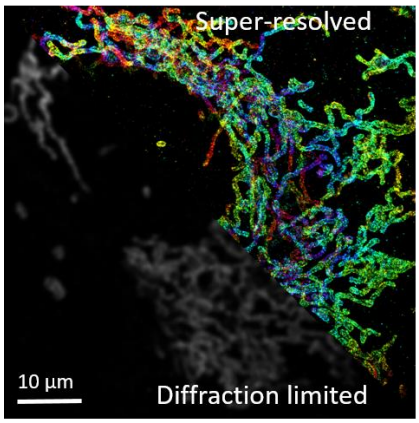
3D single molecule localization microscopy for biology

(M2 internship + PhD funding)

Keywords: single molecule, localization, microscopy, biology

Scientific description: In order to overcome the diffraction limit, new approaches in fluorescence microscopy have been developed in recent years. These discoveries, which were awarded the 2014 Nobel Prize, make it possible to observe biological samples with spatial resolutions below 20 nanometres (cf fig). In particular, single molecule localization microscopy consists of forcing fluorescent molecules to emit in a spatially and temporally shifted manner in order to determine their position with nanometric precision. This localization step is done by analysing only the spatial information of each diffraction spot observed in the image whose centre is used sought to determine the position of each molecule. Although this approach represents a revolution for biological applications that were previously inaccessible, this purely spatial analysis has important limitations such as limited acquisition speed but also sensitivity to aberrations and the difficulty of observing in depth complex samples such as tissues or spheroid models used in cancerology remains very difficult. Our group has proposed various strategies to push the performances of these nanoscopes [1-4]. However, super-resolution instruments have been developed by adapting classical microscopes, which now hampers further improvements in terms of speed, resolution and functionality. Dedicated solutions for nanoscopy are now needed.



In collaboration with the Institut Langevin, we have developed a new approach based on the introduction of a structured excitation that allows the emission of fluorescent molecules to be modulated over time. This gives a two-fold increase in resolution, but also offers the unique advantage of uniform resolution whatever the depth of observation. This technique, known as Modloc, is based in particular on an original optical detection device that is protected by a patent.

As part of this internship, we hope to push back the limits of this new approach by rethinking the optical configuration used to create the structured excitation. In order to benefit from the optimum gain for improving the axial configuration, a device using two objectives facing each other will be developed. This device will also provide increased detection thanks to the dual collection of fluorescence via the 2 objectives. The expected theoretical performance should beat the record of current devices. The performance of this new microscopy configuration will initially be evaluated on calibration samples. Secondly, biological samples will be observed with our biologist collaborators (IPSIT), in particular the organisation of the cytoskeleton (tubulin) involved in cell migration.

This project will be continued and further developed during a funded PhD position through a European funding, and in collaboration with the IPSIT, the Institut Langevin and a team from the Gottingen Institute for Nanophotonics.

[1] Jouchet et al, Nature Photonics 2021, [2] Mau et al. Nature communications 2021, [3] Cabriel et al, Nature Communications 2019, [4] Bourg et al. Nature Photonics 2015.

Techniques/methods in use: optical alignment, microscopy

Applicant skills: optics, knowledge in programming, curiosity, motivation to work at the interface between physics/biology/chemistry.

Industrial partnership: Yes, the lab is involved in a common research lab with the company Abbelight, funded by a former PhD student, the candidate will thus have the opportunity to contact and interact with this industrial partner.

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Possibility for a Doctoral thesis: Yes, PhD position funded through an ERC