Unveiling Molecular Motor Dynamics through 5D Nanometer-Scale Tracking of Position and Orientation

Keywords: Biophotonics, two-photon microscopy, molecular motors

Scientific description: Molecular motors such as myosins, kinesins, and dyneins drive intracellular transport and force generation through a succession of conformational changes occurring at the nanometer scale. While their translational motion has been extensively studied, their rotational dynamics remain much less well characterized, even though they provide a direct signature of the mechanical cycle of these motors. For example, each step of myosin V is accompanied by a 90° rotation of its transported cargo [1], while MINFLUX microscopy has recently revealed, for the first time, rotation of the kinesin stalk during motor stepping [2]. These studies highlight the importance of simultaneously measuring position and orientation to achieve a complete understanding of molecular motor function.

The objective of this project is to develop a new experimental approach capable of tracking, in real time, both the position and orientation of individual probes with levels of spatial and angular precision that have never previously been achieved simultaneously, and to use this capability to investigate molecular motor dynamics in vitro. The project will build on a two-photon tracking microscope developed at LuMIn [3]. Inspired by MINFLUX microscopy, this system uses second-harmonic-generation (SHG) nanocrystals instead of fluorophores. These probes do not undergo photobleaching, allowing continuous tracking over several tens of minutes, while simultaneously providing polarimetric information directly related to their crystallographic orientation. The microscope currently achieves a localization precision of approximately 3 nm and an angular precision close to 1°, opening up a new range of possibilities for the investigation of molecular motors.

The experimental program will proceed through progressively increasing levels of complexity. The first stage will focus on myosin II. I2BC has strong expertise in the development of biomimetic models reproducing mechanisms observed in natural systems [4]. Actin filaments will be functionalized with SHG nanocrystals, using NHS- or carboxyl-based chemistry, in order to quantitatively measure myosin-induced actin motion, including contraction and shear phenomena. The methodology will then be extended to geometries involving multiple actin filaments before moving on to processive motors. The aim will then be to reproduce and improve upon previous experiments performed on myosin V, taking advantage of the spatial, angular, and temporal performance of the microscope, and subsequently to extend this approach to dynein, whose stepping mechanisms remain far less well understood. Within the framework of this project, we will first investigate molecular motor motion in vitro under highly controlled conditions using purified proteins. These results will subsequently be validated in cellulo.

Beyond the study of molecular motors, this PhD project will contribute to the development of a new generation of tracking microscopy techniques capable of simultaneously measuring position

and orientation over very long timescales, thereby opening new perspectives for the quantitative investigation of the mechanics of individual biomolecules.

[1] Ohmachi et al., PNAS 109, 5294 (2012), doi : 10.1073/pnas.1118472109
[2] Wolff et al., Science 379, 1004 (2023), doi : 10.1126/science.ade2650
[3] F. Semmer et al., ACS Photonics 10, 3426 (2023) doi : 10.1021/acsphotonics.3c00949
[4] A.Ntadambanya et al. Angewandte Chemie 63, e202409852 (2024) doi : 10.1002/anie.202409852

Techniques/methods in use: two-photon microscopy (SHG), polarimetry, superlocalization microscopy, Protein biochemistry, nanocrystal bioconjugation, surface functionalization, and cell culture.

Applicant skills: Background in experimental optics and photonics. Experience in optical microscopy, laser-based experiments would be particularly valuable. Previous experience in biophysics, biological sample preparation, protein biochemistry, or cell biology would be an asset but is not required. Training in these techniques will be provided within the collaborating team. A strong interest in applying physical methods to biological questions is essential.

Industrial partnership: No

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Internship location: LUMIN and I2BC

Possibility for a Doctoral thesis: Yes, Funding: Doctoral school (EDOM) selection process