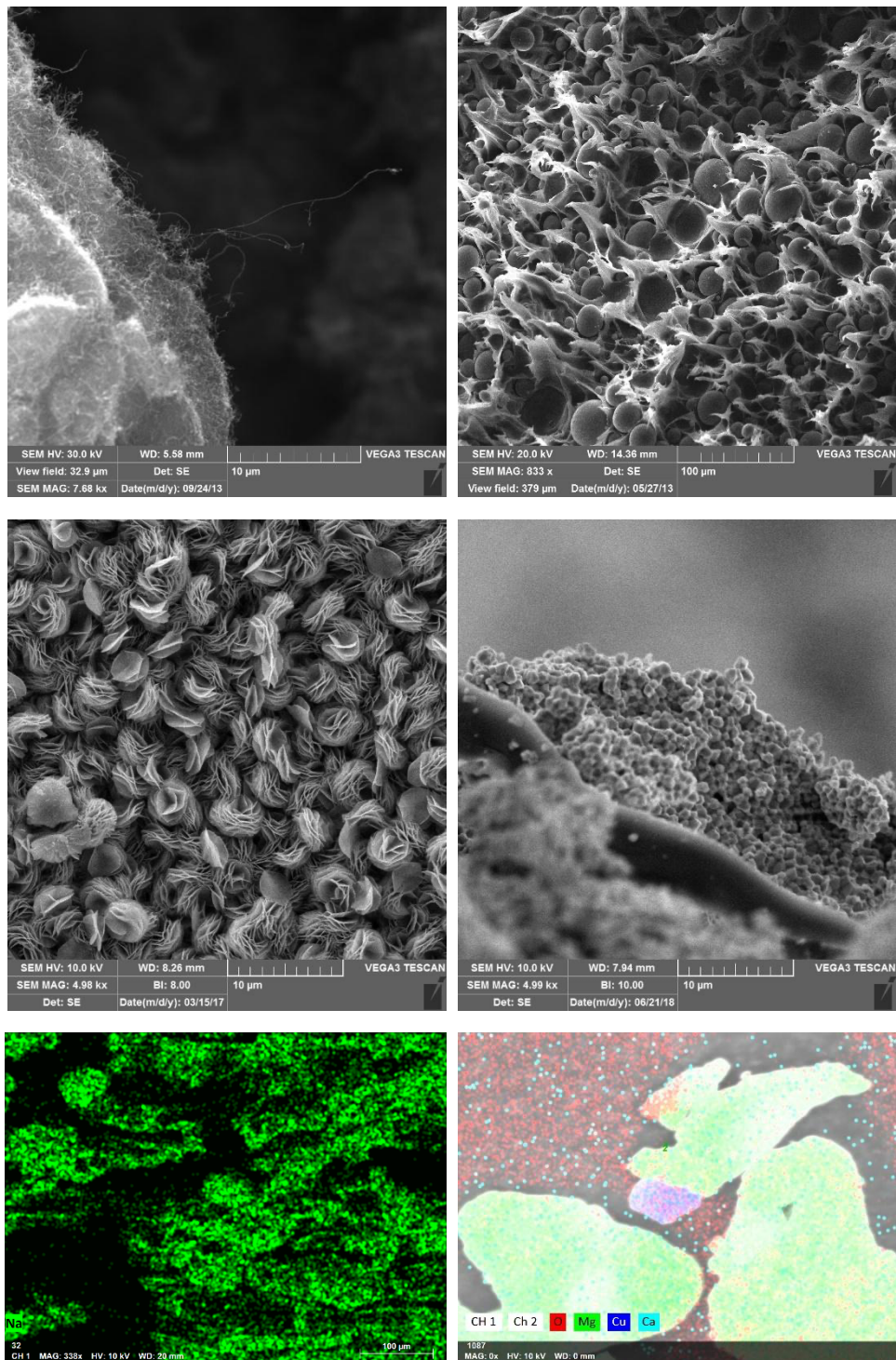


Figure 1: Some examples of the images taken by the SEM throughout its first decade.

