

**UČNI NAČRT PREDMETA / COURSE SYLLABUS**

**Predmet:** Elektrofiziološke meritve nanometrskih razsežnosti  
**Course title:** Electrophysiology in nanometer scale

Študijski program in stopnja Study programme and level	Študijska smer Study field	Letnik Academic year	Semester Semester
Interdisciplinarni doktorski študijski program BIOZNANOSTI 3. stopnja	Znanosti o celici	1,2	1,2,3,4
Interdisciplinary Doctoral Study Programme in BIOSCIENCES 3rd cycle	Cell Sciences	1,2	1,2,3,4

**Vrsta predmeta / Course type**individualno raziskovalni predmet /  
individual research course**Univerzitetna koda predmeta / University course code:**

Predavanja Lectures	Seminar Seminar	Vaje Tutorial	Klinične vaje work	Druge oblike študija	Samost. delo Individ. work	ECTS
/	15	10	/	/	100	5

**Nosilec predmeta / Lecturer:** Nosilec: akad. prof. dr. Robert Zorec**Jeziki /  
Languages:**

**Predavanja /  
Lectures:** slovenski / angleški  
Slovene / English

**Vaje / Tutorial:** slovenski / angleški  
Slovene / English

**Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:**Diploma naravoslovne smeri, opravljeni izpiti  
Temeljna fiziologija, Celična fiziologija,  
Funkcionalna biologija celice, Molekulska fiziologija**Prerequisites:**Undergraduate degree in sciences, with exams  
of Fundamental Physiology, Cell Physiology,  
Functional biology of the cell, Molecular  
Physiology**Vsebina:****Content (Syllabus outline):**

Snov je razdeljena na več delov (npr. nekaj iztočnic iz zgodovine elektrofiziologije, analogno vezje membrane, ponazoritev mirovnega membranskega potenciala, ponazoritev meritve potenciala motorične ploščice in akcijskega potenciala, meritve tokov prek membrane, metoda vpete napetosti [voltage-clamp]), meritve membranske kapacitivnosti, kombinacija optičnih metod za študij homeostaze citosolnega kalcija skupaj z elektrofiziološkimi metodami, uporaba metod molekularne biologije in elektrofiziologije skupaj z metodami za slikanje subcelularnih struktur. Študent uporabi pridobljeno znanje pri svojem delu tako, da reši naloge, ki so povezane z lastnim raziskovalnim problemom in izlušči iz literature znanstveni problem, ki ga skuša rešiti z metodami, ki jih obravnava ta predmet.

Pri predmetu sodelujejo zn. sod. dr. Jernej Jorgačevski, zn. sod. dr. Matjaž Stenovec in drugi vabljeni predavatelji.

The course is divided into several sections: Background and history of bioelectrical phenomena, analog electrical circuit of cell membrane, mechanisms and demonstration of resting membrane potential, mechanisms and demonstrations of postsynaptic potentials, action potentials, single channel recording techniques, the voltage-clamp techniques, measurements of membrane capacitance, combining electrophysiological techniques with optical techniques, measurements of cytosolic calcium activity, combined use of electrophysiology and molecular biology techniques to sample cytosol and manipulate gene expression in single cells, combining quantitative imaging techniques to study subcellular structures. Students will use the learned knowledge by using these techniques in their own research. Moreover, by studying the literature they will be able to identify physiological problems that can be addressed with the learned approaches.

Dr. Jernej Jorgačevski, Dr. Matjaž Stenovec and other invited lecturers will cooperate at the course.

#### **Temeljni literatura in viri / Readings:**

Katz B: Nerve, muscle, synapse. McGraw-Hill, 1966  
Aidley DJ: The physiology of excitable cells. Cambridge Univ. Press, 1991.  
Hille B (1992). Ionic channels of excitable membranes. Sinauer Associates.;  
Sperelakis N (2012) Cell Physiology. Academic Press.  
Zorec, R (1988). Ionski kanalčki v celični membrani. Kaj lahko raziskujemo z metodo "patch clamp"?. Med. razgl., 27, št.1 (1988), str.37-53.  
Sakmann B in Neher E (1995) Single Channel Recording 2<sup>nd</sup> ed. Plenum Press, New York

#### **Cilji in kompetence:**

Elektrofiziologija je veja fiziologije, ki se je uveljavila tako v rutinskem, kliničnem raziskovalnem (elektroencefalografija, elektrokardiografija, elektromiografija) kot v temeljnem raziskovalnem delu. V slednjem se sicer še vedno ponekod uporablja v obliki ekstracelularne, bipolarne ali unipolarne registracije, večinoma pa vse bolj kot mikroelektrofiziologija in nanoelektrofiziologija, s katero lahko študiramo lastnosti posamezne molekule ali osamljenih delov membrane ali celičnih predelkov. Tu uporabljamo različne steklene mikroelektrode, npr. za registracijo intracelularnih sinaptičnih potencialov, sinaptičnega toka (metoda *voltage-clamp*),

#### **Objectives and competences:**

Electrophysiology represents a subdivision of Physiology, which has been used in routine clinical work and clinical research (recording EEG, ECG, EMG) as well as in fundamental research. In the latter area electrophysiological techniques are used for extracellular, bipolar or unipolar signal registration, but in recent years mostly as nano and microelectrophysiological recordings for the study of single molecules or isolated membrane patches or even isolated subcellular structures. For these approaches different glass micropipettes are used. For example, for the registration of synaptic potentials with intracellular recording techniques very sharp glass microelectrodes are used. For

aktivacija in lastnosti posameznih membranskih kanalčkov (metoda *patch-clamp*) ter meritve ekso- in endocitoze hormonsko aktivnih in drugih celic z merjenjem lastnosti fuzijske pore, ki imajo premer manjši od nanometra (metoda *patch-clamp* v različici *whole-cell recording*, *cell-attached recording*, *inside-out* in *outside out recording*). Cilj predmeta je pridobitev temeljnih znanj za pristop k raziskavam membranskih procesov evkariontskih celic in njenih podceličnih predelkov. Slušatelj bo pridobil temeljne kompetence za uporabo elektrofizioloških metod pri raziskovalnem delu na posamezni celici; ki s svojim delovanjem prispevajo k delovanju tkih in višjih organizacijskih enot.

