

Course guide

230554 - EOTB - Experimental Optical Techniques in Biology

Last modified: 11/04/2025

Unit in charge: Barcelona School of Telecommunications Engineering
Teaching unit: 893 - ICFO - Institute of Photonic Sciences.

Degree: MASTER'S DEGREE IN PHOTONICS (Syllabus 2013). (Optional subject).

Academic year: 2025 **ECTS Credits:** 3.0 **Languages:** English

LECTURER

Coordinating lecturer: DAVID ARTIGAS GARCIA

Others: Segon quadrimestre:
MARÍA GARCÍA PARAJO - 10
PABLO LOZA ALVAREZ - 10

DEGREE COMPETENCES TO WHICH THE SUBJECT CONTRIBUTES

Specific:

CE8. Understand the importance of patents as the basis of a technological company and having the ability to understand and write a patent in the field of photonics.

CE2. Demonstrate the understanding of the peculiarities of the quantum model for light-matter interaction.

CE9. Ability to synthesize and present photonics research results according to the procedures and conventions of scientific presentations in English.

Generical:

CG1. Ability to project, design and implement products, processes, services and facilities in some areas of photonics, such as photonic engineering, nanophotonics, quantum optics, telecommunications and biophotonics.

CG2. Ability to modeling, calculate, simulate, develop and implement in research and technological centers and companies, particularly in research, development and innovation tasks in all areas related to Photonics.

CG4. Ability to understand the generalist and multidisciplinary nature of photonics, seeing its application, for example, to medicine, biology, energy, communications or industry

Transversal:

1. EFFECTIVE USE OF INFORMATION RESOURCES: Managing the acquisition, structuring, analysis and display of data and information in the chosen area of specialisation and critically assessing the results obtained.

2. ENTREPRENEURSHIP AND INNOVATION: Being aware of and understanding how companies are organised and the principles that govern their activity, and being able to understand employment regulations and the relationships between planning, industrial and commercial strategies, quality and profit.

3. FOREIGN LANGUAGE: Achieving a level of spoken and written proficiency in a foreign language, preferably English, that meets the needs of the profession and the labour market.

CT3. TEAMWORK. Be able to work as a member of an interdisciplinary team, either as another member, or performing management tasks in order to contribute to developing projects with pragmatism and a sense of responsibility, assuming commitments taking into account the available resources.

Basic:

CB6. Possess and understand knowledge that provides a basis or opportunity to be original in the development and/or application of ideas, often in a research context

CB7. Students should know how to apply the knowledge acquired and their problem-solving ability in new or little-known environments within broader (or multidisciplinary) contexts related to their area of study.

CB8. Students should be able to integrate knowledge and face the complexity of formulating judgments based on information that, being incomplete or limited, includes reflections on the social and ethical responsibilities linked to the application of their knowledge and judgment.

CB10. Students should possess the learning skills that allow them to continue studying in a way that will be largely self-directed or autonomous.

TEACHING METHODOLOGY

- Lectures
- Activities: Experimental part at ICFO

LEARNING OBJECTIVES OF THE SUBJECT

Optical microscopy has been for centuries a key tool to study biological systems with minimum invasiveness. The possibility of observing directly microorganisms or human cells has had a tremendous impact in the way we understand biology nowadays and has consistently resulted in major breakthroughs in the history of scientific discoveries. Although optical microscopy has continuously evolved since its invention in the 17th century, the last twenty years have witnessed a truly revolution in the development of novel optical microscopy techniques, with the most prominent example given by the Nobel prize awarding in 2014 to the inventors of super-resolution microscopy. The aim of this course is to provide a general overview of optical imaging techniques used to study biological objects, with a particular emphasis on these novel revolutionary imaging approaches. In addition, students will have the opportunity of perform hands-on training in some of the most advanced imaging techniques at ICFO.

