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

## MASTER THESIS PROPOSAL

**Dates: April 2023 – July or September 2023**

**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 nanoscopy: production, optical characterization and validation of Laguerre-Gaussian (donut) beams for parallel super-resolution microscopy

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

**Summary of the subject:**

Resolution is a major limitation of fluorescence microscopy, even for the modern confocal microscopes costing hundreds of thousands euros each. In fact, rather shockingly, today's cutting-edge fluorescence microscopes, which feature high-technology detectors and cameras, and are stuffed with lasers, precision mechanisms and exquisitely corrected optics, have no more resolving power for cells in culture than the microscope Ernst Abbe built almost 150 years ago, following scientific principles for the first time. It is then of not much surprise that the development of optical nanoscopy roughly at the turn of the century, which amply breaks the diffraction barrier by strict optical means, has brought about top recognition to their inventors: a Nobel Prize in Chemistry in 2014 to Hell, Moerner and Betzig.

Unfortunately, these amazing microscopies are incompatible with live-cell imaging, a very important application in cell biology. For example, STED microscopes employ very large laser powers (densities of MW/cm$^2$ at the focus) that are harmful to living samples and PALM-STORM is so slow that the composition of a single image takes minutes, and it is thus unable to track dynamic changes within cells. Thus, there is a clear need for optical nanoscopy techniques that can overcome the hurdles of phototoxicity and low throughput.

At the University of Barcelona we intend to find an instrumental solution to this issue based on an acousto-optic deflector (AOD) technology previously developed and patented by our group.

Our invention enables a massive parallelization of the laser illumination of a STED derivative called RESOLFT. This microscopy uses similar principles to STED in order to break the resolution limit (confinement of the fluorescence emission by a "depletion" beam) but resorts to reversible fluorophores to reduce the required laser powers down to inoffensive levels. Our technology could accelerate RESOLFT enough to reach frame rates in the range of tens of hertzs, eventually enabling the observation of living biological specimens.
To get to this ambitious goal, we need however to solve some technical problems related to the production of the depletion beams, the key element that confines the emission of fluorescence within a subwavelength region, and enables the capture of an image with a resolution of some tens of nanometres. These depletion laser beams are formally known as Laguerre-Gaussian beams and informally as optical vortices or donut beams, and feature an optical singularity at the center (a dark point of zero irradiance), which must be very carefully preserved along the optical setup in order for the microscope to reach its maximal resolution. The student choosing this project will work on characterizing and validating different ways to produce these crucial light beams, in an optical setup that parallelizes RESOLFT microscopy (Fig. 1), with the ultimate goal to capture images with resolutions of a few tens of nanometres at video rates.

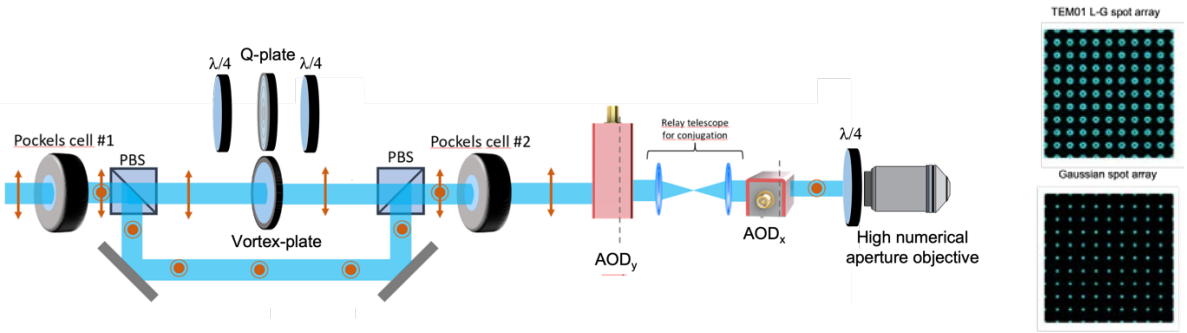


**Figure 1.** *(a) Experimental setup for donut beam creation in a parallel RESOLFT prototype and (b) spot array at the output of the AODs*

**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. To that end the experimental setup shown in Fig. 1 will be implemented. Under this setup, 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.

First, the TEM01 L-G mode characterization will be performed at the exit of the Mach Zehnder paths. This configuration allows, depending on the input polarization, to control the path the light passes through. If the light passes through the upper path the desired TEM01 L-G mode will be generated while if it passes through the lower path, the input Gaussian mode will be propagated. Secondly the generated spot array (consisting of NxN TEM01 L-G or Gaussian modes) at the output of the acousto-optic deflectors (AODs) will be analyzed. Finally, we will study the impact of the high numerical aperture objective on the quality of the TEM01 L-G

**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.