measuring synaptic currents the *voltage-clamp* method is used. Properties of single ion channels is studied with fire-polished glass pipettes with relatively wide tips under the voltage-clamp conditions (The *patch-clamp method*). Similarly, measurements of exo- and endocytosis in secretory cells and the properties of the fusion pore, with diameter at the subnanometer scale, the method of patch clamping is used (either in the *whole-cell recording*, *cell-attached recording*, *inside-out* and *outside-out recording configurations*). The aim of the course is to offer the candidates the key knowledge required to conduct the physiological research of membrane processes at the level of single cells as well as the subcellular membrane bound organelles. Thus the candidates will gain key competences and expertise to conduct their own research in this field.

#### **Predvideni študijski rezultati:**

Znanje in razumevanje:

Študent se nauči osnov sledečih metod: Metoda vpete napetosti, metode vpete napetosti krpice membrane (angl. The patch-clamp technique) za meritve tokov in napetostnih sprememb prek membrane cele celice, za meritve tokov skozi posamezni kanal, makroskopske in mikroskopske meritve membranske kapacitete, ki zrcalijo celične procese ekso- in endocitoze. Kombinirane elektrofiziološke in optofiziološke metode (metode za fotolizo vkljenjenih sekundarnih prenašalcev) z metodami za fluorescenčno označevanje subceličnih predelkov.

#### **Intended learning outcomes:**

Knowledge and understanding:

Students will gain fundamental knowledge of the: The voltage-clamp method of whole cells. The voltage-clamp method of an isolated membrane patch, i.e. the patch-clamp technique. Both are used for the measurements of currents and voltages across the membrane of whole cells and for measurements of currents through single ion channels. The latter methods are ideal also for measurements of macroscopic and microscopic changes in membrane capacitance, a parameter that is linear to the membrane area. Membrane area fluctuations reflect the processes of exo- and endocytosis. Together with electrophysiological techniques, the candidates will learn about combining these methods with optophysiological techniques (measurements of cytosolic calcium and other second messengers) with fluorescent methods to label subcellular structures.

#### **Metode poučevanja in učenja:**

#### **Learning and teaching methods:**

Kandidati bodo študirali teme, o katerih bodo opravljene predstavitve učitelj-učenec, oziroma bodo obravnavane v obliki konzultacij; pripraviti bodo morali več seminarskih nalog in praktično delo v laboratoriju.

Students will focus into the study through tutor-student consultations; work in the lab with cells and equipment, through essay work and through their own research and learning about data analysis.

<b>Načini ocenjevanja:</b>	Delež (v %) / Weight (in %)	<b>Assessment:</b>
Študent opravi izpit, ki sestoji iz pisne esejske izpitne naloge.	<b>100%</b>	Students are evaluated on the basis of their essay assignments.

**Reference nosilca / Lecturer's references:**

akad. prof. dr. Robert Zorec

KABASO, Doron, JORGAČEVSKI, Jernej, COSTA CALEJO, Ana-Isabel, FLAŠKER, Ajda, GUČEK, Alenka, KREFT, Marko, ZOREC, Robert. Comparison of unitary exocytic events in pituitary lactotrophs and in astrocytes : modeling the discrete open fusion-pore states. *Frontiers in cellular neuroscience*, ISSN 1662-5102, Apr. 2013, vol. 7.

COSTA CALEJO, Ana-Isabel, JORGAČEVSKI, Jernej, SILVA, V.S., STENOVEC, Matjaž, KREFT, Marko, GONÇALVES, Paula P., ZOREC, Robert. Aluminium-induced changes of fusion pore properties attenuate prolactin secretion in rat pituitary lactotrophs. *Neuroscience*, ISSN 0306-4522. [Print ed.], 2012, vol. 201, str. 57-66, ilustr., doi:10.1016/j.neuroscience.2011.11.015.

KABASO, Doron, GONGADZE, Ekaterina, JORGAČEVSKI, Jernej, KREFT, Marko, VAN RIENEN, Ursula, ZOREC, Robert, IGLIČ, Aleš. Exploring the binding dynamics of bar proteins. *Cellular & molecular biology letters*, ISSN 1425-8153, 2011, vol. 16, no. 3, str. 398-411 in S1-S8, ilustr.

THIEL, Gerhard, KREFT, Marko, ZOREC, Robert. Rhythmic kinetics of single fusion and fission in a plant cell protoplast. *Annals of the New York Academy of Sciences*, ISSN 0077-8923, 2009, letn. 1152, str. 1-6, doi: 10.1111/j.1749-6632.2008.03996.x.

DARIOS, Frédéric, WASSER, Catherine, SHAKIRZYANOVA, Anastasia, GINIATULLIN, Artur, JORGAČEVSKI, Jernej, KREFT, Marko, ZOREC, Robert, et al. Sphingosine facilitates SNARE complex assembly and activates synaptic vesicle exocytosis. *Neuron*, ISSN 0896-6273, 2009, letn. 62, str. 683-694, doi: 10.1016/j.neuron.2009.04.024.

VARDJAN, Nina, STENOVEC, Matjaž, JORGAČEVSKI, Jernej, KREFT, Marko, GRILC, Sonja, ZOREC, Robert. The fusion pore and vesicle cargo discharge modulation. *Annals of the New York Academy of Sciences*, ISSN 0077-8923, 2009, letn. 1152, str. 135-144, doi: 10.1111/j.1749-6632.2008.04007.x.