The course is structured in two main blocks: a theory part and a hands-on part. The theoretical part (12 hours) will establish the basic background on image formation, and different contrast mechanisms associated with transmitted light. Strong emphasis will be then placed on fluorescence microscopy as one of the most powerful techniques used by biologists. Different configurations schemes will be revised, and the fundamentals for single molecule detection will be described in detail. This theoretical part will be completed by describing novel fluorescence imaging techniques aimed at breaking the diffraction limit of light. These approaches include far-field methods such as stimulated emission depletion (STED), single molecule localization methods like PALM and STORM, and near-field (NSOM) approaches. In the second part of the course, students will be involved in three different experiments (4 hours each) using most advanced microscopic techniques. These experiments will be performed at Super-resolution Facility at ICFO. The course will be complemented with a visit to the Lakadamyali research Lab, including the NIKON Center of Excellence in STORM imaging at ICFO.

Recommendations: the course is targeted to those students willing to expand their knowledge on optical experimental techniques for biological applications. A solid background in optics acquired through their bachelor studies and/or during the first part of the Master in Photonics is highly recommended for fully benefiting from the course.

STUDY LOAD

Type	Hours	Percentage
Hours large group	24,0	32.00
Self study	51,0	68.00

Total learning time: 75 h



CONTENTS

Theory part

Description:

1. Image formation & optical techniques to increase contrast. (Garcia-Parajo, 2 hours)
 - 1.1. Lenses and image formation.
 - 1.2. Light diffraction, point spread function and resolution.
 - 1.3. Basic optical implementation
 - 1.4. Different contrast configurations: phase contrast, dark field and DIC microscopy.

2. Fluorescence microscopy. (Garcia-Parajo, 2 hours)
 - 2.1. Fundamentals of fluorescence.
 - 2.2. Basic set-up configuration.
 - 2.3. Different contrast mechanisms based on fluorescence: polarization anisotropy, lifetime imaging, FRET.
 - 2.4. Different excitation and detection schemes based on fluorescence: confocal, two-photon excitation, light sheet microscopy.

3. Single Molecule detection by means of fluorescence. (Garcia-Parajo, 2 hours)
 - 3.1. Why? Principles and challenges
 - 3.2. Different excitation and detection schemes
 - 3.3. Photophysics of individual molecules: photon bunching, anti-bunching, blinking, discrete photobleaching etc.
 - 3.4. Single molecule techniques: smFRET, single particle tracking, fluorescence correlation spectroscopy

4. Super-resolution fluorescence microscopy. (Garcia-Parajo & Lakadamyali, 6 hours)
 - 4.1. Near-field super-resolution ? principle, technical implementation, examples
 - 4.2. Far-field super-resolution methods ? principles based on fluorescence
 - 4.3. Stimulated emission depletion (STED) - principle, different technical implementations
 - 4.4. Single molecule localization methods (PALM, STORM) ? different implementations*

* Includes visit to STORM Lab @ ICFO

Full-or-part-time: 10h 30m

Theory classes: 10h 30m

Experimental Part

Description:

(to be carried out at the Super-resolution Facility @ ICFO)

- Hands-on experiment 1 (Loza-Alvarez, 4 hours): Confocal and non-linear optical microscopy: Construction and alignment of a confocal microscope. Imaging with linear and nonlinear microscopes. SHG microscopy and Polarization-based SHG microscopy of selected bio-samples.

- Hands-on experiment 2 (Loza-Alvarez, 4 hours): Light sheet microscopy: Characterization and measurement of main light sheet microscopy parameters. Imaging with linear and nonlinear regimes with Gaussian and Bessel beams. Imaging in an ultramicroscope.

- Hands-on experiment 3 (Loza-Alvarez, 4 hours): Super-resolution STED microscopy: Measurement of point spread functions for different intensity parameters of the STED beam. Imaging selected bio-samples. Two- color STED. Use of specialized algorithms for assessing the STED image.

Full-or-part-time: 12h

Theory classes: 12h



ACTIVITIES

Visit to the ICFO Super resolution laboratories

Full-or-part-time: 2h 18m

Theory classes: 2h 18m

GRADING SYSTEM

- Group Reports from the three different hands-on experiments (40%)
- Exam (60%)

BIBLIOGRAPHY

Basic:

- Hecht, E. Optics. 5th ed. San Francisco [etc.]: Addison Wesley, 2017. ISBN 9781292096933.
- Lakowicz, J.R. Principles of fluorescence spectroscopy. 3rd ed. New York: Springer, 2006. ISBN 9780387312781.

RESOURCES

Hyperlink:

- <http://www.ibiology.org>. Resource