P12: Controlled release from magnetite nanocarriers by external magnetic fields

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Flavonoids are natural antioxidants with a polyphenolic structure that exhibit therapeutic potential like anticarcinogenic, antidiabetic, anti-inflammatory, antibacterial, and antioxidant activities. However, limited bioavailability, low absorption rate, poor water solubility and instability under physiological conditions reduce their potential in the pharmaceutical industry. An effective strategy to overcome these limitations is encapsulation into nanocarriers. Exceptional candidates for flavonoid delivery are mesoporous magnetite nanocarriers due to their biodegradability, physicochemical properties and superparamagnetic nature. Structural, morphological, thermal and magnetic characterization of nanoparticles was carried out using different experimental techniques. Encapsulation of flavonoids was carried out by the adsorption method, and encapsulation efficiency was determined by UV-Vis spectroscopy. *In vitro* kinetics of flavonoid release from magnetic nanocarriers were controlled by a combination of external permanent and oscillating magnetic fields and quantified by UV-Vis spectroscopy. This study confirmed that the mesoporous magnetite nanoparticles present universal and excellent drug delivery nanocarriers particularly able to load and release with higher efficiency flavonoids of different physicochemical and/or structural properties. By optimizing parameters such as temperature, frequency of the oscillating magnetic field, strength of the permanent magnetic field and the choice of medium, the desired kinetic profile of the flavonoid release is ensured.

Keywords: drug release, external magnetic fields, flavonoids, magnetite nanoparticles

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P13: Automatic detection of microplastic particles in cosmetics using Raman microscopy

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The presence of microplastic particles in the environment has been an essential topic of discussion in the public sphere during the last decade. Likewise, intentionally added microplastics in various cosmetic products have recently been recognized as an issue due to their potential effect on human health. To address this, a new EU regulation 2023/2055 has been published, which aims to regulate the use of synthetic polymer microparticles in the production of cosmetic products [1]. Because of this, the need for a reliable method for detecting and characterizing microplastic particles has become apparent.

Vibrational spectroscopy is a technique that uses a spectrum obtained due to the interaction between a sample and light of a particular wavelength. It allows gaining information on the sample material's identity and/or chemical properties. Raman microscopy is one of these techniques, and it combines the analytical potential of both Raman spectroscopy and visual microscopy, making it a good technique of choice for obtaining information regarding the number, size, shape and chemical identity of individual polymer microparticles [2]. Appropriate sample preparation and setting up working laser conditions are crucial to facilitate these. In this work, we aimed to develop and validate a method for extracting microplastics in different cosmetic products, with their subsequent automatic detection and identification on a silicon filter using Raman microscopy. The technique involves using several other extraction protocols, depending on the type of cosmetic samples, including a novel microwave-assisted digestion procedure. The validation has been performed on two different sample matrices (gel and cream) with two other polymers (polyethylene and polypropylene), yielding extraction efficiencies above 90 %.

Keywords: microparticles, polymers, spectroscopy, validation

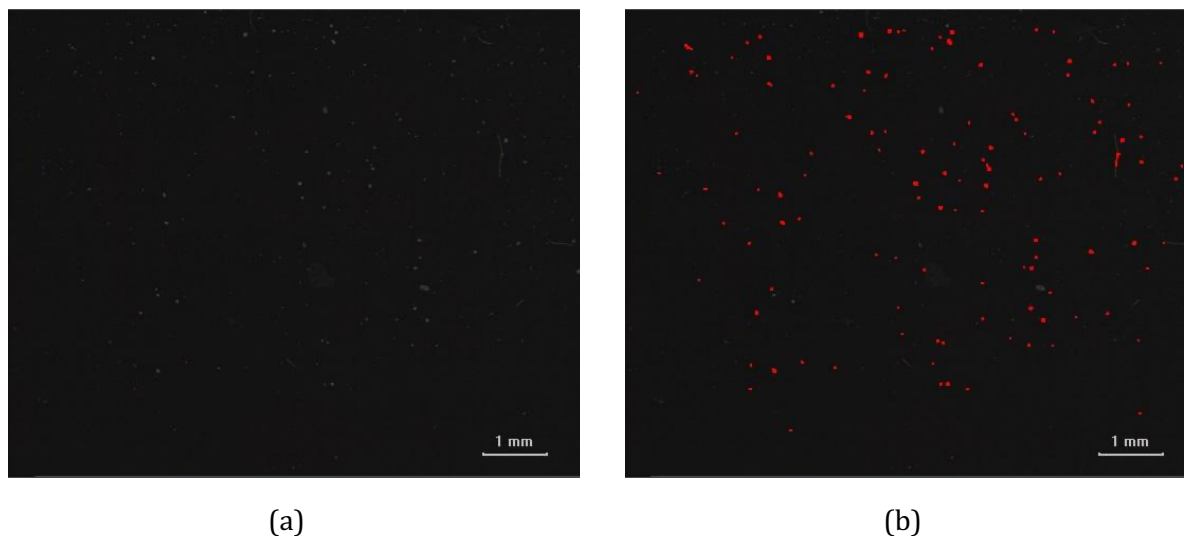


Figure 1: (a) Silicon filter with unknown microplastic particles, (b) successfully identified polyethylene particles marked on the same filter.

