Course guide
230554 - EOTB - Experimental Optical Techniques in Biology

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: 2022 ECTS Credits: 3.0 Languages: English

DEGREE COMPETENCES TO WHICH THE SUBJECT CONTRIBUTES

Specific:
CE8. (ENG) Máster 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) Máster en Fotònica:
Demostrar que comprende las peculiaridades que comporta el modelo cuántico para la interacción luz-materia.
CE9. (ENG) Máster en Fotònica:
Capacidad para sintetizar y exponer los resultados de investigación en fótónica según los procedimientos y convenciones de las presentaciones científicas en inglés.

Generical:
CG1. (ENG) Máster 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) Máster 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 Fotònica.
CG4. (ENG) Máster en Fotònica:
Capacidad para entender el carácter generalista y multidisciplinario de la fótónica viendo su aplicación por ejemplo a la medicina, biología, energía, comunicaciones o la industria
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

<table>
<thead>
<tr>
<th>Type</th>
<th>Hours</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Hours large group</td>
<td>24,0</td>
<td>32.00</td>
</tr>
<tr>
<td>Self study</td>
<td>51,0</td>
<td>68.00</td>
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**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.


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:

RESOURCES

Hyperlink:
- [http://www.ibiology.org](http://www.ibiology.org), Resource