Testi del Syllabus

Resp. Did. **THALHAMMER AGNES** **Matricola: 038054**

Docente **THALHAMMER AGNES, 3 CFU**

Anno offerta: **2023/2024**
Insegnamento: **675SM - MICROSCOPIA OTTICA IN BIOLOGIA CELLULARE**
Corso di studio: **SM53 - GENOMICA FUNZIONALE**
Anno regolamento: **2023**
CFU: **3**
Settore: **BIO/06**
Tipo Attività: **D - A scelta dello studente**
Anno corso: **1**
Periodo: **Secondo Semestre**
Sede: **TRIESTE**

Testi in italiano

Lingua insegnamento	Inglese
Contenuti (Dipl.Sup.)	The purpose of the course is to give an overview of microscopy techniques used in cellular biology with an introduction to digital image processing and analysis. Topics of the course are: • The historical evolution of microscopy and the scientific basis of currently used technologies. • The theory behind image formation and light diffraction. • The importance of resolution vs magnification. • The methods used to generate contrast in microscopy based on transmitted light. • The importance and the use of fluorescence microscopy. • The current state of the art of microscopy: the super resolution systems overcoming the classical resolution limits. • The basic knowledge necessary to design an experiment requiring usage of microscopy. • The Digital imaging and quantitative microscopy
Testi di riferimento	Murphy, D. B. and Davidson, M. W. (2012) References, in Fundamentals of Light Microscopy and Electronic Imaging, Second Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9781118382905.refs O'Farrell, M. (2006) Basic Light Microscopy, in Cell Biology Protocols (eds J. R. Harris, J. Graham and D. Rickwood), John Wiley & Sons, Ltd, Chichester, UK. doi: 10.1002/0470033487.ch1 (2013) Fluorescence Microscopy, in Fluorescence Microscopy: From Principles to Biological Applications (ed U. Kubitscheck), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. doi: 10.1002/9783527671595.ch3 All text are downloadable using the "Wiley Online Books", freely

	accessible using an account belonging to the University of Trieste
Obiettivi formativi	The aim of the course can be summarized as follows; 1) knowledge and understanding: The course is designed to familiarize the students with the scientific possibilities given by microscopy techniques with emphasis on the recently developed methodologies. 2) Applying knowledge and understanding: The practical and theoretical lectures aim at explaining the optimal usage of different microscopes. The students will be able to choose the microscopy technique that best allows the study of a given biological problem in terms of spatial resolution, temporal dynamics, interaction and sample damage and spectral and compositional chemical information, mechanical and morphological structure. 3) Making judgements: The students will have to acquire independence in the evaluation of the best protocols to perform scientific relevant image acquisition and quantitative microscopy. 4) Communication skills: The students are highly encouraged to engage in the revision part of the lessons (first 20 min of a lesson are dedicated to discussion of the content of the previous lesson) to allow familiarity and active usage of technical language. 5) Learning skills: The student, given a biological question, will be invited to design and present an experiment and the necessary microscopy setup to achieve the most relevant results.
Prerequisiti	Basic courses (from a previous degree) in physics, cell biology and histology
Metodi didattici	1) Frontal lectures with power point slide projections and short movies 2) Practical sessions with "hands on" laboratory microscopes 3) Demonstration of confocal, superresolution and 2 photon microscopes 4) Practical session at computer using ImageJ for Image processing and analysis with provided example images
Altre informazioni	The detailed program and the ppt presentations used to support teaching will be available on Moodle2 website of the course
Modalità di verifica dell'apprendimento	Written exam (Moodle on line)+ facultative Oral exam The written exam will produce a note up to 30 cum laude. The maximum note "cum laude" can be achievable only with the written exam. The written exam is based on a series of open and "multiple choice" questions distributed on the main topics presented. A result under 18/30 will be considered as not passed. Any results from 18/30 to 29/30 can be discussed with an oral session of the exam. The students will be invited to evaluate their written exam with focus on the open (not correct answers) points or topics. With the oral session it will be possible to modify the written note for a maximum of three points. Upon specific request, it is possible to perform the exam all in oral session. The following criteria will be used for assessment and marking: -Excellent (30 -30 cum laude): excellent knowledge of the topics, excellent usage of the technical language, brilliant application of theoretical knowledge to concrete cases. -Very good (27 -29): good knowledge of the topics, remarkable usage of the technical language, the student is able to correctly apply theoretical knowledge to concrete cases. -Good (24-26): good knowledge of the main topics, good command of the technical language; the student shows an adequate ability to apply theoretical knowledge to concrete cases. - Satisfactory (21-23): the student does not show full command of the main topics of teaching, while possessing the fundamental knowledge; however, he shows satisfactory knowledge of the technical language and sufficient ability to apply theoretical knowledge to concrete cases.

