

INTERNSHIP PROPOSAL

(One page maximum)

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Thesis possibility after internship: YES/NO
Funding: YES/NO If YES, which type of funding: ENS

Photoactive Tags for Highly Multiplexed Fluorescence Imaging

Summary (half a page maximum)

Immunofluorescence imaging of biological tissues routinely requires the simultaneous detection of multiple biomarkers (e.g. for genetic profiling that aims to adapt personal cure in the context of cancer treatment). However, spectral crowding limits conventional multiplexing to a handful of colors, and current high-plex strategies (e.g., Exchange-PAINT, iterative staining with fluid exchange) sacrifice temporal resolution and simplicity: markers are read sequentially rather than simultaneously.

Independent research threads have recently expanded the multiplexing capacity beyond the spectral dimension.^{1,2} In particular, our group has recently contributed to a complementary thread: exploiting the photoejection regime of FAST-like tags (small protein scaffolds that reversibly bind synthetic fluorogenic dyes and turn them bright), in which illumination transiently ejects the fluorogen from the binding pocket, turning non-covalent fluorescence labeling tags into reversibly photoconvertible fluorescent tags (see Figure).^{3,4}

Unexploited opportunities lie at the intersection of these threads. Meeting the demand of modern tissue imaging for several tens simultaneous markers per round requires labels whose signatures are much richer than a single color or a single photocycle. The FAST scaffolds submitted to properly tailored time- and spectrally-modulated light excitation are prone to deliver fluorescence time-traces that can form a fundamentally richer observable than anything reported so far.

The overarching goal of the internship is to deliver the proof of principle that FAST scaffolds are relevant to deliver highly multiplexable fluorescent labels. The specific objectives are: Objective 1 — Numerically generate the multispectral fluorescence responses of FAST scaffolds in the presence of fluorogens submitted to tailored time- and spectrally-modulated light excitation; Objective 2 — Exploit tools of machine learning for delivering kinetic fingerprints from the multispectral fluorescence FAST response; Objective 3 — Numerically explore sequences of modulated illumination for optimizing the separability of the kinetic fingerprints of FAST mutants; Objective 4 — Exploit optimal illumination sequences experimentally implemented at Leuven for highly multiplexed imaging of FAST mutants in fluorescence imaging. This internship is embedded in an active collaboration between the hosting team and the teams of Prof. Arnaud Gautier (Sorbonne University) and Prof. Peter Dedeker (Leuven University).

1. R. Chouket et al, Extra kinetic dimensions for label discrimination, Nat. Commun., 2022, 13, 1482; **2.** O. K. Wade et al., 124-Color Super-resolution Imaging by Engineering DNA-PAINT Blinking Kinetics, Nano Lett., 2019, 19, 2641; **3.** M. Mandal et al, Non-Covalent Reversibly Photoconvertible Fluorescent Tags for Wash-Free Protein Labeling, Advanced Science, 2026, e78041; **4.** Y. Shpinov et al, Kinetic and Mechanistic Characterization of RSpFAST, a Non-Covalent Versatile Chemogenetic Platform for Fluorescence Imaging, J. Am. Chem. Soc., 2026, 148, 37874-37883

Please, indicate which speciality(ies) seem(s) to be more adapted to the subject:

Condensed Matter Physics: YES/NO Soft Matter and Biological Physics: YES/NO
Quantum Physics: YES/NO Theoretical Physics: YES/NO