Plasmonics Meets Far-Field Optical Nanoscopy

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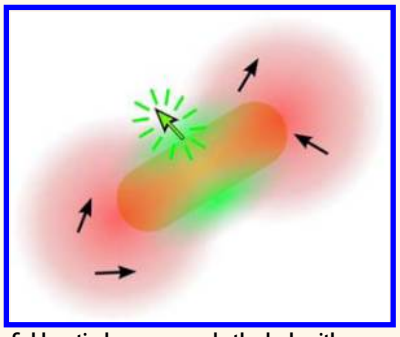
ABSTRACT



Plasmonics and near-field optical nanoscopy both deal with expanding optics into the subwavelength regime. However, these two fields have so far followed parallel paths of development and only recently have researchers started to explore combinations of their concepts with potential synergy. In this Perspective, we provide an up-to-date summary of the successful combinations reported and give insight into some new possibilities.

Contrary to the well-known diffraction limit of traditional optical microscopes, the fluorescence microscope is, in principle, capable of unlimited resolution. In the past decade, outstanding activity in the field of optical imaging has powered the transition of far-field fluorescence microscopes into nanoscopes that provide resolution that competes with that of electron microscopes. Yet, these systems maintain the simplicity and versatility of optical systems relying on conventional lenses. Today, this concept has already become widely accepted, and a number of methods denominated under the names of super-resolution fluorescence microscopy or far-field optical nanoscopy (the term we will use in the rest of this Perspective) have been established.[1] All optical nanoscopy methodologies discern the fluorescent features or molecules with subdiffraction proximities by switching their emission on and off so that they emit sequentially in time.

Explaining the working principles of all these techniques[1] is beyond the scope of this Perspective. However, we will briefly mention that far-field optical nanoscopy methods can be grouped into two families. In the first family, a coordinate-controlled sequential on–off switching of fluorophores is performed with illumination schemes, such as in stimulated emission depletion (STED)[2,3] or saturated structured illumination microscopy (SSIM).[4,5] The second family of methods makes use of stochastic on–off switching of fluorophores, such as in stochastic optical reconstruction microscopy (STORM), photoactivation localization microscopy (PALM),[6] or super-resolution optical fluctuation imaging (SOFI).[7,8]

Plasmonics and Nanophotonics. An electromagnetic wave propagating in a material media polarizes the medium and thus excites mechanical motion of the charges within. In turn, the moving charges radiate and thus the field and charge motion are coupled. This coupled excitation is called a polariton. In the case of a metal, the field can couple to electron motion, producing plasmon polaritons. If the right conditions of frequency and wave vector are met, (plasmon) polaritons can be excited resonantly. Particularly interesting cases are (i) surface plasmon polaritons that correspond to electron density waves propagating along a plane metal–dielectric interface and (ii) localized surface plasmons that correspond to electron oscillations constrained to the geometry of a nanoparticle. The science and technology involving surface plasmons is known as plasmonics and has recently attracted considerable attention from numerous fields of research.[9]

In large part, this new interest in plasmonics is linked to the strong development of nanophotonics, that is, the study and manipulation of light at the nanometer scale. The electromagnetic fields associated with plasmonic resonances are surface bound and decay exponentially from the metal

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surface, and present wave vectors much larger than those of propagating light of the same frequency, and, in the case of localized surface plasmons, are further confined by the nanoparticle size and geometry.[10] These characteristics make them a powerful tool for the development of subwavelength light sources,[11] for example, in near-field optical microscopy;[12] compact, high-speed, and interference-free active and passive optical devices;[13] as well as optical nanoantennas.[14,15]

Although plasmonics and far-field optical nanoscopy both deal with expanding optics into the subwavelength regime, they have followed separate development paths. Only recently have researchers started to explore combinations of plasmonics and optical nanoscopy concepts with potential synergies. Essentially, there are two main possibilities: (i) plasmons can be used to enhance or to enable new methods of optical nanoscopy; and (ii) optical nanoscopy can be used to characterize plasmonic structures and fields in great detail. Below, we present examples of successful combinations and suggest new possibilities.

