



Univerza v Mariboru

Fakulteta za naravoslovje  
in matematiko

### UČNI NAČRT PREDMETA / COURSE SYLLABUS

<b>Predmet:</b>	<b>Napredni opto- in elektrofiziološki pristopi v biologiji</b>
<b>Course title:</b>	<b>Advanced electro- and optophysiological approaches in biology</b>

Študijski program in stopnja Study programme and level	Študijska smer Study field	Letnik Academic year	Semester Semester
Doktorski študij Ekološke znanosti, 3. stopnja		1. ali 2.;	1.- 4.;
Doctoral Study Ecological Sciences, 3rd degree		1st or 2nd	1st-4th

Vrsta predmeta / Course type

Izbirni/Elective

Univerzitetna koda predmeta / University course code:

Predavanja Lectures	Seminar Seminar	Vaje Tutorial	Lab. vaje Laboratory work	Terenske vaje Field work	Samost. delo Individ. work	ECTS
5	5		20		150	6

Nosilec predmeta / Lecturer:

Jurij Dolenšek

Jeziki /

Predavanja / Lectures: slovenski / Slovene

Languages:

Vaje / Tutorial: slovenski / Slovene

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

Pogojev ni.

**Prerequisites:**

None.

**Vsebina:**

- a) Izolacija in priprava tkiv
  1. Izolacija trebušne slinavke in priprava tkivnih rezin.
  2. Izolacija nadledvične žleze in priprava tkivnih rezin.
  3. Izolacija gladkih mišičnih celic in priprava tkivnih rezin.
- b) Napredne elektrofiziološke metode
  4. Klasična elektrofiziološka

**Content (Syllabus outline):**

- a) Tissue isolation and preparation
  1. Isolation of pancreas and tissue slice preparation
  2. Isolation of adrenal gland and tissue slice preparation.
  3. Isolation of smooth muscle and tissue slice preparation.
- b) Advanced electrophysiologic methods
  4. Classical electrophysiological

<p>karakterizacija ionskih kanalov.</p> <p>4.1. Metoda vpete krpice membrane, vpeta napetost, protokoli stimulacije celice za razlikovanje med ionskimi kanali.</p> <p>4.2. Metoda vpete krpice membrane, vpeti tok, z injiciranim tokom ali s kemični agonisti povzročena aktivacija ionskih kanalov in posledična električna aktivnost celice.</p> <p>5. Napreden pristop raziskovanja mehanizma eksocitoze</p> <p>5.1. Merjenje kapacitivnosti celične membrane, s katero lahko določimo zlivanje posameznih veziklov.</p> <p>5.2. Dializa celice z vkleščenim kalcijem, kontrolirano sproščanje kalcija in sprožanje eksocitoze v odvisnosti od koncentracije kalcija.</p> <p>c) Napredne optofiziološke meritve</p> <p>6. Merjenje dinamike znotrajcelične koncentracije kalcija.</p> <p>6.1. Uporaba ultra hitrih in občutljivih kamer v kombinaciji z barvili, ki so občutljiva na kalcij.</p> <p>6.2. Konfokalna mikroskopija v kombinaciji z barvili, ki so občutljiva na kalcij.</p> <p>7. Določanje 3D strukture tkiva</p> <p>7.1. Barvanje celičnih membran in s pomočjo konfokalne mikroskopije 3D rekonstrukcija strukture tkiva.</p> <p>d) Napredne metode obdelave podatkov: zahtevnejša uporaba programskih orodij Matlab/Phyton za analizo časovnih vrst in serij slik. Kompleksni prikazi rezultatov obdelave.</p>	<p>characterization of ion channels</p> <p>4.1. Patch-clamp, voltage clamp, stimulation protocols for discrimination between ion channels.</p> <p>4.2. Patch-clamp, current clamp, ion channel activation by either current injection or chemical agonists, subsequent electrical activity.</p> <p>5. Advanced approach to studying mechanisms of exocytosis.</p> <p>5.1. Measuring membrane capacitance, used to determine fusion of individual vesicles.</p> <p>5.2. Cell dialysis with caged-calcium solution, controlling increase of intracellular calcium concentration, and subsequent calcium-dependent exocytosis.</p> <p>c) Advanced optophysiological measurements</p> <p>6. Measuring intracellular calcium concentration dynamics.</p> <p>6.1. Usage of ultra-fast and sensitive CCD cameras in conjunction with calcium-sensitive dyes.</p> <p>6.2. Usage of confocal microscopy in conjunction with calcium-sensitive dyes.</p> <p>7. Determining tissue 3D structure.</p> <p>7.1. Membrane labelling, using confocal microscopy to make 3D tissue reconstruction.</p> <p>d) Advanced methods of data analysis: Complex usage of softwares Matlab/Phyton for analysis of time series and series of images. Advanced presentation of analysed results.</p>
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**Temeljni literatura in viri / Readings:**

