Master in Photonics – "PHOTONICS BCN"
Master ERASMUS Mundus "EuroPhotonics"

MASTER THESIS PROPOSAL

Dates: April 2024 – July or September 2024

Laboratory: Optical Trapping Lab-Grup de Biofotònica, Departament de Física Aplicada
Institution: Universitat de Barcelona (UB)
City, Country: Barcelona, Spain

Title of the master thesis: Live-cell optical microscopy: study, optical characterization and validation of different microscopy arquitectures for parallel super-resolution microscopy using Laguerre-Gaussian (donut) beams

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Keywords: Super-resolution microscopy, depletion (STED and RESOLFT) microscopy, fluorescence microscopy, vortex beams, donut beams

Summary of the subject:
Advanced optical microscopy techniques are widely present within the field of life sciences. In particular, fluorescence microscopy is an essential tool for studying biological processes that occur at the cellular and molecular scales. However, optical microscopy has limitations in terms of spatial resolution, due to the physical phenomenon of diffraction, structures smaller than the subcellular scale (200 nm) cannot be observed. To overcome this limitation, various solutions have been proposed since the mid-20th century, based on non-optical technologies, such as pioneering electron microscopy or more recently atomic force microscopy. However, these technologies lack the versatility of visible light-based microscopy, which is more compatible with molecular biology labeling methods.
The development of super-resolution microscopy in the early 21st century managed to overcome the diffraction barrier using only an all-optical approach. The initial proposals for super-resolution microscopy, such as STED or PALM-STORM, although they achieve resolutions on the order of nanometers, are not well-suited for use in live cells. While STED microscopes use high-power pulsed lasers that can be harmful to live samples, PALM-STORM

microscopes are very slow and, therefore, incompatible with real-time observations of cellular dynamics.

To address these limitations, it is necessary to develop super-resolution technologies that are compatible with the observation of live cells in real-time. In the last five years, RESOLFT microscopy has been proposed as a derivative of STED microscopy, using photoactivatable fluorophores and TEM01 Laguerre-Gauss mode (doughnut-shaped) illumination beams, which allow for super-resolution imaging with low light intensities. Therefore, RESOLFT is a promising candidate for obtaining high-resolution images of live cells, as the illumination requirements minimize cellular damage. However, image reconstruction from point-by-point scanning of the sample does not allow for video-speed imaging. In this project, we propose the use of acousto-optic deflectors (AODs) for the parallelization of multiple beams in the illumination and scanning of various biological samples. Currently, this technology is fully implemented in more conventional microscopes, such as structured light and confocal microscopes developed under the project 'Super Fast and Flexible Digital Confocal Microscope,' SUFFICE, PID2019-109225RB-I00.

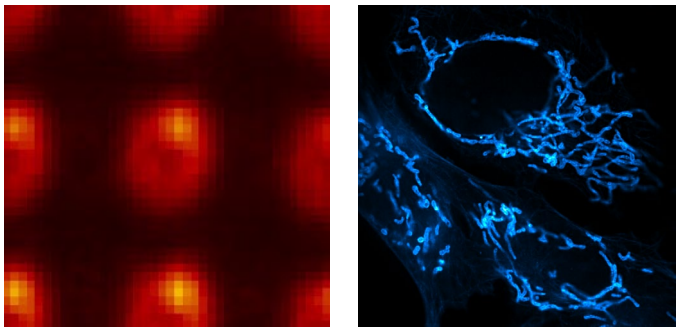

Figure 1. *(a) Image of the doughnut beam at the camera plane. (b) Reconstructed image using subtraction microscopy.*

Objectives:

The goal of this TFM is the production, characterization, and validation of the optical quality of a TEM01 Laguerre-Gauss mode (optical vortex or doughnut beam) for parallel super-resolution microscopy.

First, two different approaches to generate the TEM01 L-G mode, either using a vortex plate or a programmable q-switch plate, will be studied and compared. Secondly the generated spot array (consisting of NxN TEM01 L-G or Gaussian modes) will be analyzed different parallelization architectures of doughnut modes to achieve the minimum possible aberrations to ensure optimal optical performance. In this TFM we will implement the subtraction microscopy technique, an approach to super-resolution microscopy easier to implement than RESOLF since no different wavelengths for switching on the fluorophores are needed.

Additional information:

* Required skills: Disposition for experimental work in a microscopy lab, interested in biophotonics, proficient in a computer language with preference for Matlab, Python and LabVIEW.
* Miscellaneous: Early incorporation is possible.