230554 - IMAGBIO – Experimental Optical Techniques in Biology

Coordinating unit: 230 - ETSETB Barcelona School of Telecommunications Engineering
Teaching unit: 893 - ICFO - Institute of Photonic Sciences
Academic year: 2015 - 2016
Degree: Master's Degree in Photonics
Erasmus Mundus Master's Degree in Photonics Engineering, Nanophotonics and Biophotonics
ECTS credits: 3 **Teaching languages:** English

Academic staff

Coordinator: Maria Garcia-Parajo (ICFO) maria.garcia-parajo@icfo.es
Other professors: Pablo Loza-Alvarez (ICFO) pablo.loza@icfo.es
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Degree competences to which the subject contributes

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. 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.
3. 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.
4. TEAMWORK: Being able to work in an interdisciplinary team, whether as a member or as a leader, with the aim of contributing to projects pragmatically and responsibly and making commitments in view of the resources that are available.

Teaching methodology

Lectures
Activities:
- Experimental Part (to be carried out at the Super-resolution Facility @ICFO)

Objectives and short description of the course

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

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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

Total learning time: 75h	Hours large group:	22.5h	30%
	Hours medium group:	0h	0%
	Hours small group:	0h	0%
	Guided activity:	2.25h	3%
	Self study:	50.25h	67%

Course index

Theory part (12 hours)

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

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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

Experimental part (12 hours) – (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.

Qualification system

Group Reports from the three different hands-on experiments (50%)
Exam (50%)

Bibliography

- Optics. Eugene Hecht. Addison Wesley
- Principles of Fluorescence Spectroscopy. Joseph Lakowicz. Springer
- Website: http://www.ibiology.org
- Specific comprehensive review papers will be recommended to the students according to the topic.