**UČNI NAČRT PREDMETA / COURSE SYLLABUS**

**Predmet:** Mikroskopija in analiza slike bioloških vzorcev - projektno delo  
**Course title:** Microscopy and image analysis of biological samples - project work

Študijski program in stopnja Study programme and level	Študijska smer Study field	Letnik Academic year	Semester Semester
Interdisciplinarni doktorski študijski program BIOZNANOSTI 3. stopnja	Znanosti o celici	1, 2	1, 2, 3, 4
Interdisciplinary Doctoral Study Programme in BIOSCIENCES 3rd cycle	Cell Sciences	1, 2	1, 2, 3, 4

**Vrsta predmeta / Course type**

individualno raziskovalni predmet / individual research course

**Univerzitetna koda predmeta / University course code:**

Predavanja Lectures	Seminar Seminar	Vaje Tutorial	Klinične vaje work	Druge oblike študija	Samost. delo Individ. work	ECTS
/	20	/	/	30	200	10

**Nosilec predmeta / Lecturer:** prof. dr. Rok Kostanjšek

**Jeziki / Languages:**  
**Predavanja / Lectures:** slovenski, angleški / slovene, english  
**Vaje / Tutorial:** slovenski, angleški / slovene, english

**Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:** **Prerequisites:**

Splošni pogoji za vpis na doktorski študij

General conditions for enrolment in doctoral studies

**Vsebina:**

- nadgradnja osnovnih svetlobno-mikroskopskih tehnik (mikroskopija v temnem polju, fazno-kontrastna mikroskopija, interferenčno kontrastna-DIC mikroskopija in fluorescenčna mikroskopija) s predstavitvijo njihovih prednosti in uporabe pri opazovanju bioloških vzorcev
- predstavitev postopkov zajemanja

**Content (Syllabus outline):**

- Upgrading of student's basic knowledge on light microscopy techniques (e.g. microscopy in a dark field, phase-contrast microscopy, interference contrast-DIC microscopy and fluorescent microscopy), their advantages and applications on biological samples
- Procedures for image acquisition, processing, and quantitative analysis of micrographs

mikroskopske slike, obdelovanja in kvantitativne analize slike bioloških vzorcev

- predstavitev naprednih svetlobno-mikroskopskih tehnik za izdelavo optičnih rezin ter osnovami 3D-rekonstrukcije slik bioloških vzorcev
- predstavitev presevne elektronske mikroskopije (TEM) za strukturno analizo različnih bioloških vzorcev (tkiva in celice, bakterije, virusi, liposomi...) s poudarkom na razumevanju in praktični izvedbi postopkov za pripravo bioloških vzorcev ter interpretaciji mikrografij
- spoznavanje tehnik vrstične elektronske mikroskopije (SEM), praktično seznanjanje s postopki priprave bioloških vzorcev in interpretacijo SEM mikrografij
- predstavitev naprednih elektronsko-mikroskopskih tehnik (HRTEM, EELS) in spektroskopskih metod (EDXS, EELS) bioloških vzorcev
- seznanjanje s sodobnimi tehnikami in pristopi za vizualizacijo bioloških vzorcev

- advanced light microscopic techniques with demonstration of optical sectioning and 3D reconstruction on biological samples
- Principles and techniques of transmission electronic microscopy (TEM) for structural analysis of biological samples (tissues, cells, bacterial, viral particles, liposomes...) with special emphasis on understanding of methodology, interpretation of micrographs and practical preparation of biological samples
- Principles and demonstration of scanning electron microscopy (SEM), its application on biological samples, interpretation of micrographs and practical approaches in sample preparation.
- Principles and examples of high resolution transmission electron microscopy (HRTEM) and spectroscopic methods (EDXS, EELS) of biological samples
- Principles of advanced microscopic techniques and approaches for visualization of biological samples

#### Temeljni literatura in viri / Readings:

- Hayat M. A. (2000) Principles and techniques of electron microscopy – Biological applications. 4th Edition. Cambridge University Press, Cambridge, UK, 543 str., ISBN 0-521-63287-0 (izbrana poglavja str. 367-388, 400-436)
- Bozzola J.J., Russell L.D. (1999): Electron microscopy – Principles and Techniques for Biologists. 2nd Edition. Jones and Bartlett Publishers, Sudbury, Massachusetts, USA, 670 str., ISBN 0-7637-0192-0 (str. 16-46, 120-146, 148-201, 262-278, 368-394, 442- 475, 480-608).
- Beatty B., Mai S., Squire J. (2002) Fluorescence in situ hybridization – Practical approach. Oxford University Press, New York, USA, 255 str., ISBN 0-19-963884-5 (poglavje 2, str. 5-27)
- Allen T.D. (ur.) (2008) Introduction to Electron Microscopy for Biologists, Methods in Cell Biology 88, izbrana poglavja D.B. Williams, C.B. Carter, Transmission Electron Microscopy, Plenum Press, 1996, izbrana poglavja
- Russ J.C. (2011) The Image Processing Handbook, 6th Edition. CRC Press, USA, 885 str., ISBN 978-1-4398-4045-0, izbrana poglavja
- Izbrani članki iz strokovnih revij:
- Microscopy and microanalysis online journal; Wiley and Sons
- Journal of Microscopy, Blackwell publishing

#### Cilji in kompetence:

- nadgradnja znanja o mikroskopskih metodah in analizi slike
- poznavanje z lastnostmi (prednostmi in morebitnimi omejitvami) različnih mikroskopskih tehnik
- poznavanje postopkov priprave bioloških vzorcev za opazovanje z različnimi mikroskopskimi tehnikami

#### Objectives and competences:

- upgrade of basic knowledge on microscopic methods
- acquaintance with properties (benefits and potential drawbacks) of various microscopic techniques
- acquaintance with preparation procedures of biological samples or with various microscopic techniques

- poznavanje procesov zajema, obdelave, analize in interpretacije mikrografij  
 - razvijanje lastne iniciativnosti pri zasnovi poskusov, ki vključujejo vizualizacijske pristope

