



Univerza v Mariboru

Fakulteta za naravoslovje
in matematiko

UČNI NAČRT PREDMETA / COURSE SYLLABUS

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

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.; 1st or 2nd	1.- 4.; 1st-4th
Doctoral Study Ecological Sciences, 3rd degree			

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

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

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.

Cilji in kompetence:

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.

Objectives and competences:

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:

Znanje in razumevanje:

Teoretično znanje o modernih tehnikah fizioloških raziskav in praktično usposabljanje naprednih metod, ki omogočajo raziskovanje delovanja tkiv (metoda vpete krpice, lock-in ojačevalci, konfokalno slikanje). Poznavanje prednosti in pasti teh tehnik. Znanje kompleksnejših metod računalniške obdelave in prikaza fizioloških podatkov.

Prenesljive/ključne spretnosti in drugi atributi:

- Komunikacija
- Vodenje in upravljanje
- Raziskovanje
- Timsko delo

Intended learning outcomes:

Knowledge and understanding:

Theoretical knowledge of modern techniques in physiological research and practical learning of advanced techniques that are in use in research of tissue physiology (patch-clamp, lock-in amplifiers, confocal imaging). Knowing advantages and pitfalls of these techniques. Knowing complex computer-based methods to analyse and present physiological data.

Transferable/Key Skills and other attributes:

- Communication
- Leadership and management
- Research
- Teamwork

- Samoupravljanje	- Self-management
-------------------	-------------------

Metode poučevanja in učenja:
Learning and teaching methods:

Interaktivna predavanja. E-učenje. Problem-based seminarji. Praktične vaje.	Interactive frontal method. E-learning. Problem-based seminars. Practicals.
--	--

Delež (v %) /

Načini ocenjevanja:

Weight (in %)

Assessment:

Ustni kolokvij iz praktičnih veščin	50 %	Oral examination based on practicals.
Ustni izpit	50 %	Oral examination.

Reference nosilca / Lecturer's references:

Jurij Dolenšek, Viljem Pohorec, Marjan Slak Rupnik and Andraž Stožer. Pancreas Physiology, Challenges in Pancreatic Pathology, Dr. Andrada Seicean (Ed.), InTech, 2017, DOI: 10.5772/65895,

DOLENŠEK, Jurij, RUPNIK, Marjan, STOŽER, Andraž. Structural similarities and differences between the human and the mouse pancreas. *Islets*, ISSN 1938-2022, 2015, vol. 7, iss. 1, 16 str,

DOLENŠEK, Jurij, ŠPELIČ, Denis, SKELIN, Maša, ŽALIK, Borut, GOSAK, Marko, RUPNIK, Marjan, STOŽER, Andraž. Membrane potential and calcium dynamics in beta cells from mouse pancreas tissue slices : theory, experimentation, and analysis. *Sensors*, ISSN 1424-8220, 2015, vol. 15, iss. 11, str. 27393-27419,

DOLENŠEK, Jurij, STOŽER, Andraž, SKELIN, Maša, MILLER, Evan, RUPNIK, Marjan. The relationship between membrane potential and calcium dynamics in glucose-stimulated beta cell syncytium in acute mouse pancreas tissue slices. *PloS one*, ISSN 1932-6203, 2013, vol. 8, iss. 12, str. 1-16,

Markovic R., Stozer A, Gosak M, **Dolenšek J**, Marhl M, Slak Rupnik M. Progressive glucose stimulation of islet beta cells reveals a transition from segregated to integrated modular functional connectivity patterns. *Sci Rep* 5: 7845, 2015,

DOLENŠEK, Jurij, SKELIN, Maša, RUPNIK, Marjan. Calcium dependencies of regulated exocytosis in different endocrine cells. *Physiological research*, ISSN 0862-8408, 2011, vol. 60, iss. Suppl. 1, str. S29-S38,

STOŽER, Andraž, **DOLENŠEK, Jurij**, SKELIN, Maša, RUPNIK, Marjan. Cell physiology in tissue slices : studying beta cells in the islets of Langerhans = Celična fiziologija v tkivnih rezinah : preučevanje celic beta v Langerhansovih otočkih. *Acta medico-biotechnica*, ISSN 1855-5640, 2013, vol. 6, [no.] 1, str. 20-32,

SKELIN, Maša, **DOLENŠEK, Jurij**, STOŽER, Andraž, RUPNIK, Marjan. Measuring exocytosis in endocrine tissue slices. V: THORN, Peter (ur.). *Exocytosis methods*, (Neuromethods, ISSN 0893-

2336, Vol. 83). New York [etc.]: Springer, 2014, str. 127-146,

STOŽER, Andraž, **DOLENŠEK, Jurij**, RUPNIK, Marjan. Glucose-stimulated calcium dynamics in Islets of Langerhans in acute mouse pancreas tissue slices. *PloS one*, ISSN 1932-6203, 2013, vol. 8, iss. 1, str. 1-13.