UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

# COURSE SYLLABUS

# Biophotonics Laboratory II

**2324-1-F1701Q124**

## Aims

Introduce the students to some experimental activities to be carried out at the Biophotonics laboratory of the Physics department and follow them in carrying out and analyzing the data obtained..

The aim is to foster knowledge in the field of fluorescence optical microscopy with image analysis using quantitative methods for applications in biology and nanotechnology.

## Contents

Linear and non linear excitation optical fluorescence microscopy. Image correlation methods. Digital Holography and Optical Tweezers. Automatic image processing techniques

## Detailed program

Confocal optical microscopy: radial and axial resolution, images acquired in different modalities, spectral imaging of fixed samples. Cellular systems incubated with metal nanoparticles: internalization. Image correlation on complex systems: diffusion in hydrogels and in cells. Flux measurements in microchannels, Superresolution microscopy (STED) with 20 nm nanospheres. Identification of single molecules (fluorescent proteins).

Non linear fluorescence microscopy: radial and axial resolution. Measurements of fixed tissue samples at different excitation wavelengths. Measurements of yeast cells with membrane and nuclear dyes. Measurements of cells with nanoparticles. Second harmonic generation (SHG). Measures of Urea crystal at different laser polarizations. Measures of tumor tissues samples and analysis by means of phasors methods to retrieve the collagen fibers orientation.

We will also perform a simple experiment to introduce the concepts at the basis of digital holography.
We will similarly approach the technique of optical tweezers by means of a simple optical trapping experiment.

All the experiments will be introduced with 2 hours each of theoretical introduction and with a few hours of examples of the optical components and the data analysis. The data analysis will be based on Python codes and the optical components will be studied with practical design examples based on OpticStudio software.

Image processing techniques: python programming and introduction to the most common image pre-processing and image analysis techniques: filtering, noise reduction, image segmentation, automatic extraction of quantitative parameters.

## Prerequisites

The topics covered in the different courses of the Bachelor Degree in Physics.

Recommended: the Biophotonics course of the Master Degree in Physics and/or the Experiments of Biophotonics course of the Bachelor Degree in Physics.

## Teaching form

Experiments in the research labs of the Biophysics group, rooms 4054-4052-4051.

## Textbook and teaching resource

Textbook and teaching resource

R.Rigler, M.Orrit, T.Basche', "Single Molecule Spectroscopy", Sprinter Verlag, Chemical Physics series, 2012.

M.V.Klein, T.E.Furtak, "Optics", Wiley and Sons Inc, 1986.

A. Diaspro, "Confocal and two-photon microscopy : foundations, applications, and advances", edited by A.Diaspro, Wiley, 2002.

## Semester

II semester.

***During the Covid-19 emergency period, the course will be carried out according to modalities to be defined and which will partly take advantage of the possibility of numerical simulations and the analysis of experimental data collected in the laboratory by small subgroups of students. The exams will be carried out using the WebEx platform and on the e-learning page of the course there will be a public link for access to the examination of possible virtual spectators.***

## Assessment method

Students are required to write in English a relation describing the experiments performed and it will be the object of the discussion during the final oral exam.

The final score will be determined by the evaluation of the relation, of the knowledge of the different topics covered in the lab, of the experimental data analysis and of the student's behavior overall the lab course. The assessement of the knowledge of the basci contents and the capabiity to solve experimental problems will be done by oral discussion.

## Office hours

Always, on request.

## Sustainable Development Goals

QUALITY EDUCATION