- acquiring of basic skills on acquisition, processing, analysis and interpretation of images of biological samples  
 - development of self-initiative approach in designing of experiment, which include visualization approaches

**Predvideni študijski rezultati:**

Znanje in razumevanje:  
 - razumevanje fizikalnih osnov mikroskopskih tehnik  
 - sposobnost izbire ustreznih mikroskopskih tehnik pri zasnovi poizkusov  
 - poznavanje osnovnih postopkov priprave bioloških vzorcev za svetlobno in elektronsko mikroskopijo  
 - pridobitev znanj za zajemanje, obdelavo in analizo slike  
 - sposobnost interpretacije mikrografij bioloških vzorcev pridobljenih z različnimi mikroskopskimi tehnikami in prepoznavanje artefaktov kot posledice priprave vzorcev

**Intended learning outcomes:**

Knowledge and understanding:  
 - understanding of physical background of microscopic techniques  
 - choosing of appropriate microscopic approach in experiment design  
 - knowledge on basic procedures of biological sample preparation for light and electron microscopy  
 - basic knowledge on image acquisition, processing and analysis  
 - ability of interpretation of micrographs depicting biological samples

**Metode poučevanja in učenja:**

Konzultacije, seminarji (kot osnova projektnega dela) in individualno projektno delo, ki bo izvedeno v laboratorijih za svetlobno in elektronsko mikroskopijo na Oddelku za biologijo Biotehniške fakultete

**Learning and teaching methods:**

Consultations, seminars (as background of individual project) and individual project

**Načini ocenjevanja:**

Pisni izpit iz tem predavanj  
 Predstavitev individualnega projekta

Delež (v %) /  
 Weight (in %)

**50%**  
**50%**

**Assessment:**

Examination  
 Project presentation

**Reference nosilca / Lecturer's references:**

**KOSTANJŠEK Rok**

**KOSTANJŠEK, Rok**, PAŠIĆ, Lejla, DAIMS, Holger, SKET, Boris. Structure and community composition of sprout-like bacterial aggregates in a Dinaric Karst subterranean stream. *Microbial ecology*, ISSN 0095-3628, 2013, vol. 66, issue 1, str. 5-18, ilustr. <http://link.springer.com/content/pdf/10.1007%2Fs00248-012-0172-1>, doi: [10.1007/s00248-012-0172-1](https://doi.org/10.1007/s00248-012-0172-1). [COBISS.SI-ID [2711375](https://www.cobiss.si/id/2711375)]

BAVDEK, Andrej, **KOSTANJŠEK, Rok**, ANTONINI, Valeria, LAKEY, Jeremy H., DALLA SERRA, Mauro, GILBERT, Robert J., ANDERLUH, Gregor. pH dependence of listeriolysin O aggregation and pore-forming ability. *FEBS journal*, ISSN 1742-464X, 2012, vol. 279, iss. 1, str. 126-141. <http://onlinelibrary.wiley.com/doi/10.1111/j.1742-4658.2011.08405.x/pdf>, doi: [10.1111/j.1742-4658.2011.08405.x](https://doi.org/10.1111/j.1742-4658.2011.08405.x)

[4658.2011.08405.x](#). [COBISS.SI-ID [4881690](#)]

VODOVNIK, Maša, **KOSTANJŠEK, Rok**, ZOREC, Maša, MARINŠEK-LOGAR, Romana. Exposure to Al<sub>2</sub>O<sub>3</sub> nanoparticles changes the fatty acid profile of the anaerobe *Ruminococcus flavefaciens*. *Folia microbiologica*, ISSN 0015-5632. [Print ed.], 2012, vol. 57, no. 4, str. 363-365. <http://www.springerlink.com/content/n44j862418163777/fulltext.pdf>, doi: [10.1007/s12223-012-0143-4](https://doi.org/10.1007/s12223-012-0143-4). [COBISS.SI-ID [3037576](#)]

KNEŽEVIĆ, Petar, OBREHT, Dragana, CURCIN, S., PETRUŠIĆ, Milivoje, ALEKSIĆ, Verica, **KOSTANJŠEK, Rok**, PETROVIC, O., et al. Phages of *Pseudomonas aeruginosa*: response to environmental factors and in vitro ability to inhibit bacterial growth and biofilm formation. *Journal of applied microbiology*, ISSN 1364-5072, 2011, issue 1, vol. 111, str. 245-254, doi: [10.1111/j.1365-2672.2011.05043.x](https://doi.org/10.1111/j.1365-2672.2011.05043.x). [COBISS.SI-ID [2392399](#)]

VITTORI, Miloš, ŽNIDARŠIČ, Nada, **KOSTANJŠEK, Rok**, ŠTRUS, Jasna. Microscopic anatomy of male tegumental glands and associated cuticular structures in *Titanethes albus* (Crustacea: Isopoda). *Arthropod structure & development*, ISSN 1467-8039, 2012, vol. 41, issue 2, str. 133-144. <http://dx.doi.org/10.1016/j.asd.2011.08.004>, doi: [10.1016/j.asd.2011.08.004](https://doi.org/10.1016/j.asd.2011.08.004). [COBISS.SI-ID [2468943](#)]

AMBROŽIČ, Jerneja, ŽGUR-BERTOK, Darja, **KOSTANJŠEK, Rok**, AVGUŠTIN, Gorazd. Isolation and characterization of a novel violacein-like pigment producing psychrotrophic bacterial species *Janthinobacterium svalbardensis* sp. nov. *Antonie van Leeuwenhoek*, ISSN 0003-6072, 2013, vol. 103, issue 4, str. 763-769. [http://download.springer.com/static/pdf/897/art%253A10.1007%252Fs10482-012-9858-0.pdf?auth66=1354969235\\_038af70ca72300adad29e7303bd340f3&ext=.pdf](http://download.springer.com/static/pdf/897/art%253A10.1007%252Fs10482-012-9858-0.pdf?auth66=1354969235_038af70ca72300adad29e7303bd340f3&ext=.pdf), doi: [10.1007/s10482-012-9858-0](https://doi.org/10.1007/s10482-012-9858-0). [COBISS.SI-ID [3147912](#)]