Plasmonics Aiding Optical Nanoscopy. Let us first examine some reported examples using the simplest plasmonic structure, a metallic thin film. Wu *et al.* reported a simple and clever method for super-resolution imaging of dielectric structures on top of a metallic film.[16] Instead of using light or chemistry to switch molecules between a fluorescent and a nonfluorescent state, they utilize the dynamic adsorption of fluorescent molecules at the liquid–solid interface. Only fluorophores that adsorb on the surface remain fixed for the amount of time needed for localization, while the rest are blurred out by fast diffusion to a uniform background. In addition, a metallic film substrate is implemented below the dielectric structures. Fluorophores that adsorb onto the metallic film do not contribute to the super-resolution image because they experience efficient energy transfer to the metal that completely quenches their emission. The same scheme could be implemented in a complementary fashion, as well, metallizing only the regions of interest. A further improvement using evanescent illumination by surface plasmon resonance excitation has been reported recently.[17]

An alternative approach to exploit the plasmonic enhancement of the STED field that is not limited to two dimensions is suggested by Sivan *et al.* in this issue of *ACS Nano*.

Wei and Liu[18] demonstrated the use of plasmon interference patterns in order to improve structured illumination microscopy (SIM). The much larger wave vectors of surface plasmons lead to interference patterns with much higher spatial frequency than any possible pattern formed by propagating light of the same frequency. In addition, the spatial frequency of the surface plasmon interference is not limited by the numerical aperture of the objective. The reconstructed images using two different plasmonic designs showed a 3- and 4-fold resolution improvement in comparison to epi-fluorescence microscopy.[18] Of course, a spacer layer of a few tens of nanometers between the object and the metal film is necessary in order to avoid complete fluorescence quenching. A straightforward extension of this scheme, which to our knowledge has not yet been implemented, would involve the use of the plasmonic field enhancement at the metal–dielectric interface for saturated structured illumination microscopy (SSIM).[4,5] Such enhancement was indeed used recently to achieve the field intensities necessary for large-field STED in a nonlinear structured illumination scheme;[19] first results and simulations indicate that 30 nm resolution can be achieved with this method. Because the propagation of plasmons becomes shorter as their wave vector increases, it is necessary to find a useful compromise when developing actual designs.

Besides the various advantages, all of the aforementioned techniques based on propagating plasmons on metal surfaces are intrinsically two-dimensional (2D) and can only form high-resolution images of objects close to the metal surface. An alternative approach conceived to exploit a plasmonic enhancement of the STED field that is not limited to 2D is suggested by Sivan *et al.* in this issue of *ACS Nano*.[20] Specifically, they propose the use of nanoparticles consisting of a metallic shell holding fluorophores in their interior. Ideally, the metallic shell should be transparent to the wavelength of fluorescence excitation and detection and have its plasmonic resonance matched to the STED wavelength. The calculations indicate that such a hybrid label would improve all aspects of STED imaging, simply because the metallic nanoparticle provides a near-field enhancement of the STED field at the fluorophore position. An experimental realization would require fine-tuning of the plasmon resonance in order to amplify the STED field inside the shell, without completely quenching the fluorescence emission. Although challenging, all of the components and know-how for the fabrication of such a hybrid label are readily available.

