



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

### **MASTER THESIS PROPOSAL**

**Full time from April 2026** (it can start part time from February 2026)  
**Presentation date to be chosen: end of July or beginning of September 2026**

**Note:** The main Master Thesis supervisor has to be a professor of the Master in Photonics program. One co-supervisor (internal or external) can be defined. Main Supervisor is responsible for the subject of the proposal and has to give continuous support to the student (research development, Report writing and presentation preparation). For external proposals a co-supervisor from the Master program and a collaboration agreement with UPC are needed. You can find all information about the Master Thesis process in [our webpage](#).

**Laboratory: Super-resolution light microscopy and nanoscopy (SLN)**

**Institution: ICFO, The Institute of Photonic Sciences.**

**City, Country: Castelldefels, Spain**

**Title of the master thesis: Development of an Open-Source Python Tool for the Analysis of Fluorescence Lifetime Imaging Microscopy (FLIM) Data**

**Name and affiliation of the master thesis supervisor: Nicolas Mateos Estévez, ICFO, Pablo Loza, ICFO**

**Name and affiliation of the co-supervisor (if any):**

(for external proposals a co-supervisor chose among the Master Program professors and a collaboration agreement with UPC is needed)

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**Keywords:**

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

Fluorescence Lifetime Imaging Microscopy (FLIM) is a powerful and increasingly adopted technique in biological imaging. It measures the fluorescence lifetime — the average time a fluorophore remains in its excited state before emitting a photon — typically on the nanosecond scale. This lifetime depends not only on the intrinsic properties of the fluorophore but also on its microenvironment (e.g., pH, viscosity, molecular binding, or FRET interactions). As such, FLIM provides a contrast mechanism independent of fluorophore concentration and is invaluable in studying molecular interactions, metabolic states, and cellular environments.



At the Super-Resolution Light Microscopy and Nanoscopy (SLN) Facility, we operate two commercial FLIM systems: the Leica SP8 and Abberior Infinity Line, both based on time-domain detection. These systems excite samples using short picosecond laser pulses and record the photon arrival times to build fluorescence decay curves per pixel. Despite their robustness, data analysis remains challenging, as it requires precise calibration, model selection, and signal correction. Moreover, proprietary vendor software (e.g., Leica LAS X, Abberior Lightbox, SymPhoTime 64) limits flexibility, reproducibility, and interoperability with other tools.

Open-source alternatives exist — such as FLIMJ (ImageJ plugin), FLUTE, and Napari-Phasor — but none fully meet the needs of modern research facilities:

- **FLIMJ** integrates lifetime fitting in FIJI and supports OME data structures. *Strengths*: user-friendly interface, integration with ImageJ ecosystem. *Limitations*: limited to specific data formats, Java-based backend makes extension and performance optimization difficult.
- **FLUTE** offers accurate curve-fitting algorithms and decay modeling. *Strengths*: powerful fitting routines and validation. *Limitations*: limited interactivity, only phasor analysis and unstable.
- **Napari-flim-phasor-plotter** focuses on visualizing phasor plots from FLIM data in Napari. *Strengths*: interactive visualization, modern Python environment. *Limitations*: supports only basic phasor transformations, lacks robust fitting and preprocessing modules.

Currently, no open-source solution unifies phasor analysis, lifetime fitting, pre-processing, and instrument calibration for Leica and Abberior systems in a single Python environment.

Within the framework of the QUANT-FLIM project, this Master's thesis aims to bridge this gap by developing the first Python-based, open, and extensible FLIM analysis toolkit tailored to our facility's data — FLIM4all. The tool will be built around the Napari ecosystem for interactivity and modularity, paving the way for a user-friendly plugin that can later be expanded to other microscopy systems.

## 2. Objectives (maximum 1 page):

The overarching goal is to develop an open-source, Python-based analysis pipeline for time-domain FLIM data, from calibration and acquisition to visualization and quantitative analysis. The project will involve both experimental and computational work in close collaboration with the microscopy facility staff.

1. **Develop a Python-based analysis framework for FLIM data.** Create a modular codebase capable of importing data from Leica and Abberior systems, performing lifetime extraction, and visualizing results interactively.
2. **Establish a full analysis pipeline.** Implement pre-processing routines (background subtraction, IRF correction, binning), decay fitting using multiple algorithms (least squares, maximum likelihood, phasor transformation), and phasor plot computation and visualization.
3. **Experimental calibration and acquisition.** Gain hands-on experience with FLIM sample preparation, calibration using standard fluorophores, and image acquisition with the Leica and



Abberior systems. Establish reliable calibration procedures linking acquisition parameters with analysis outputs.

4. **Lay the groundwork for a Napari plugin: FLIM4all.** Build the foundation for an interactive Napari plugin integrating the full FLIM analysis workflow. The plugin will emphasize compatibility, transparency, and reproducibility, and provide an intuitive interface for facility users.
5. **Literature and software review.** Conduct a comprehensive review of current FLIM analysis algorithms and open-source tools, identifying best practices and gaps to inform the FLIM4all design.

### Expected Outcomes:

- A validated Python-based pipeline for FLIM data analysis.
- A prototype Napari plugin for phasor and fitting analysis.
- A set of calibration protocols for FLIM experiments at the facility.
- Documentation and example datasets for future facility users and developers.