VITTORI, Miloš, **KOSTANJŠEK, Rok**, ŽNIDARŠIČ, Nada, ŠTRUS, Jasna. Molting and cuticle deposition in the subterranean trichoniscid *Titanethes albus* (Crustacea, Isopoda). V: ŠTRUS, Jasna (ur.), TAITI, Stefano (ur.), SFENTHOURAKIS, Spyros (ur.). *Advances in Terrestrial Isopod Biology*, (Zookeys, Vol. 176, special issue). Sofia: Pensoft Publishers, 2012, vol. 176, special issue, str. 23-38. [http://www.pensoft.net/J\\_FILES/1/articles/2285/2285-G-3-layout.pdf](http://www.pensoft.net/J_FILES/1/articles/2285/2285-G-3-layout.pdf), doi: [10.3897/zookeys.176.2285](https://doi.org/10.3897/zookeys.176.2285). [COBISS.SI-ID [2550351](#)]

MILATOVIČ, Maša, **KOSTANJŠEK, Rok**, ŠTRUS, Jasna. Ontogenetic development of *Porcellio scaber*: staging based on microscopic anatomy. *Journal of crustacean biology*, ISSN 0278-0372, 2010, vol. 30, no. 2, str. 225-234. [COBISS.SI-ID [2163535](#)]

VALANT, Janez, DROBNE, Damjana, SEPČIĆ, Kristina, JEMEC, Anita, KOGEJ, Ksenija, **KOSTANJŠEK, Rok**. Hazardous potential of manufactured nanoparticles identified by in vivo assay. *Journal of hazardous materials*, ISSN 0304-3894. [Print ed.], 2009, issues 1-3, vol. 171, str. 160-165, ilustr. <http://dx.doi.org/10.1016/j.jhazmat.2009.05.115>, doi: [10.1016/j.jhazmat.2009.05.115](https://doi.org/10.1016/j.jhazmat.2009.05.115). [COBISS.SI-ID [6013049](#)]

KNEŽEVIĆ, Petar, **KOSTANJŠEK, Rok**, OBREHT, Dragana, PETROVIC, Olga. Isolation of *Pseudomonas aeruginosa* specific phages with broad activity spectra. *Current microbiology*, ISSN 0343-8651, 2009, vol. 59, str. 173-180. [COBISS.SI-ID [2009679](#)]



**UČNI NAČRT PREDMETA / COURSE SYLLABUS**

<b>Predmet:</b>	<b>Specialne tehnike v elektronski mikroskopiji</b>
<b>Course title:</b>	<b>Special techniques of electron microscopy</b>

<b>Študijski program in stopnja</b> Study programme and level	<b>Študijska smer</b> Study field	<b>Letnik</b> Academic year	<b>Semester</b> Semester
Interdisciplinarni doktorski študijski program BIOZNANOSTI 3. stopnja	<b>Znanost o celici</b>	<b>1,2</b>	<b>1,2,3,4</b>
Interdisciplinary Doctoral Study Programme in BIOSCIENCES 3rd cycle	<b>Cell sciences</b>	<b>1,2</b>	<b>1,2,3,4</b>

**Vrsta predmeta / Course type** individualno raziskovalni predmet / individual research course

**Univerzitetna koda predmeta / University course code:**

<b>Predavanja</b> Lectures	<b>Seminar</b> Seminar	<b>Vaje</b> Tutorial	<b>Klinične vaje</b> work	<b>Druge oblike študija</b>	<b>Samost. delo</b> Individ. work	<b>ECTS</b>
/	5	10	/	10	100	5

**Nosilec predmeta / Lecturer:** Nosilec: prof. dr. Rok Romih

<b>Jeziki / Languages:</b>	<b>Predavanja / Lectures:</b>	slovenski / angleški Slovene / English
	<b>Vaje / Tutorial:</b>	slovenski / angleški Slovene / English

**Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:** **Prerequisites:**

Splošni pogoji za vpis na doktorski študij

General terms for doctor study.

**Vsebina:** **Content (Syllabus outline):**

Predstavitev novosti v presewni in vrstični elektronski mikroskopiji in možnosti uporabe novejših metod v bioznanostih. Pregled kriometod s poudarkom na teoretskih osnovah zamrzovanja kot načina fiksacije bioloških vzorcev, uporabi zmrzovalnega lomljenja in jedkanja za študij strukture membran, študiju celične ultrastrukture s tehniko zamrzovanja pri visokem pritisku in hladne izmenjave ter pripravi krioultratankih rezin. Predstavitev metod za lokalizacijo celičnih sestavin na ultrastrukturnem nivoju: spoznavanje principov imunocitokemije in posebnosti v elektronski mikroskopiji, primerjava metod označevanja antigenov pred vklapljanjem vzorcev in po vklapljanju. Uporaba elektronsko gostih označevalcev za sledenje membranskih transportov. Princip elektronske tomografije: izdelava tridimenzionalnih rekonstrukcij in modelov celičnih struktur. Praktični primeri kombinacij navednih metod pri študiju celične organizacije in funkcije.

Introduction to recent advances in transmission and scanning electron microscopy and their application in life sciences. Overview of cryo methods with emphasis on theory of freezing and its comparison with chemical fixation, on the use of freeze-fracturing and freeze-etching for studying cell membranes, on the studies of ultrastructure by high pressure freezing and freeze-substitution methods and on preparation of cryo-ultrathin sections. Introduction to methods for ultrastructural localization of molecules: the basic principles of immunocytochemistry and considerations in the field of electron microscopy, comparison of pre-embedding and post-embedding labelling. Markers of intracellular membrane traffic. Principles of electron tomography: production of three dimensional reconstructions and models of cellular structures. Examples of application of various methods to study cell organisation and function.