	-Sufficient (18-20): minimum knowledge of the main subjects of teaching and of the technical language, limited ability to adequately apply theoretical knowledge to concrete cases. - Insufficient (<18): the student does not have an acceptable knowledge of the contents of the various topics of the program.
Programma esteso	1) Introduction Overview of the Course What Can You Learn with a Light Microscope? Early History of Microscopy 2) Image Formation Lenses and Image Formation Microscope Imaging and Koehler Illumination Objectives and Eyepieces Diffraction and Point Spread Function 3) Resolution , What is Light? How to Focus and setting up Koehler Illumination 4) Contrast Generation for Transmitted Light Darkfield and Phase Contrast Microscopy Polarized Light and Polarization Microscopy Differential Interference Contrast (DIC) Microscopy 5) Fluorescence Microscopy Introduction to Fluorescence Microscopy Fluorescent Probes / Fluorescent Proteins Optical Sectioning and Confocal Microscopy Light Sheet Sectioning 6) Super-Resolution: Total Internal Reflection Fluorescence (TIRF) Microscopy Overview and Stimulated Emission Depletion (STED) Localization Microscopy Structured Illumination Microscopy (SIM) 7) Photobleaching and Photoactivation Förster Resonance Energy Transfer (FRET) Microscopy Fluorescence Lifetime Imaging Microscopy 8) Designing a Fluorescence Microscopy Experiment Labeling Proteins with Fluorescent Probes Correlating Fluorescence with Electron Microscopy 9) Quantitative Analysis of Biological imaging Microscopy Cameras and Detectors I: How Do They Work? 10) Introduction to Digital Images and their processing 11) Image Analysis / Deconvolution Microscopy 12) Practical lessons: hands on experience in Microscopy laboratory and with image processing and analysis
Obiettivi Agenda 2030 per lo sviluppo sostenibile	

Obiettivi per lo sviluppo sostenibile

Codice	Descrizione
3	Salute e benessere
9	Industria, innovazione e infrastrutture

Testi in inglese

	English
	The purpose of the course is to give an overview of microscopy techniques used in cellular biology with an introduction to digital image processing and analysis. Topics of the course are:

	• The historical evolution of microscopy and the scientific basis of currently used technologies. • The theory behind image formation and light diffraction. • The importance of resolution vs magnification. • The methods used to generate contrast in microscopy based on transmitted light. • The importance and the use of fluorescence microscopy. • The current state of the art of microscopy: the super resolution systems overcoming the classical resolution limits. • The basic knowledge necessary to design an experiment requiring usage of microscopy. • The Digital imaging and quantitative microscopy
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	Basic courses (from a previous degree) in physics, cell biology and histology
	1) Frontal lectures with power point slide projections and short movies 2) Practical sessions with "hands on" laboratory microscopes 3) Demonstration of confocal, superresolution and 2 photon microscopes 4) Practical session at computer using ImageJ for Image processing and analysis with provided example images
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	Written exam (Moodle on line)+ facultative Oral exam The written exam will produce a note up to 30 cum laude. The maximum note "cum laude" can be achievable only with the written exam. The written exam is based on a series of open and "multiple choice" questions distributed on the main topics presented. A result under 18/30 will be considered as not passed.

Any results from 18/30 to 29/30 can be discussed with an oral session of the exam.
The students will be invited to evaluate their written exam with focus on the open (not correct answers) points or topics.
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- Insufficient (<18): the student does not have an acceptable knowledge of the contents of the various topics of the program.

1) Introduction
Overview of the Course
What Can You Learn with a Light Microscope?
Early History of Microscopy
2) Image Formation
Lenses and Image Formation
Microscope Imaging and Koehler Illumination
Objectives and Eyepieces
Diffraction and Point Spread Function
3) Resolution , What is Light?
How to Focus and setting up Koehler Illumination
4) Contrast Generation for Transmitted Light
Darkfield and Phase Contrast Microscopy
Polarized Light and Polarization Microscopy
Differential Interference Contrast (DIC) Microscopy
5) Fluorescence Microscopy
Introduction to Fluorescence Microscopy
Fluorescent Probes / Fluorescent Proteins
Optical Sectioning and Confocal Microscopy
Light Sheet Sectioning
6) Super-Resolution:
Total Internal Reflection Fluorescence (TIRF) Microscopy
Overview and Stimulated Emission Depletion (STED)
Localization Microscopy
Structured Illumination Microscopy (SIM)
7) Photobleaching and Photoactivation
Förster Resonance Energy Transfer (FRET) Microscopy
Fluorescence Lifetime Imaging Microscopy
8) Designing a Fluorescence Microscopy Experiment
Labeling Proteins with Fluorescent Probes
Correlating Fluorescence with Electron Microscopy
9) Quantitative Analysis of Biological imaging Microscopy
Cameras and Detectors I: How Do They Work?
10) Introduction to Digital Images and their processing
11) Image Analysis / Deconvolution Microscopy
12) Practical lessons: hands on experience in Microscopy laboratory and with image processing and analysis

Obiettivi per lo sviluppo sostenibile

Codice	Descrizione
3	Good health and well-being
9	Industries, innovation and infrastructure