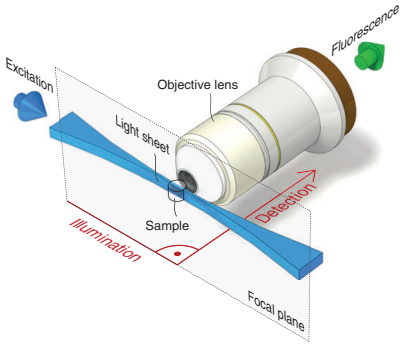
Simple single-plane illumination for single-molecule microscopy

Supervisors: Ignacio Izeddin (Institut Langevin, ESPCI), Antoine Coulon (Institut Curie)
Host institute: Institut Langevin, ESPCI Paris, 1 rue Jussieu, 75005 Paris
https://www.institut-langevin.espci.fr/, http://www.coulonlab.org/

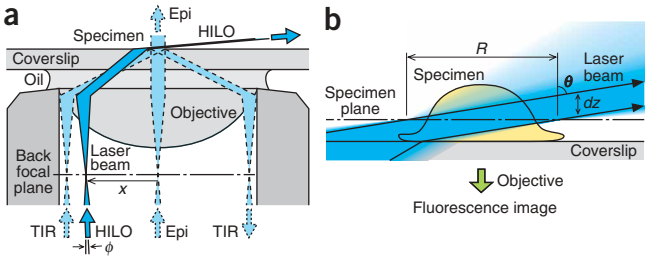
With the help of wavefront shaping techniques, we propose to develop an innovative and elegant microscopy architecture that uses a single objective for both selective plane illumination and signal collection in the context of single-molecule fluorescence microscopy.

One of the biggest drawbacks in epifluorescence microscopy, where the whole sample is illuminated, arises from the out-of-focus background noise. A solution to this problem consists in using a second objective to selectively illuminating only a thin slice of the sample perpendicularly to the direction of observation. Selective plane illumination microscopy (SPIM), also known as light-sheet illumination microscopy, has been widely used in developmental biology but its implementation in single-molecule microscopy remains challenging due to the short working distance and high numerical aperture objectives necessary for detecting single molecules. Although single-objective SPIM architectures have been proposed (e.g. Galland et al, Nat. Meth. 2015), these rely on complex illumination schemes and the use of micromirrors that need to be properly placed on the sample.



Principle of SPIM microscopy, adapted from Huisken and Stainier, Development (2009)

A simple and cheap alternative for selective plane illumination compatible with SM microscopy consists in highly inclined and laminated optical sheet (HILO) microscopy, leading to an increase up to a factor of 8 in signal-to-noise ratio. The major limitation of this approach is that the illumination and detection planes are not the same.



Principle of HILO microscopy, adapted from Tokunaga et al, Nat. Meth. (2008)

In this internship, we propose to implement an elegant solution to match the detection plane to that of the illumination by HILO microscopy by placing a wavefront shaping element in the emission path of the microscope. The candidate will be in charge of building the set-up around a commercial microscope and perform the first proof-of-principle experiments with a deformable mirror or a spatial light modulator, as well as performing simulations prior to the fabrication of a phase mask.

The internship will take place at the Institut Langevin, ESPCI, under the supervision of Ignacio Izeddin, in close collaboration with Antoine Coulon, UMR3664 and UMR168, Institut Curie. The aim is to use this new approach to the study of genome organization and its influence in gene expression at the molecular level.

For more information about this project, contact Ignacio Izeddin at ignacio.izeddin@espci.fr or Antoine Coulon at antoine.coulon@curie.fr.