#### **Temeljni literatura in viri / Readings:**

- Bozzola JJ, Russell LD. 1999. Electron Microscopy, Second Edition. Jones and Bartlett Publishers, ISBN 0-7637-0192-0.
- Revijalni članki s področja in tekoča periodika. / Review articles from selected journals.

#### **Cilji in kompetence:**

- pridobivanje znanj s področja elektronske mikroskopije;
- seznanitev z najsodobnejšimi metodami elektronske mikroskopije v bioznanostih;
- spoznati možnosti izbire ustrezne metodike za reševanje znanstvenih vprašanj;
- zmožnost interpretacije rezultatov.

#### **Objectives and competences:**

- learning in the field of electron microscopy;
- to get an overview of recent advances in electron microscopy for life sciences;
- to know possibilities of choosing appropriate method for answering scientific questions;
- to develop the ability to interpret results.

#### **Predvideni študijski rezultati:**

Znanje in razumevanje:  
Študenti spoznajo aktualne metode elektronske mikroskopije in dobijo vpogled v razvoj področja.  
Študenti znajo izbrati ustrezne metode, ki omogočajo študij določenih problemov v bioznanostih.  
Študenti znajo interpretirati rezultate,

#### **Intended learning outcomes:**

Knowledge and understanding:  
- of the state-of-the-art electron microscopy and its perspectives;  
- students are able to differentiate and select suitable methods for their research in life sciences;  
- students can interpret results of different methods;

pridobljene z različnimi metodami.  
Študenti samostojno prepoznajo napake (artefakte), ki so posledica metodoloških omejitev metod v elektronski mikroskopiji.

- students recognise artefacts of specimen preparations in electron microscopy.

#### Metode poučevanja in učenja:

Predavanja, demonstracije, praktično delo v laboratoriju, predstavitev seminarjev in konzultacije.

#### Learning and teaching methods:

Lectures, demonstrations, practical work, seminars and consultations.

#### Načini ocenjevanja:

Delež (v %) /  
Weight (in %)

#### Assessment:

Izpit in izdelava seminarja.

100 %

Exam and seminar preparation.

#### Reference nosilca / Lecturer's references:

##### Rok Romih

1. HUDOKLIN, Samo, JEZERNIK, Kristijan, NEUMÜLLER, Josef, PAVELKA, Margit, **ROMIH, Rok**. Electron tomography of fusiform vesicles and their organization in urothelial cells. *PloS one*, ISSN 1932-6203, 2012, vol. 7, iss. 3, str. [1-8], e32935.
2. ZUPANČIČ, Daša, ZAKRAJŠEK, Maja, ZHOU, Ge, **ROMIH, Rok**. Expression and localization of four uroplakins in urothelial preneoplastic lesions. *Histochemistry and cell biology*, ISSN 0948-6143, 2011, vol. 136, iss. 4, str. 491-500.
3. HUDOKLIN, Samo, JEZERNIK, Kristijan, NEUMÜLLER, Josef, PAVELKA, Margit, **ROMIH, Rok**. Urothelial plaque formation in post-Golgi compartments. *PloS one*, ISSN 1932-6203, 2011, vol. 6, issue 8, str. e23636.
4. DERGANČ, Jure, BOŽIČ, Bojan, **ROMIH, Rok**. Shapes of discoid intracellular compartments with small relative volumes. *PloS one*, ISSN 1932-6203, 2011, vol. 6, issue 11, str. [1-9]. e26824.
5. HUDOKLIN, Samo, ZUPANČIČ, Daša, **ROMIH, Rok**. Maturation of the Golgi apparatus in urothelial cells. *Cell and tissue research*, ISSN 0302-766X, 2009, letn. 336, št. 3, str. 453-463.
6. KRALJ, Slavko, ROJNIK, Matija, **ROMIH, Rok**, JAGODIČ, Marko, KOS, Janko, MAKOVEC, Darko. Effect of surface charge on the cellular uptake of fluorescent magnetic nanoparticles. *Journal of nanoparticle research*, ISSN 1388-0764, 2012, vol. 14, no. 10, str. 1151-1-1151-14

**UČNI NAČRT PREDMETA / COURSE SYLLABUS**

<b>Predmet:</b>	<b>Študij živih celic- praktično delo</b>
<b>Course title:</b>	<b>Live Cell Imaging – practical course</b>

<b>Študijski program in stopnja</b> <b>Study programme and level</b>	<b>Študijska smer</b> <b>Study field</b>	<b>Letnik</b> <b>Academic year</b>	<b>Semester</b> <b>Semester</b>
Interdisciplinarni doktorski študijski program BIOZNANOSTI 3. stopnja	<b>Znanost o celici</b>	<b>1,2</b>	<b>1,2,3,4</b>
Interdisciplinary Doctoral Study Programme in BIOSCIENCES 3rd cycle	<b>Cell sciences</b>	<b>1,2</b>	<b>1,2,3,4</b>

**Vrsta predmeta / Course type** teoretični predmet / theoretical course

**Univerzitetna koda predmeta / University course code:**

<b>Predavanja</b> <b>Lectures</b>	<b>Seminar</b> <b>Seminar</b>	<b>Vaje</b> <b>Tutorial</b>	<b>Klinične vaje</b> <b>work</b>	<b>Druge oblike študija</b>	<b>Samost. delo</b> <b>Individ. work</b>	<b>ECTS</b>
/	5	/	/	45	200	10

**Nosilec predmeta / Lecturer:** Nosilec: prof. dr. Peter Veranič

<b>Jeziki / Languages:</b>	<b>Predavanja / Lectures:</b>	slovenski / angleški Slovene / English
	<b>Vaje / Tutorial:</b>	slovenski / angleški Slovene / English

**Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:** **Prerequisites:**

Študent potrebuje osnovno znanje celične biologije, biokemije in molekularne genetike. Študent je moral opraviti izpit iz celične biologije v dodiplomskem študiju.

