



## Course guides

# 230554 - EOTB - Experimental Optical Techniques in Biology

Last modified: 03/06/2020

**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).  
ERASMUS MUNDUS MASTER'S DEGREE IN PHOTONICS ENGINEERING, NANOPHOTONICS AND BIOPHOTONICS (Syllabus 2010). (Optional subject).

**Academic year:** 2020    **ECTS Credits:** 3.0    **Languages:** English

### LECTURER

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**Coordinating lecturer:** David Artigas, UPC.

**Others:** Pablo Loza-Alvarez, ICFO.  
María García-Parajo, ICFO.  
Melike Lakadamyali, ICFO.

### DEGREE COMPETENCES TO WHICH THE SUBJECT CONTRIBUTES

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#### Specific:

CE8. (ENG) Màster en Fotònica:

Comprender la importancia de las patentes como base de la empresa tecnológica y tener la capacidad para entender y redactar una patente en el ámbito de la fotónica

CE2. (ENG) Màster en Fotònica:

Mostrar que comprende las peculiaridades que comporta el modelo cuántico para la interacción luz-materia.

CE9. (ENG) Màster en Fotònica:

Capacidad para sintetizar y exponer los resultados de investigación en fotonica según los procedimientos y convenciones de las presentaciones científicas en inglés.

#### General:

CG1. (ENG) Màster en Fotònica:

Capacidad para proyectar, diseñar e implantar productos, procesos, servicios e instalaciones en algunos ámbitos de la fotónica como los relacionados con la ingeniería fotónica, la nanofotónica, la óptica cuántica, las telecomunicaciones y la biofotónica

CG2. (ENG) Màster en Fotònica:

Capacidad para la modelización, cálculo, simulación, desarrollo e implantación en centros de investigación, centros tecnológicos y empresas, particularmente en tareas de investigación, desarrollo e innovación en todos los ámbitos relacionados con la Fotónica.

CG4. (ENG) Màster en Fotònica:

Capacidad para entender el carácter generalista y multidisciplinario de la fotonica viendo su aplicación por ejemplo a la medicina, biología, energía, comunicaciones o la industria

**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. (ENG) Màster en Fotònica:

TRABAJO EN EQUIPO. Ser capaz de trabajar como miembro de un equipo interdisciplinar ya sea como un miembro más, o realizando tareas de dirección con la finalidad de contribuir a desarrollar proyectos con pragmatismo y sentido de la responsabilidad, asumiendo compromisos teniendo en cuenta los recursos disponibles

**Basic:**

CB6. (ENG) Poseer y comprender conocimientos que aporten una base u oportunidad de ser originales en el desarrollo y/o aplicación de ideas, a menudo en un contexto de investigación

CB7. (ENG) Que los estudiantes sepan aplicar los conocimientos adquiridos y su capacidad de resolución de problemas en entornos nuevos o poco conocidos dentro de contextos más amplios (o multidisciplinares) relacionados con su área de estudio.

CB8. (ENG) Que los estudiantes sean capaces de integrar conocimientos y enfrentarse a la complejidad de formular juicios a partir de una información que, siendo incompleta o limitada, incluya reflexiones sobre las responsabilidades sociales y éticas vinculadas a la aplicación de sus conocimientos y juicio.

CB10. (ENG) Que los estudiantes posean las habilidades de aprendizaje que les permitan continuar estudiando de un modo que habrá de ser en gran medida autodirigido o autónomo.

**TEACHING METHODOLOGY**

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- Lectures
- Activities: Experimental part at ICFO

**LEARNING OBJECTIVES OF THE SUBJECT**

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

**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 (50%)
- Exam (50%)

## BIBLIOGRAPHY

**Basic:**

- Hecht, E. Optics. 4th ed. San Francisco [etc.]: Addison Wesley, 2002. ISBN 0321188780.
- Lakowicz, J.R. Principles of fluorescence spectroscopy. 3rd ed. New York: Springer, 2006. ISBN 9780387312781.

## RESOURCES

**Hyperlink:**

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