Single-molecule fluorescence lifetime imaging nanoscopy for biophysics and thermoplasmonics

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The field of optical fluorescence microscopy has been revolutionized with the emergence of **super-resolution** imaging, recognized with the Nobel Prize in Chemistry 2014. This set of techniques allows us to image objects with a resolution at the nanometer length scale (~10 nm), well below the classical limit imposed by the diffraction of light (typically ~200 nm). Among these, **single-molecule localization microscopy (SMLM)**[1] approaches (such as PALM[2], STORM[3], etc.) are based on the capacity of detecting single-molecules and the ability of *switching on* and *off* fluorescent emitters. **At the Institut Langevin, ESPCI Paris PSL, we have further developed such concept and conceived a novel microscopy system capable of simultaneously detecting single fluorescent molecules as well as their fluorescence lifetime, and thus obtaining super-resolved fluorescence lifetime images (smFLIM)**[4,5]**.**

smFLIM lies at the confluence between biophysics and nanophotonics with multiple applications in both fields[6] . It achieves the ultimate spatio-temporal resolution and gives access to multiple scales from 10 µm to 10 nm and from s to ps.
So far, we have applied our system to study light-matter interactions in plasmonic and dielectric nanostructures and obtained super-resolved cartographies of the local density of electromagnetic states (LDOS) of silver nanowires, gold nanocones[7] and GaP nanodimers[8] (Fig. 1a, b). However, this new approach opens up new and exciting applications not only in the fields of materials science and nanophotonics, but also for biological imaging and biophysics.

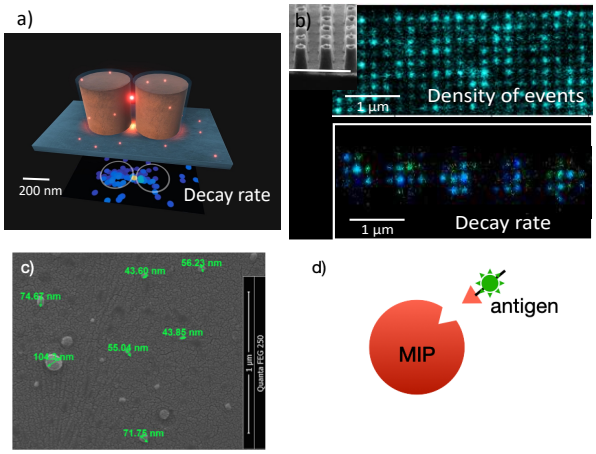

Figure 1. a) Super-resolved decay rate map of single molecules labeling a nanoantenna composed of two GaP disks. b) Super-resolved map of the density of events and decay rate of single molecules in the near-field of an array of truncated gold nanocones (shown in the SEM image). c) SEM image of some MIPs spread on a coverslip. d) Sketch of a single MIP to antigen binding.

We are looking for a motivated postdoctoral fellow to work with us to explore different applications of smFLIM. The first application (1) is the study of antigen-antibody recognition at the single-molecule level. To this aim, we will study, in collaboration with the Enzyme and Cell Engineering Laboratory led by Prof. K. Haupt (University of Technology of Compiègne), an emerging class of biomimetic nanomaterials: molecularly imprinted polymer-based synthetic antibodies which will be in our case structured in the form of nanoparticles. The second application (2) is the study of temperature increase in nanostructured samples based on single-molecule fluorescence modification. We are also open to any other suggestion of potential applications of smFLIM from potential candidates.

(1) Molecularly imprinted polymers (MIP) mimic, in synthetic matrices, the molecular recognition phenomena occurring in Nature[7] (Fig. 1c,d). They are able to recognize target molecules (the 'antigen') with high specificity, selectivity and affinity. MIPs are promising materials to replace antibodies in many fields, including bioseparation, bioanalysis, bioimaging, and even medical treatment[8]. We collaborate on this topic with the group of Prof. Karsten Haupt, who is one of the world leader in the field.
While the properties of MIPs have been studied at the macroscopic level, the investigation of single MIPs through the characterization of single binding events is still missing. We will extend smFLIM to **single-**

molecule FLIM-FRET (Fluorescence Lifetime Imaging Microscopy by Förster Resonance Energy Transfer) to study molecular interactions in single MIPs on distances up to ~10 nm. In smFLIM-FRET, the interaction between a MIP nanoparticle and an antigen, labeled with *donor* and *acceptor* fluorophores respectively, will be monitored via changes in the fluorescence lifetime.

(2) During the past two decades, the ability of remotely control the temperature of a plasmonic nanoparticle with light has seen important applications in several fields, going from nanomedicine and biology to photothermal chemistry and solar light harvesting [9]. The quantity which is usually measured is a global enhancement of the temperature over all the nanoparticles. However, new insights could be gained with a local mapping of the temperature, with nanometric resolution. smFLIM has the potential to answer this challenge, based on the use of stochastically photoactivatable molecules that have a fluorescence response which is temperature dependent[10]. The sensitivity of single-molecules' fluorescence to this parameter will be explored in this part of the project and its application to photothermal chemistry will be investigated.

Profile

We are accepting applications from post-docs with different background and motivation, ranging from optics and nanophotonics to physical-chemistry and biophysics, and from fundamental research to applied sciences interests. A background in fields such as thermoplasmonics, single fluorescent emitters studies, and machine learning will be particularly appreciated.

The post-doctoral fellow will work on a daily basis with a PhD student who already masters the smFLIM setup and will help the supervision. She/He will also be following the work of another PhD student of the group, working on thermal sources and scattering media.

The post-doctoral fellow will participate to weekly group meetings joining our group with the group lead by Yannick De Wilde. Group topics cover experiments on light emission in scattering media in the fluorescence and thermalized regimes, including emission by nanosources in complex environments, time-resolved fluorescence, near-field thermal radiation, optical spectroscopy, second harmonic generation. Fluency in written and spoken English is essential.

Forecast starting date: October 2024 to December 2024

Contract duration: 1 year

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