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Optoplasmonics tweaks for pushing the limits of superresolution fluorescence microscopy

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Scientific discoveries are frequently stimulated by the invention of new instruments and tools. One of these fascinating tools is fluorescence microscopy, today an integral part of natural and life sciences. Another tool is based on photonic and plasmonic nanostructures that can amplify and confine light. For biological imaging, one challenge is to combine these tools using simple and biocompatible nanostructure designs. Here, I show how biocompatible plasmonic coatings on standard microscope cover glasses can positively influence and control single molecule switching and localization – the key for super-resolved visualization of molecular architecture and dynamics. Recently, my research group improved the resolution of a direct stochastic optical reconstruction microscopy (dSTORM) experiment; the enhanced signal-to-noise ratio sharpens the location precision by a factor of 2 or more.

Beyond lateral super-resolution, axial super-resolution is the true Achilles heel in modern microscopy. Fortunately, tailored nano-coatings also enable axial super-resolution by interference and surface plasmon effects near the structure surface. Consequently, it is possible to translate spectral information of fluorescent markers into spatial information. Thus, fast and precise imaging becomes possible reaching an axial localization precision of 10 nm and below when the distance-dependent spectral “fingerprint” of the emitter is used to monitor its relative distance from the nanostructure.

Such optoplasmonics approaches solely rely on nanocoated coverslips, and do not require any additional hardware. This makes them a versatile booster for many other fluorescence methods including Fluorescence Correlation Spectroscopy and Förster Resonance Energy Transfer (FRET). The latter is often the method of choice for probing biomolecular interactions or conformational changes. I will show how optoplasmonics can increase the FRET efficiency of a classic donor-acceptor pair, and comment on this controversially discussed topic. Our latest experiments show that metal coatings allow reinforcing the otherwise forbidden donor-acceptor energy transfer by virtual optimization of the dipole orientation. This increases the dynamic range of an FRET experiment, and opens new perspectives for experiments on samples that exhibit only weak energy transfer.

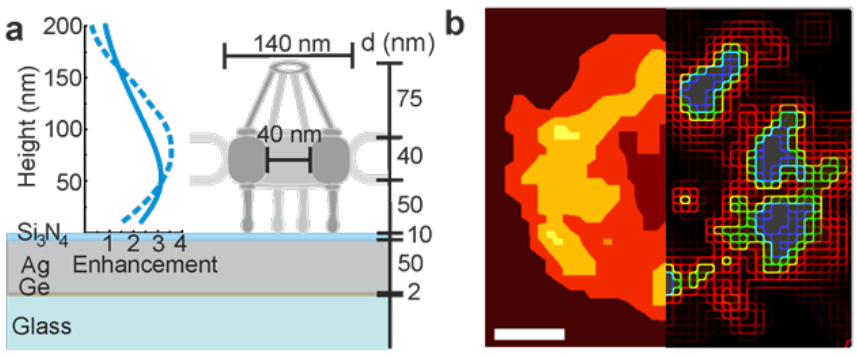

*Figure: Resolving the Nuclear Pore Complex (NPC): (**a**) Optimized metal-dielectric substrate designed to exhibit the strongest enhancement field of emission (solid line) and excitation (dashed line) of the red emitting dye A647 at the axial position of the NPC ring structure labeling the pore anchoring protein gp210. (**b**) Experimental dSTORM images of a single NPC ring on uncoated (left) and coated (right) glass surface. Scale bar: 50 nm.*