

Testi del Syllabus

Resp. Did. **BAJ GABRIELE** **Matricola: 012048**

Docente **BAJ GABRIELE, 3 CFU**

Anno offerta: **2020/2021**

Insegnamento: **675SM - MICROSCOPIA OTTICA IN BIOLOGIA CELLULARE**

Corso di studio: **SM53 - GENOMICA FUNZIONALE**

Anno regolamento: **2020**

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 English

Contenuti (Dipl.Sup.) The purpose of the course is to give an overview of microscopy techniques used in cellular biology. 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 also aim at explain the different microscopic set up and their optimal usage.

3) Making judgements: The students will have to acquire independence in the evaluation of the best protocols to perform scientific relevant images and quantitative microscopy.

4) Communication skills and 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

Modalità di verifica dell'apprendimento

Written exam (+ 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 16/30 will be considered as not passed.

Any results from 16/30 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.

Any changes to these indications, which may become necessary to ensure the application of safety protocols related to the COVID19 emergency, will be communicated on the Department's and Degree Course websites and Lecture course Moodle page

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
 11) Image Analysis / Deconvolution Microscopy
 Extra lessons Microscopy laboratory hands on



Testi in inglese

	English
	<p>The purpose of the course is to give an overview of microscopy techniques used in cellular biology. Topics of the course are:</p> <ul style="list-style-type: none"> • 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
	<p>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</p> <p>All text are downloadable using the "Wiley Online Books", freely accessible using an account belonging to the University of Trieste</p>
	<p>The aim of the course can be summarized as follows;</p> <ol style="list-style-type: none"> 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 also aim at explain the different microscopic set up and their optimal usage. 3) Making judgements: The students will have to acquire independence in the evaluation of the best protocols to perform scientific relevant images and quantitative microscopy. 4) Communication skills and 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.
	Basic courses (from a previous degree) in physics, cell biology and histology
	<ol style="list-style-type: none"> 1) Frontal lectures with power point slide projections and short movies 2) Practical sessions with "hands on" laboratory microscopes
	<p>Written exam (+ 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. Any changes to these indications, which may become necessary to ensure the</p>

application of safety protocols related to the COVID19 emergency, will be communicated on the Department's and Degree Course websites and Lecture course Moodle page

The written exam is based on a series of open and "multiple choice" questions distributed on the main topics presented.

A result under 16/30 will be considered as not passed.

Any results from 16/30 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.

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

11) Image Analysis / Deconvolution Microscopy

Extra lessons Microscopy laboratory hands on