Ogden D. Microelectrode Techniques: The Plymouth Workshop Handbook. Second Edition. The Company of Biologists 1994.

Hille B. Ion Channels of Excitable Membranes. Third edition. Sinauer 2001.

Molleham A. Patch Clamping An Introductory Guide to Patch Clamp Electrophysiology. Wiley & Sons 2003.

Pawley J. Handbook of Biological Confocal Microscopy. Third edition. Springer, 2006.

Dolensek, J., et al., Membrane Potential and Calcium Dynamics in Beta Cells from Mouse Pancreas Tissue Slices: Theory, Experimentation, and Analysis. *Sensors (Basel)*, 2015. 15(11): p. 27393-419.

Špelič, D., et al., Improved analysis of membrane potential oscillations in the network of cells from islet of Langerhans. *GraphiCon'2013 Conference Proceedings*, 2013: p. 339.

Klemen, M., et al., Measuring Exocytosis in Endocrine Tissue Slices, in *Exocytosis Methods*, P. Thorn, Editor 2014, Humana Press. p. 127-146.

Stozer A, Dolensek J, Krizancic Bombek L, Pohorec V, Slak Rupnik M, and Klemen MS. Confocal Laser Scanning Microscopy of Calcium Dynamics in Acute Mouse Pancreatic Tissue Slices. *J Vis Exp* 2021

STOŽER, Andraž, DOLENŠEK, Jurij, KRIŽANČIĆ BOMBEEK, Lidija, GOSAK, Marko, SKELIN, Maša. Calcium imaging. V: WALTER, Andreas (ur.), MANNHEIM, Julia G. (ur.), CARUANA, Carmel J. (ur.). *Imaging modalities for biological and preclinical research. Volume 1, Ex vivo biological imaging*. Bristol: IOP Publishing, 2021. Str. i.1.e-1-i.1.e-13, ilustr. IPEM-IOP series in physics and engineering in medicine and biology

SKELIN, Maša, DOLENŠEK, Jurij, VALLADOLID-ACEBES, Ismael, STOŽER, Andraž, LIPOVŠEK DELAKORDA, Saška. Application of transmission electron microscopy to detect changes in pancreas physiology. V: MHADHBI, Mohsen (ur.). *Electron microscopy*. London: IntechOpen, 2022. Str. 1-22

PARADIŽ, Eva, STOŽER, Andraž, SKELIN, Maša. Sklopitev med spodbujanjem in izločanjem v celicah [beta]: nevrohormonska ojačitvena pot = Stimulus secretion coupling in pancreatic [beta] cells: the neurohormonal amplifying pathway. *Medicinski razgledi : [medicinski pregledni, strokovni in raziskovalni članki]*. [Tiskana izd.]. 2019, letn. 58, št. 3, str. 301-312.

PARADIŽ, Eva, SKELIN, Maša, STOŽER, Andraž. Sklopitev med spodbujanjem in izločanjem v celicah [beta]: sprožilna in presnovna ojačitvena pot = Stimulus secretion coupling in pancreatic [beta] cells: the triggering and the metabolic amplifying pathway. *Medicinski razgledi : [medicinski pregledni, strokovni in raziskovalni članki]*. [Tiskana izd.]. 2019, letn. 58, št. 3, str. 289-299,

STOŽER, Andraž, DOLENŠEK, Jurij, GOSAK, Marko, KRIŽMARIĆ, Miljenko, KOROŠAK, Dean, CMOR, Nino, MLAKAR, Jan, PETRESKI, Tadej, PEKLAR, Rok, ŠTANC, Rok, MURKO, Jakob, VIDRIH, Nejc, POKERŽNIK, Žan. *QuantiPhy : interactive physiological simulator*. <https://phy.percipio-big-data.com/>.