The applicant student should have the basic knowledge in cell biology, biochemistry and molecular genetics. The student must have passed the examine of the course of Cell biology at the under graduate level.

**Vsebina:**  **Content (Syllabus outline):**

Uvod v sodobne metode, ki omogočajo morfološko analizo živih celic. Poudarek je na svetlobnomikroskopskih metodah, ki omogočajo dolgotrajno vzdrževanje in opazovanje celic pod mikroskopom (pomen temperature pH, vlažnosti, slikanje v časovnem zaporedju, analiza gibanja). Mehanizem in uporaba fluorescenčne označbe (GFP, Dil, Koleratoksin...) pri študiju celičnega gibanja, preraščanja in vitro poškodovanega področja, vpostavljanja medceličnih stikov, celične diferenciacije, dinamiki molekul membran, medcelične komunikacije, odzivanja na toksine...). Vzpodbujanje fluorescence povzroča tvorbo prostih radikalov, kar povzroča bledenje fluorokromov. Pregled metod, ki zmanjšajo fototoksičnost med opazovanjem fluorescenčno označenih celic (mikroskop z vrtečim se diskom, CLEM, antioksidanti). Razlaga artefaktov kot posledica fototoksičnosti.

Introduction of current methods, which enable observation and analysis of events appearing in live cells. The emphasis is on methods of light microscopy, which enable long-term maintaining and observation of cells in a microscope (temperature, pH and humidity controlled environment, time laps imaging, relocation of cells with collocate grids...). The mechanisms and the use of fluorescence labels (GFP, Dil, cholera toxin...), will be introduced to follow cell migration, transformation during regrowth of in vitro injuries, intercellular contact formation, rearrangement of molecules during cell differentiation, molecular dynamics in membranes, intercellular communication, cell response to drugs and toxins... During excitation of fluorochromes the free radicals are produced, which can damage cells and cause fading of labelled structures. A survey of methods for decreased phototoxicity will be given (spinning disc microscopy, CLEM, antioxidative agents) and of possible artefacts as a result of phototoxicity.

#### Temeljni literatura in viri / Readings:

1. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P. (2008) Molecular biology of the cell, 5th edition.
2. revijalni članki s področja, tekoča periodika, druga učna gradiva

#### Cilji in kompetence:

- poznavanje metod celične biologije (fluorescenčno označevanje, sekvenčno slikanje, ...)
- sposobnost interpretacije procesov v živi celici (spreminjanje arhitekture citoskeleta, vezikularnega transporta, dinamike membranskih molekul)
- razumevanje celične dinamike in vitro (epitelijsko mezenhimska transformacija, vzpostavljanje medceličnih stikov...)
- razumevanje razlogov za artefakte zaradi fototoksičnosti...)

#### Objectives and competences:

- acquaintance to methods of cell biology (time lapse imaging, fluorescence labelling of molecules in live cells)
- ability of interpretation of processes in live cells (reorganisation of cytoskeleton in migrating cells, vesicular transport and molecular dynamics in membranes)
- understanding of the cell dynamics in *in vitro* conditions (epithelio – mesenchymal transition, intercellular contact formation...)
- understanding the reasons for the artefacts caused by phototoxicity as a result of illumination of photochromes

**Predvideni študijski rezultati:**

Znanje in razumevanje:

študent:

- razume dinamika celičnih struktur
- pozna mehanizme delovanje celice
- pozna metode označevanja celic
- pozna razloge fototoksičnosti

**Intended learning outcomes:**

Knowledge and understanding:

- The student
- understands the dynamics of cell structures
  - is familiar with basic mechanisms of cell functioning
  - is familiar with methods of live cell labelling
  - is familiar with reasons of photodamage and resulted artefacts

**Metode poučevanja in učenja:**

Razprava o teoretičnih principih in praktičnem delu v laboratoriju, priprava bioloških vzorcev, analiza mikrofotografij, seminarsko delo, konzultacije

**Learning and teaching methods:**

Discussion on theoretical principles, practical work in laboratory, preparation of biological specimens, microscopy, analysis of micrographs, seminar work, consultations

**Načini ocenjevanja:**Delež (v %) /  
Weight (in %)**Assessment:**

predstavitve seminarja, razprava	Razprava/discussion 50% Seminar 50%	seminar presentation, discussions
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**Reference nosilca / izvajalcev / Lecturer's references:****Peter Veranič**

1. BLANGO, Matthew G., OTT, Elizabeth M., ERMAN, Andreja, VERANIČ, Peter, MULVEY, Matthew A. Forced resurgence and targeting of intracellular uropathogenic *Escherichia coli* reservoirs. *PloS one*, ISSN 1932-6203, Mar. 2014, vol. 9, iss.

3. <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0093327>, doi: [10.1371/journal.pone.0093327](https://doi.org/10.1371/journal.pone.0093327). [COBISS.SI-ID [31235545](https://www.cobiss.si/id/31235545)],

