



## **Master in Photonics – “PHOTONICS BCN” Master ERASMUS Mundus “EuroPhotonics”**

### **MASTER THESIS PROPOSAL**

**Starting full time from April 2025**

**Presentation at the end of July or beginning of September 2025**

**Laboratory:** Super resolution Light microscopy and Nanoscopy (SLN)

**Institution:** ICFO (The Institute of Photonic Sciences)

**City, Country:** Barcelona, Spain

**Title of the master thesis:** Phasor Analysis for Hyperspectral Imaging Demixing Overlapping Fluorescent Components Using Sine and Cosine Filters

**Name of the master thesis supervisor and co-supervisor:**

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**Keywords:** Hyperspectral Imaging, Phasor Analysis, Fluorescence, Overlapping Spectra, Microscopy

**Summary of the subject (maximum 1 page):**

Fluorescent microscopy plays a pivotal role in understanding complex biological systems by visualizing cellular processes. However, the spectral overlap of fluorescent components in multi-label experiments poses a significant challenge to conventional imaging methods. Phasor analysis, a Fourier-based technique, offers a powerful tool to address this challenge by simplifying spectral data representation.

This master's thesis focuses on developing and implementing phasor analysis combined with sine and cosine (S/C) filters to demix overlapping fluorescent components in hyperspectral images. The method maps spectral data into a phasor plot, where distinct components can be separated even when their emission spectra overlap. By validating this approach with hyperspectral data, we aim to establish its effectiveness in separating overlapping fluorophores.

The purpose of this thesis is to explore the application of this method to biological problems with at least three overlapping components and investigate its boundaries when addressing increasingly complex systems. The student will use algorithms and hyperspectral images from well-characterized fluorophores to generate reference phasor plots for the fluorescence components and implement the S/C filters in an epifluorescent microscope. Then, a comparison of the phasor positions obtained from the experimental S/C filter analysis with the reference phasor positions provided by hyperspectral imaging is required to define the limits of this setup.



## Objectives:

The following specific goals will be addressed:

- Study algorithms for phasor-based spectral demixing.
- Define well-characterized fluorophores to test and validate phasor analysis with the studied algorithms. This will be achieved by processing hyperspectral images from the fluorophores to simulate S/C images, with the assistance of the studied algorithms.
- Implement and test S/C filters in an epi-fluorescent microscope setup.
- Acquire S/C images from the pre-defined fluorophores and compare them with the simulated hyperspectral images and FLIM data.
- Test the method's ability to demix overlapping and non-overlapping fluorophores.
- Establish the limits of S/C analysis for different fluorophore conditions.