COURSE UNIT (MODULE) DESCRIPTION

Course unit (module) title	Code
Quantitative fluorescence microscopy	

Lecturer (s)	Department (s)
Coordinator: dr. Marijonas Tutkus **Lab. supervisors: doc. Aurimas Kopūstas and dr. Marijonas Tutkus**	Department of Neurobiology and Biophysics, Life Sciences Centre

Cycle	Level of the course unit	Type of the course unit
1st stage (Bachelor's studies)	-	Facultative

Mode of delivery	Period of delivered	Language(s) of instruction
Lectures/laboratory	Autumn semester	Lithuanian/English

Prerequisites and corequisites	
Prerequisites: For undergraduate students in Biology, Chemistry, Physics, Nanoengineering, Health and Medical Sciences, and Life Sciences.	**Corequisites (if any):** -

Number of credits allocated to the course unit (module)	Total student's workload	Contact hours	Self-directed learning hours
5	130	48	82

Purpose of the course unit (module): programme competencies to be developed
• The general objective of the course is to provide practical skills for the analysis of biological samples by fluorescence microscopy and to perform the analysis/processing of recorded data to obtain quantitative information on the behavior of biological molecules in cells and artificial systems. • Upon completion of the course, students will acquire the following knowledge: ◦ Basic knowledge of optical and fluorescence microscopy, ◦ Fundamental knowledge required to extract quantitative information from microscopic data ◦ Basic knowledge of data processing and analysis. • Upon completion of the course, students will acquire the following skills:

- How to perform classical microscopy experiments
- How to perform fluorescence measurements at the level of single molecules

Learning outcomes of the course unit (module)	Teaching and learning methods	Assessment
To provide basic and advanced knowledge of imaging of quantitative fluorescence microscopy of biological samples and processing and analysis of recorded data.	Lectures, Self-directed learning.	The first part of the exam: a test from the theoretical part.
1. To learn the methods of fluorescence microscopy data processing and analysis applicable to samples from the cellular level to individual molecules. 2. Learn how to properly record data using fluorescence microscopes and sample preparation methods for fluorescence microscopy.	Laboratory works, seminars, Self-directed learning.	The second part of the exam: evaluation of lab report and its oral defense

Content: breakdown of the topics	Contact hours							Self-study work: time and assignments	
	Lectures	Tutorials	Seminars	Exercises	Laboratory work	Internship/ work placement	**Contact hours**	**Self-study hours**	**Assignments**
1. Microscope optical circuit, conjugated planes, diffraction and imaging, resolution and digital aperture, contrast techniques.	2						**2**	**4**	Reading the literature on the topic
2. Molecular fluorescence, dyes, filters and dichroic mirrors, excitation sources, point spread function (PSF), diffraction limit.	2						**2**	**4**	Reading the literature on the topic
3. Microscope types, an overview of methods, wide-field, confocal, total internal reflection (TIRF), super-resolution microscopes.	2						**2**	**4**	Reading the literature on the topic
4. Introduction to TIRF microscopy and practical exercises using TIRF: critical angle, evolutionary field, polarization. Oxygen removal, triplet quenching.	2						**2**	**4**	Reading the literature on the topic
5. Continuation of TIRF microscopy: superresolution (STORM, PALM), introduction to image recording - cameras (CCD) and Avalanche photodiodes (APD).	2						**2**	**4**	Reading the literature on the topic