2. SKOČAJ, Matej, RESNIK, Nataša, GRUNDNER, Maja, OTA, Katja, ROJKO, Nejc, HODNIK,

- Vesna, ANDERLUH, Gregor, SOBOTA, Andrzej, MAČEK, Peter, VERANIČ, Peter, SEPČIČ, Kristina. Tracking cholesterol/sphingomyelin-rich membrane domains with the ostreolysin A-mCherry protein. *PloS one*, ISSN 1932-6203, Mar. 2014, vol. 9, iss. 3, str. 1-12, ilustr., doi: [10.1371/journal.pone.0092783](https://doi.org/10.1371/journal.pone.0092783). [COBISS.SI-ID [3072591](#)],
3. ERMAN, Andreja, KEREK KOS, Mojca, ŽAKELJ, Simon, RESNIK, Nataša, ROMIH, Rok, VERANIČ, Peter. Correlative study of functional and structural regeneration of urothelium after chitosan-induced injury. *Histochemistry and cell biology*, ISSN 0948-6143, Nov. 2013, vol. 140, iss. 5, str. 521-531, ilustr., doi: [10.1007/s00418-013-1088-7](https://doi.org/10.1007/s00418-013-1088-7). [COBISS.SI-ID [30654169](#)]
4. ERMAN, Andreja, LAKOTA, Katja, MRAK POLJŠAK, Katjuša, BLANGO, Matthew G., KRIŽAN-HERGOUTH, Veronika, MULVEY, Matthew A., SODIN-ŠEMRL, Snežna, VERANIČ, Peter. Uropathogenic escherichia coli induces serum amyloid A in mice following urinary tract and systemic inoculation. *PloS one*, ISSN 1932-6203, Mar. 2012, vol. 7, iss. 3, str. [1-7], e32933. <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0032933>, doi: [10.1371/journal.pone.0032933](https://doi.org/10.1371/journal.pone.0032933). [COBISS.SI-ID [29697497](#)]
5. IMANI, Roghayeh, KABASO, Doron, ERDANI-KREFT, Mateja, GONGADZE, Ekaterina, PENIČ, Samo, ELERŠIČ, Kristina, KOS, Andrej, VERANIČ, Peter, ZOREC, Robert, IGLIČ, Aleš. Morphological alterations of T24 cells on flat and nanotubular TiO<sub>2</sub> surfaces. V: *Proceedings of the RBC 2012, Regional Biophysics Conference 2012, Kladovo-Beograd, Serbia, September 03-07, 2012*, (Croatian medical journal, ISSN 0353-9504, vol. 53, no. 6, 2012). Zagreb: Medicinska naklada, 2012, vol. 53, no. 6, str. 577-585, doi: [10.3325/cmj.2012.53.577](https://doi.org/10.3325/cmj.2012.53.577). [COBISS.SI-ID [26403623](#)],
6. RESNIK, Nataša, SEPČIČ, Kristina, PLEMENITAŠ, Ana, WINDOFFER, Reinhard, LEUBE, Rudolf, VERANIČ, Peter. Desmosome assembly and cell-cell adhesion are membrane raft-dependent processes. *The Journal of biological chemistry*, ISSN 0021-9258, 2011, vol. 286, issue 2, str. 1499-1507, ilustr., doi: [10.1074/jbc.M110.189464](https://doi.org/10.1074/jbc.M110.189464). [COBISS.SI-ID [27616729](#)]
7. LAKOTA, Katja, RESNIK, Nataša, MRAK-POLJŠAK, Katjuša, SODIN-ŠEMRL, Snežna, VERANIČ, Peter. Colocalization of serum amyloid A with microtubules in human coronary artery endothelial cells. *Journal of Biomedicine and Biotechnology (Online)*, ISSN 1110-7251, 2011, str. [1-8], 528276. <http://www.hindawi.com/journals/jbb/2011/528276>, doi: [10.1155/2011/528276](https://doi.org/10.1155/2011/528276). [COBISS.SI-ID [29065689](#)]
8. ERMAN, Andreja, VERANIČ, Peter. Time- and temperature-dependent autolysis of urinary bladder epithelium during ex vivo preservation. *Protoplasma*, ISSN 0033-183X, 2011, vol. 248, issue 3, str. 541-550, doi: [10.1007/s00709-010-0201-1](https://doi.org/10.1007/s00709-010-0201-1). [COBISS.SI-ID [27356121](#)],
9. BATISTA, Urška, GARVAS, Maja, NEMEC, Marjanca, SCHARA, Milan Valter, VERANIČ, Peter, KOKLIČ, Tilen. Effects of different detachment procedures on viability, nitroxide reduction kinetics, and plasma membrane heterogeneity of V-79 cells. *Cell biology international*, ISSN 1065-6995, 2010, letn. 34, št. 6, str. 663-668, doi: [10.1042/CBI20090276](https://doi.org/10.1042/CBI20090276). [COBISS.SI-ID [26810841](#)]
10. LOKAR, Maruša, IGLIČ, Aleš, VERANIČ, Peter. Protruding membrane nanotubes : attachment of tubular protrusions to adjacent cells by several anchoring junctions. *Protoplasma*, ISSN 0033-183X, 2010, vol. 246, št. 1/4, str. 81-87, doi: [10.007/s00709-010-0143-7](https://doi.org/10.007/s00709-010-0143-7). [COBISS.SI-ID [26823641](#)],
11. SCHARA, Karin, JANŠA, Vid, ŠUŠTAR, Vid, DOLINAR, Drago, PAVLIČ, Janez Ivan, LOKAR, Maruša, KRALJ-IGLIČ, Veronika, VERANIČ, Peter, IGLIČ, Aleš. Mechanisms for the formation of

membranous nanostructures in cell-to-cell communication. *Cellular & molecular biology letters*, ISSN 1425-8153, 2009, vol. 14, no. 4, str. 636-656, ilustr. [COBISS.SI-ID [7232852](#)],

12. VERANIČ, Peter, ERMAN, Andreja, KEREK KOS, Mojca, BOGATAJ, Marija, MRHAR, Aleš, JEZERNIK, Kristijan. Rapid differentiation of superficial urothelial cells after chitosan induced desquamation. *Histochemistry and cell biology*, ISSN 0948-6143, 2009, vol. 131, no. 1, str. 129-139. <http://www.springerlink.com/content/d10j1841401g1332/fulltext.pdf>, doi: [10.1007/s00418-008-0492-x](https://doi.org/10.1007/s00418-008-0492-x). [COBISS.SI-ID [24682457](#)],