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 **Doctoral Study Ecological Sciences, 3rd degree**		**1. ali 2.;** **1st or 2nd**	**1.- 4.;** **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: Maša SKELIN KLEMEN

Jeziki / Languages:	Predavanja / Lectures:	slovenščina / Slovene
	Vaje / Tutorial:	slovenščina / Slovene

Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:	Prerequisites:
Pogojev ni.	None.

Vsebina:	Content (Syllabus outline):

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
	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 koncetracije 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: zahtevnešja uporaba programskih orodij Matlab/Phyton za analizo časovnih vrst in serij slik. Kompleksni prikazi rezultatov obdelave.

characterization of ion channels
- 4.1. Pacth-clamp, voltage clamp, stimulation protocols for dicrimination 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 exocxytosis.
- 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:

Pawley J. Handbook of Biological Confocal Microscopy. Third edition. Springer, 2006. Dostopno na: https://link-springer-com.ezproxy.lib.ukm.si/book/10.1007/978-0-387-45524-2

Dodatna literatura/Additional reading:
Ogden, D. (1994). *Microelectrode techniques. The Plymouth Workshop Handbook* (D. Ogden, Ed. Vol. Second). The Company of Biologists Ltd. https://doi.org/https://plymsea.ac.uk/id/eprint/7954/
Hille, B. (2001). *Ion channels of excitable membranes* (3rd ed.). Sunderland (Mass.) : Sinauer associates. http://lib.ugent.be/catalog/rug01:002054346
Molleman, A. (2003). *Patch Clamping: An Introductory Guide to Patch Clamp Electrophysiology*. Wiley. https://books.google.si/books?id=JBY9NUsq2DkC
Dolensek, J., Spelic, D., Klemen, M. S., Zalik, B., Gosak, M., Rupnik, M. S., & Stozer, A. (2015). Membrane Potential and Calcium Dynamics in Beta Cells from Mouse Pancreas Tissue Slices: Theory, Experimentation, and Analysis. *Sensors (Basel)*, *15*(11), 27393-27419. https://doi.org/10.3390/s151127393
Špelič, D., Dolenšek, J., Stožer, A., Slak Rupnik, M., Žalik, B., & Mongus, D. (2013). Improved analysis of membrane potential oscillations in the network of cells from islet of Langerhans. *GraphiCon'2013 Conference Proceedings*, 339.
Klemen, M., Dolenšek, J., Stožer, A., & Rupnik, M. (2014). Measuring Exocytosis in Endocrine Tissue Slices. In P. Thorn (Ed.), *Exocytosis Methods* (Vol. 83, pp. 127-146). Humana Press. https://doi.org/10.1007/978-1-62703-676-4_7
Stožer, A., Dolenšek, J., Križančić Bombek, L., Pohorec, V., Slak Rupnik, M., & Klemen, M. S. (2021). Confocal Laser Scanning Microscopy of Calcium Dynamics in Acute Mouse Pancreatic Tissue Slices. *JoVE*(170), e62293. https://doi.org/doi:10.3791/62293
Stožer, A., Dolenšek, J., Križančić Bombek, L., Gosak, M., & Skelin Klemen, M. (2021). Calcium imaging. In *Imaging Modalities for Biological and Preclinical Research: A Compendium, Volume 1* (pp. I.1.e-1-I.1.e-13). IOP Publishing. https://doi.org/10.1088/978-0-7503-3059-6ch5
Skelin Klemen, M., Dolenšek, J., Valladolid-Acebes, I., Stožer, A. & Lipovšek, S. (2022). Application of Transmission Electron Microscopy to Detect Changes in Pancreas Physiology. In M. Mhadhbi (Ed.), *Electron Microscopy*. IntechOpen. https://doi.org/10.5772/intechopen.104807
Paradiž, E., Skelin, M., & Stožer, A. (2019). 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*, *58*(3), 289–299. http://www.dlib.si/details/URN:NBN:SI:doc-KKG4SVSS
Paradiž, E., Stožer, A., & Skelin, M. (2019). Sklopitev med spodbujanjem in izločanjem v celicah [beta]: nevrohormonska ojačitvena pot = Stimulus secretion coupling in pancreatic [beta] cells: the neurohormonal amplifying pathway. *Medicinski razgledi*, *58*(3), 301–312. http://www.dlib.si/details/URN:NBN:SI:doc-3TX6KF25 Stožer, A., Dolenšek, J., Gosak, M., Križmarić, M., Korošak, D., Cmor, N., Mlakar, J., Petreski, T., Peklar, R., Štanc, R., Murko, J., Vidrih, N., & Pokeržnik, Ž. (prib. 2017). *QuantiPhy: interactive physiological simulator*. s. n. 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 reasearcher 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 measurment of intracellular calcium concentration dynamics. Advanced methods of computer-based data analysis and presentation of experimental data.

Predvideni študijski rezultati: **Intended learning outcomes:**

Študentje bodo znali uporabiti teoretično znanje o moderniih 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.	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: **Learning and teaching methods:**

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

Načini ocenjevanja:	Delež (v %) / Weight (in %)	Assessment:
Projekt	100 %	Project

Reference nosilca / Lecturer's references:

1.)
Skelin Klemen, M., Dolensek, J., Krizancic Bombek, L., Pohorec, V., Gosak, M., Slak Rupnik, M., & Stozer, A. (2023). The effect of forskolin and the role of Epac2A during activation, activity, and deactivation of beta cell networks. *Front Endocrinol (Lausanne)*, *14*, 1225486.

https://doi.org/10.3389/fendo.2023.1225486

2.)
Skelin Klemen, M., Dolenšek, J., Valladolid-Acebes, I., Stožer, A. & Lipovšek, S. (2022). Application of Transmission Electron Microscopy to Detect Changes in Pancreas Physiology. In M. Mhadhbi (Ed.), *Electron Microscopy*. IntechOpen. https://doi.org/10.5772/intechopen.104807

3.) Stožer, A., Dolenšek, J., Križančić Bombek, L., Pohorec, V., Slak Rupnik, M., & Klemen, M. S. (2021). Confocal Laser Scanning Microscopy of Calcium Dynamics in Acute Mouse Pancreatic Tissue Slices. *JoVE*(170), e62293. https://doi.org/doi:10.3791/62293