**Cilji in kompetence:**

**Objectives and competences:**

Poglavitni cilj predmeta je pridobitev modernih teoretičnih znanj in praktičnih veščin novejših tehnik, ki jih potrebuje sodoben raziskovalec fiziolog v laboratoriju. Znanja in veščine obsegajo od priprave tkivnih preparatov do merjenja ionskih kanalov, merjenja kapacitivnosti kot mere izločanja, modernih tehnik konfokalnega slikanja 3D strukture tkiva, dinamičnih sprememb znotrajcelične spremembe kalcijeve koncentracije.

Napredne metode računalniške obdelave in prikaza v poskusih pridobljenih rezultatov.

The major aim of the course is to gain knowledge and practical skills of modern techniques that are nowadays needed by a researcher in a physiological laboratory. Knowledge and skills cover tissue preparation, measuring ion channel conduction, membrane capacitance as a measure for exocytosis, modern techniques of confocal 3D imaging and confocal measurement of intracellular calcium concentration dynamics.

Advanced methods of computer-based data analysis and presentation of experimental data.

**Predvideni študijski rezultati:**

Študentje bodo znali uporabiti teoretično znanje o modernih tehnikah fizioloških raziskav in se praktično usposabili za napredne metode, ki omogočajo raziskovanje delovanja tkiv (metoda vpete krpice, lock-in ojačevalci, konfokalno slikanje). Bodo poznali prednosti in pasti teh tehnik. Bodo znali uporabiti kompleksnejše metode računalniške obdelave in prikaza fizioloških podatkov.

**Intended learning outcomes:**

Students will be able to apply theoretical knowledge of modern physiological research techniques and will be trained in advanced methods that allow the study of tissue function (patch-clamp method, lock-in amplifiers, confocal imaging). They will be familiar with the advantages and pitfalls of these techniques. They will be able to use more complex methods of computer processing and display of physiological data.

**Metode poučevanja in učenja:**

Interaktivna predavanja.  
E-učenje.  
Problem-based seminarji.  
Praktične vaje.

**Learning and teaching methods:**

Interactive frontal method.  
E-learning.  
Problem-based seminars.  
Practicals.

**Načini ocenjevanja:**

Ustni kolokvij iz praktičnih veščin  
Ustni izpit

Delež (v %) /

Weight (in %)

**Assessment:**

Oral examination based on practicals.  
Oral examination.

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

1. Dolenšek J, Kos T, Stožer A, and Špernjak A. Teachers perception of the use on a low-cost pulse rate sensor for biology education. *Advances in Physiology Education* 46: 238-245, 2022.  
2. Marolt U, Paradiž Leitgeb E, Pohorec V, Lipovšek S, Venglovecz V, Gál E, Ébert A, Menyhárt I, Potrč S, Gosak M, Dolenšek J, and Stožer A. Calcium imaging in intact mouse acinar cells in acute

pancreas tissue slices. PLOS ONE 17: e0268644, 2022.

3. Pohorec V, Križančić Bombek L, Skelin Klemen M, Dolenšek J, and Stožer A. Glucose-Stimulated Calcium Dynamics in Beta Cells From Male C57BL/6J, C57BL/6N, and NMRI Mice: A Comparison of Activation, Activity, and Deactivation Properties in Tissue Slices. *Frontiers in endocrinology* 13: 2022.

4. Serdinšek T, Lipovšek S, Leitinger G, But I, Stožer A, and Dolenšek J. A Novel in situ Approach to Studying Detrusor Smooth Muscle Cells in Mice. *Scientific reports* 10: 2685, 2020.

5. Stožer A, Klemen MS, Gosak M, Bombek LK, Pohorec V, Rupnik MS, and Dolenšek J. Glucose-dependent activation, activity, and deactivation of beta cell networks in acute mouse pancreas tissue slices. *Am J Physiol-Endoc M* 321: E305-E323, 2021.