References:

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P14: Synthesizing a spatially arranged network of gold micro- and nanoclusters on silicon substrate

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We report a simple method for arranging gold micro- and nanoclusters in a line pattern using photolithography and thermal annealing. The samples were made on a $5 \times 5 \text{ mm}^2$, thermally oxidized silicon chips. Line pattern was acquired using lift-off. The resulting gold lines were 10 nm in thickness, and 2 μm in width, which was accomplished by deposition via sputter coater. The samples were then thermally annealed in two ways. First by keeping a constant annealing time and changing the temperature of the annealing, and the other by keeping the annealing temperature constant and varying the annealing time. The samples were then examined using scanning electron (SEM) and atomic force microscopes (AFM), and the images were processed using ImageJ and Gwyddion. Distributions of clusters by area and by height are presented. We notice that the clusters begin to form at $T = 800 \text{ }^\circ\text{C}$ when annealing time is $t = 1 \text{ h}$, and after $t = 2 \text{ h}$ when annealing temperature is $T = 600 \text{ }^\circ\text{C}$.

Keywords: AFM, Gwyddion, ImageJ, photolithography, SEM

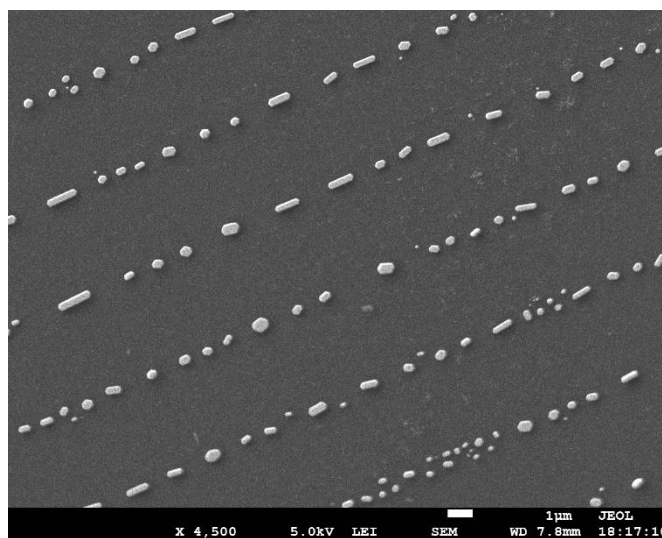


Figure 1: The figure shows an SEM micrograph in LEI mode of a sample annealed at a temperature $T = 1000 \text{ }^\circ\text{C}$ for 1 h.



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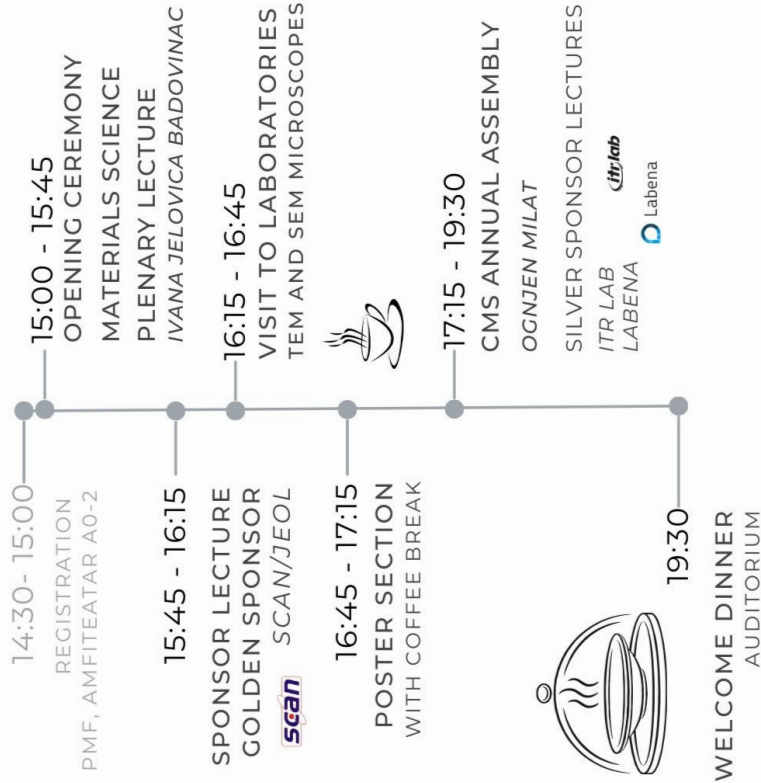
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