UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

**Master's degree in Biology – Chemistry-Biology Department**

# Master 2 internship project
# Year 2023-2024

**Laboratory/Institute:** IBS
**Director:** Winfried WEISSENHORN
**Team:** I2SR/Pixel
**Head of the team:**

**Name and status of the scientist in charge of the project:** D. Bourgeois **HDR: yes** ☒ **no** ☐
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**Program of the Master's degree in Biology:**

☐ Microbiology, Infectious Diseases and Immunology ☒ Structural Biology of Pathogens
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

**Title of the project:**

## The nuclear pore complex as a tool to optimize super-resolution fluorescence microscopy with photoconvertible fluorescent proteins.

Objectives (up to 3 lines):

We study the performance of fluorescent proteins used as markers in super-resolution microscopy. We will use the nuclear pore complex as a template to evaluate the quality of super resolved images as a function of (i) the employed fluorescent protein (ii) environmental parameters and (iii) data acquisition schemes.

Abstract (up to 10 lines):

Super-resolution fluorescence microscopy has become an essential tool to image biological samples at nanometric resolution (Chemistry Nobel Prize 2014). A very popular super-resolution approach is PALM (PhotoActivated Localization Microscopy). PALM is a single-molecule detection technique, and relies on the use of fascinating fluorescence markers called "photoconvertible" fluorescent proteins" (PCFPs). PCFPs change color upon UV illumination, for example from green to red, a property fundamental to the PALM concept. Different types of PCFPs exist but none is ideal. Moreover, the precise photophysical behavior of PCFPs is strongly dependent on environmental conditions such as pH, oxygen or redox potential. The quality of super-resolved images also depend on the precise illumination scheme used to collect the data. Recently, the nuclear pore complex (NPC), one of the biggest protein complex found in eukaryotic cells, has been demonstrated to be an exquisite quantitative reference structure to assess the quality of PALM super-resolved images. The student will be involved in imaging NPCs labeled with various PCFPs using PALM under a variety of experimental conditions, and in processing the data. The aim will be to optimize the way PALM can be conducted with PCFPs.

Methods (up to 3 lines):

This project stands at the interface between cell biology, imaging and physics. NPC-labeled cells have already been produced using the CRISPR-Cas9 technology. The project will involve cell culture, super resolution data collection and processing. The student will be supervised by Jip Wulffele (jip.wulffele@ibs.fr)

Up to 3 relevant publications of the team:

J. V. Thevathasanet al, "Nuclear pores as versatile reference standards for quantitative superresolution microscopy", Nature Methods, (2019) 16, 1045-1053.
E. De Zitter et al, "Mechanistic Investigations of Green mEos4b Reveal a Dynamic Long-Lived Dark State" J. Am. Chem. Soc., (2020), 142, 10978–10988. Doi: 10.1021/jacs.0c01880
J. Wulffele, D.Thédié, O. Glushonkov & D. Bourgeois, "mEos4b Photoconversion Efficiency Depends on Laser Illumination Conditions Used in PALM", J. Phys. Chem. Lett. 2022, 13, 5075−5080

Requested domains of expertise (up to 5 keywords):

Cell biology, interest for single-molecule imaging and data analysis.