Development of innovative thin film-based substrates for improving TIRF microscopy sensitivity and lateral resolution

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PhD topic context:

The plasma membrane is the point to enter or exit living cells for many intracellular pathogens (bacteria, viruses…). These processes are spatially regulated at the scale of a few molecules and have a precise time scale. Thus, measuring the dynamics of these constituents at the membrane of living cells during the assembly or the penetration of pathogens allows increasing the understanding of these mechanisms. To this end, multimodal fluorescence microscopy techniques have been implemented based on total internal reflection fluorescence microscopy (TIRF-M) to gain in axial resolution. However, this technique suffers from low sensitivity and needs improvements in lateral resolution.

In this project, we aim at improving both issues by the mean of optimized and original 1D to 3D micro to nano-structured optical components. We therefore want to improve TIRF-M spatial and temporal resolution without developing new microscopes/detectors, without modifying photophysics of existing dyes and in order to work at low concentration, i.e. in physiological conditions. Finally, the developed optical components will be designed to be adapted to any commercial microscope and ease of use for the end-users.

PhD description:

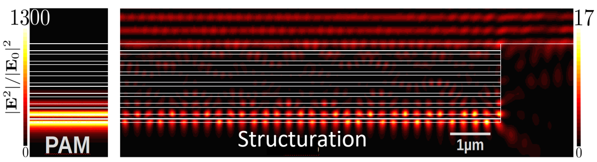

Figure 1: Preliminary numerical results showing field distribution within a micro-structured PAM.

We have already developed at the Institut Fresnel, resonant dielectric multi-layers that can be optimized to support field enhancements up 10^4 at the free interface [1] when working under total internal reflection as in TIRF-M. These resonant multi-layers can be designed whatever illumination conditions, making them flexible and adaptable to biological imaging. However, we have largely improved the design of such component by designing resonant **Planar All-dielectric Multi-layers (PAM)** with a lower field enhancement more suitable with the dyes photophysics used in TIRF-M but with a resonance with better angular acceptance to match the microscope constraints given by its high numerical aperture.

During this PhD project, we want to go further by improving the PAM design and introducing micro- to nano-structuration or other optical functions into the PAM.

The different tasks during this PhD thesis will be:

- first pushing our one-color PAM concept to numerically design 2-colors PAM for enhanced 2-colors TIRF-M imaging. This first step will permit the candidate to get into thin optical design and optimization as a first step in our development.

- second investigating numerically various micro- to nano-structuration patterns of the PAM. The numerical optimizations of the envisioned structures will be carried out using appropriate (2D/3D) finite element formulations [2]. The computational method will be an opensource implementation of finite element method (relying on Gmsh, GetDP and python). Clear modal pictures of the resonant mechanisms at stake will be established.

- After each numerical study, optimized structures will be fabricated within the Espace Photonique for the thin film depositions. The structuration will be done within the MIMENTO platform with our ANR partner in Besançon. The candidate will be involved at each step.

- Finally, before testing the developed components with biological imaging, the developed component will be characterized by scattering-scanning near field optical microscopy. Mappings of the near field amplitude and phase will be then obtained and compared to the numerical predictions.

The ultimate goal being viruses imaging, the PhD candidate could participate to the testing part over TIRF-M with the IRIM our ANR partner in Montpellier. To sum up, even though, the full ANR project is interdisciplinary, the PhD topic is dedicated to optical design and numerical simulations, and optical components fabrication and characterization.

We are looking for an excellent candidate with a strong background and interest in optics with numerical skills and experimental tastes.

[1] A. L. Lereu, F. Lemarchand, M. Zerrad, D. Niu, V. Aubry, A. Passian and C. Amra, “Sensitivity of resonance properties of all-dielectric multilayers driven by statistical fluctuations,” Opt. Exp. 27, 030654 (2019).
[2] G. Demésy, F. Zolla, A. Nicolet, M. Commandré, and C. Fossati, "The finite element method as applied to the diffraction by an anisotropic grating," Opt. Express **15**, 18089-18102 (2007)

Candidate profile:

We are looking for a highly motivated candidate with a solid background in physics/optics. As this project involves numerical simulations and experimental realizations, we seek a candidate with strong skills in programming and interest in experimental work and fabrication.
Please contact G. Demesy and A. Lereu with CV, motivations and if possible Master grades.