Conceptually, STED microscopy is particularly interesting because the resolution limit can be pushed simply by increasing the intensity of the STED field. Higher STED field intensities leave smaller and smaller regions where the stimulated emission is not saturated, and thus the excitation probability is not negligible. It has been proposed that by using extremely strong laser intensities, the stimulated-emission-free

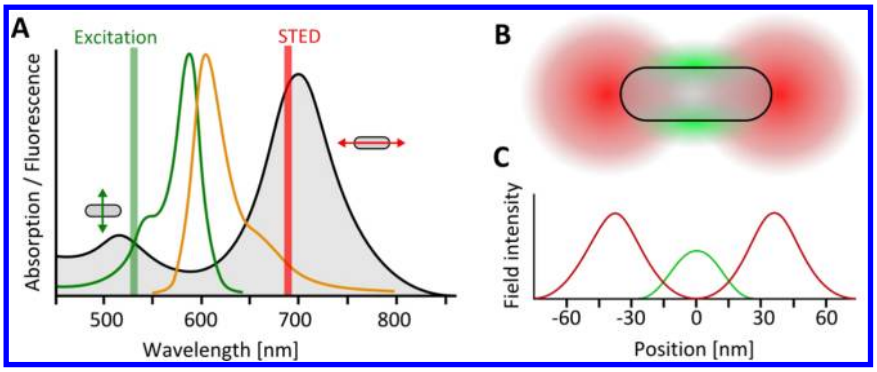

Figure 1. Near-field illumination scheme for stimulated emission depletion (STED) based on the plasmon resonances of a gold nanorod. (A) Spectra of all the components. Extinction spectrum of a gold nanorod 25 nm diameter × 70 nm length (black). Absorption (green) and emission (orange) spectra of the fluorescent dye Abberior STAR 580. Excitation and STED wavelengths are shaded at 532 and 690 nm, respectively. (B) Near-field intensity distribution for both resonances of the nanorod. (C) Intensity profile along the region near the surface of the nanorod.

regions close to the zero intensity point of the STED field could reach the subnanometer size and below, so that intramolecular information could be obtained.[1] For example, the probability of excitation, stimulated emission, photoconversion, or photoisomerization could be measured as a function of the orbital structure; the role of different molecular parts in certain transitions could be determined. Plasmonic structures may play a key role in accomplishing this because they can provide the extra light concentration necessary to bridge the length scale gap between optical diffraction and molecular sizes. It is possible to think of metallic nanostructures designed to confine and to locally enhance both excitation and STED fields to specific nanometric regions. The simplest of such structures is a metallic nanorod. Typically, small metallic nanorods present two plasmonic resonances corresponding to the transverse and the longitudinal dipolar modes. The spectral positions of these resonances depend on the geometry of the rods, mainly on their aspect ratios,[9] which gives a facile means to tune the resonances to the spectral regions suitable for STED. For example, a gold nanorod with a diameter of 25 nm and a length of 70 nm presents a transverse resonance at about 535 nm and a longitudinal resonance at about 700 nm (Figure 1A). Illuminating this rod with a laser at 532 nm polarized transverse to the long axis of the rod and a 690 nm laser polarized along the main axis of the rod produces a near-field distribution like the one shown schematically in Figure 1B. The green (excitation) field is concentrated at the sides of the rod, whereas the near-infrared (STED) field is confined to regions at the tips of the rod. The fact that the longitudinal resonance is dipolar is important because it assures a zero of the field intensity at the rod center. The field intensity profiles along the rod (Figure 1C) show that the nanorod constitutes a near-field analogue of a far-field STED microscopy illumination scheme, with the difference in this case being that the maxima of the STED field are only about 70 nm apart.

Plasmonic structures like metallic rods have already been fabricated and used at the tips of near-field scanning optical microscopes.[14] This, in combination with further localization due to stimulated emission saturation, could make it possible to interrogate optically subnanometer regions of a material or molecule.

Optical Nanoscopy To Characterize Plasmonic Structures. Far-field optical nanoscopy is a powerful tool to characterize plasmonic structures and their associated subwavelength fields. Cang *et al.* realized this in a simple and elegant experiment. Individual fluorophores were localized with nanometric precision as they were excited by the enhanced field of a 15 nm plasmonic "hot spot" of a rough metallic surface.[21] Stranahan and Willets used the same approach, but instead, localized individual molecules from their Raman signal.[22] The level of resolution provided by their optical nanoscopy revealed an offset of a few tens of nanometers between the origin of the Raman signal and the nanoparticle position. This is an interesting experimental observation that had previously been predicted, but until the work of Stranahan and Willets had never been observed; we will explain it below.