6. Introduction to confocal microscopy and practical exercises using this method: sectioning, pinhole, laser scanning, and rotating disk microscope schemes.	2						**2**	**4**	Reading the literature on the topic
7. Introduction to dyes for monitoring molecular interactions: dye selection, FRET, fluorescence quenching, filter selection.	2						**2**	**4**	Reading the literature on the topic
8. Introduction to the practical sessions: DNA and protein interactions (types of DNA restriction enzymes, monitoring of interactions using FRET of single molecules).	2						**2**	**4**	Reading the literature on the topic
9. Introduction to practical classes: research of transmembrane proteins in liposomes (introduction to transmembrane proteins, light-absorbing complexes in LHCII liposomes and research of their function).	2						**2**	**5**	Reading the literature on the topic
10. Practical classes: sample preparation, oxygen removal, immobilization of test objects on surfaces.					3		**3**	**5**	Reading the literature on the topic
11. Practical classes: acquaintance with TIRF and confocal microscopes, their management, the most important details.					3		**3**	**5**	Reading the literature on the topic
12. Quantitative microscopy using a confocal microscope: Measurement of protein density in cells.					3		**3**		Reading the literature on the topic
13. Quantitative microscopy using the TIRF microscope: In vitro measurements of DNA and restriction enzyme interactions at the level of single molecules.					3		**3**		Reading the literature on the topic
14. Quantitative microscopy using a confocal microscope: in vitro measurement of protein density.					3		**3**		Reading the literature on the topic
15. Data analysis: introduction to the extraction of quantitative information from microscopic images (thresholding, background detection, and dot overlap).	2						**2**	**5**	Reading the literature on the topic
16. Data analysis: extraction of quantitative information from microscopic images (intensity integration, detection of intensity change points).	2						**2**	**5**	Reading the literature on the topic
17. Practical classes: analysis of recorded data (cells).			3				**3**	**5**	Reading the literature on the topic
18. Practical classes: analysis of recorded data (DNA restriction).			3				**3**	**5**	Reading the literature on the topic
19. Practical classes: analysis of recorded data (of LHCII liposomes).			3				**3**	**5**	Reading the literature on the topic
21. Independent study and preparation for the presentation of the description of laboratory work.								**5**	Reading the literature on the topic
22. Independent study and preparation for the presentation of the description of laboratory work.								**5**	Reading the literature on the topic

23. Presentation of the description of laboratory works and oral presentations.			2				**2**		Reading the literature on the topic
Total									

Assessment strategy	**Weight, %**	**Assessment period**	**Assessment criteria**
Implementation of laboratory works, lab report and oral presentations.	70 %	During the semester	A student is allowed to take an exam if laboratory and practical work has been completed and at least 50% of the possible points have been obtained in the oral and written presentation of their lab report. The performance of laboratory work is evaluated with 3 points. The maximum evaluation of the laboratory report is 2 points. The maximum grade for oral presentations is also 2 points.
Exam	30%	During the exam session	20 question test. The correct answer to one question is evaluated with 0.15 points. Final evaluation: the sum of the evaluations received for all test questions + evaluation of the performance of laboratory work + evaluation of the lab report + evaluation of oral presentations.

Author	**Year of publication**	**Title**	**Issue of a periodical or volume of a publication**	**Publishing place and house or weblink**
Compulsory reading				
David A. Roas, Constantinos Pitris, Nimmi Ramanujam.	2011	Handbook of biomedical optics		CRC Press
Partha Pratim Mondal, Alberto Diaspro.	2014	Fundamentals of Fluorescence Microscopy: Exploring Life with Light		Springer
Spencer L. Shorte, Friedrich Frischknecht	2007	Imaging Cellular and Molecular Biological Functions	ISBN-13: 978-3-540-71330-2	Springer-Verlag Berlin Heidelberg
R. Rotomskis, E. Žurauskas, E. Žurauskienė, S. Bagdonas, V. Žalgevičienė.	2008	Fluorescencinis vaizdinimas biomedicinoje		VU Onkologijos institutas, VĮ Mokslotyros institutas
Optional reading				
Joseph R. Lakowicz.	2007	Principles of Fluorescence Spectroscopy		Springer Science & Business Media.
Bruce Alberts, Alexander Johnson, Julian Lewis, Mar-	2007	Molecular Biology of the Cell		Garland Science

tin Raff, Keith Roberts, Peter Walter.				
Paul R. Selvin, Taekjip Ha	2008	Single-molecule Techniques: A Laboratory Manual		CSHL Press