Metallic nanoparticles may act as efficient antennas for optical radiation.[23] They can efficiently collect far-field radiation and confine it to subwavelength regions and reciprocally couple light from a local (near-field coupled) source into the far-field. Like in any radio- or microwave antenna, the interaction with far-field radiation follows an angular pattern determined by the properties of the antenna (material and geometry). Let us now consider a Raman-active or fluorescent molecule as a local source close to a metallic nanoparticle. Molecular transitions are ruled by a transition dipole moment, and thus the emission of light follows a dipolar angular distribution (Figure 2A). The phase center of that emission is located at the position of the molecule, and a far-field image can be used to determine that position with high accuracy, as is done in STORM or

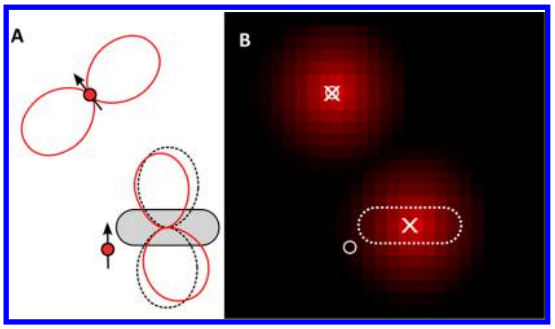

Figure 2. Antenna effect of plasmonic nanoparticles. (A) Angular emission patterns of a free fluorophore (top, red line), a dipolar longitudinal plasmonic resonance of a metallic nanorod (bottom, black dotted line), and a fluorophore with its emission coupled to the resonant mode of nanorod (bottom, red line). The angular patterns are plotted at the phase center. (B) Schematic far-field image of the free fluorophore and the fluorophore coupled to the nanorod. The circles indicate fluorophore position, and the crosses indicate localization obtained from the far-field images.

PALM. If the molecule has an emission spectrum that overlaps with a plasmonic resonance of the metallic nanoparticle and is located and oriented so that it is able to excite the plasmonic resonance in the near-field, then the light emission will be determined by the antenna mode.[14,24] Not only will the angular pattern of the emission be dominated by the antenna mode (Figure 2A) but also a far-field image will report on the antenna mode and provide no information about the position of the molecule. For example light emitted by a fluorescent molecule coupled to the dipolar resonance of a metallic nanorod appears in the far-field as coming from the center of the nanorod (Figure 2B). This is a key aspect to take into account when studying molecules coupled to plasmonic structures and has implications for all methodologies of optical nanoscopy. Figure 2 illustrates this effect.

In order to characterize the function of plasmonic structures, it is important to determine the positions of both the source and the structure with subwavelength resolution. One way to achieve this could be through the use of multicolor labels that would enable the spectral separation of photons that either are or are not coupled to the plasmonic structure.

Far-field optical nanoscopy is a powerful tool to characterize plasmonic structures and their associated subwavelength fields.

FUTURE OUTLOOK

Additional possibilities exist that we could not cover in this Perspective. For example, metallic nanoparticles also exhibit plasmon-mediated one-[25,26] and two-photon luminescence.[27,28] Although the quantum efficiency of these processes is low, it is compensated by the large excitation cross sections that metallic nanoparticles have near the plasmon resonances, leading to a number of detected photons comparable to the case of a single fluorophore. It is currently under investigation whether this luminescence can be used for any methodology of far-field optical nanoscopy. We believe the concepts of plasmonics and far-field optical nanoscopy will meet in synergistic configurations with increasing frequency, providing exciting and unprecedented information about nanoscale phenomena.

Conflict of Interest: The authors declare no competing financial interest.

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