Optical antennas focus in on biology

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Biological processes often involve multimolecular interactions on a nanometre scale or at very large molecular concentrations, making them difficult to visualize. Optical antennas have the potential to become powerful tools for nanobioimaging by enhancing optical fields on this tiny scale.

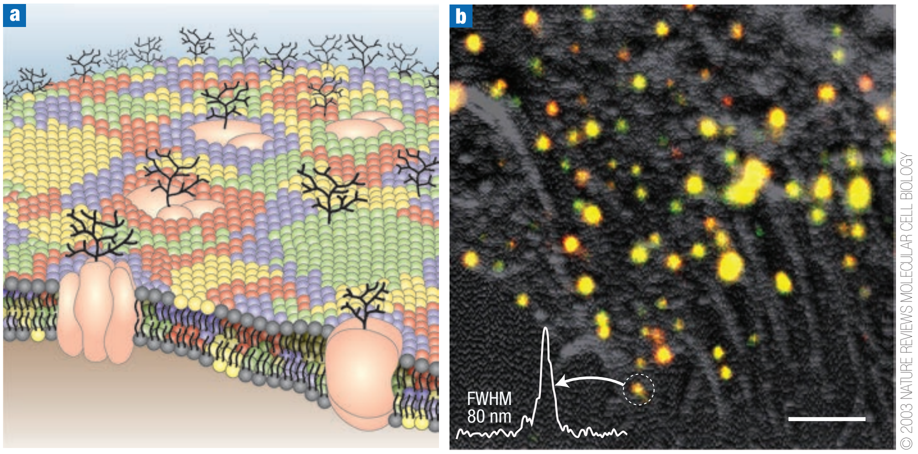

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Figure 1 Imaging a cell. **a**, A cartoon illustrating the nanometre-scale complexity of the cell membrane. The membrane has a largely heterogeneous composition. However, multiple components of the membrane organize in domains and clusters of nanometre-scale dimensions (reprinted from ref. 25). **b**, Simultaneous topography (grey) and near-field optical imaging (colour spots, which indicate domains) of a dendritic cell expressing the pathogen recognition receptor DC-SIGN. Without the organization of DC-SIGN in domains, viruses like HIV-1 cannot be bound and internalized by the cell[2,7]. The sizes of the domains vary from 80 nm to 180 nm. The scale bar is 500 nm.

One of the ultimate challenges in biology is to understand the relationship between the structure, function and dynamics of biomolecules in their natural environment, the living cell. Although modern molecular biology has made enormous progress in identifying different cell components, both inside and at the cell membrane, observing molecular processes in living cells is still a major goal. Key multimolecular interactions that dictate cell functionality occur at the nanometre scale[1,2] (Fig. 1a), a size regime not accessible by classical optical techniques owing to the diffraction of light. Also, biologically relevant processes, such as transient interactions between proteins and nucleic acids or between enzymes and their ligands, occur at very high ligand concentrations[3,4]. To be able to investigate processes between individual units, the detection volume must be reduced by at least three orders of magnitude. Advances in nanophotonics are now taking a leap towards subdiffraction-limited optical resolution. Indeed, these approaches might become key techniques in modern biology by providing tools for studying processes both *in vitro* and *in vivo* at relevant spatial scales and physiological concentrations.

Creative designs using sharp metallic tips, nanoparticles and plasmonic resonances have become extremely important in nanophotonic research. The main idea in this emerging field is to localize and enhance the optical radiation in a small region (on the nanometre scale), in a way very similar to how electromagnetic antennas convert propagating radiation into a confined zone, the so-called feedgap. Unsurprisingly, these metal-based nanostructures have been named optical nanoantennas. If the promises of nanometre-scale optical confinement and enhancement are achieved, they would certainly have a tremendous impact in a number of different fields of science, including bioimaging, and bio- and chemical sensing.

One of the earliest examples of an optical antenna is the subwavelength-aperture probe, as used in near-field scanning optical microscopy (NSOM). Obviously, in terms of antenna concepts, a simple circular hole is far from optimum. Nevertheless, the technique can deliver detailed information at the nanometre scale of complex systems such as cell membranes under real conditions[5–8] (Fig. 1b). Unfortunately the fraction of light that can be detected using ‘aperture-type’ NSOM is limited (10^{-4}–10^{-6} for a 70-nm aperture). This low light throughput and the finite skin depth of the metal restrict the practical resolution of NSOM to about 50 nm.

In recent years, more elegant approaches towards the high-precision fabrication of efficient optical antennas

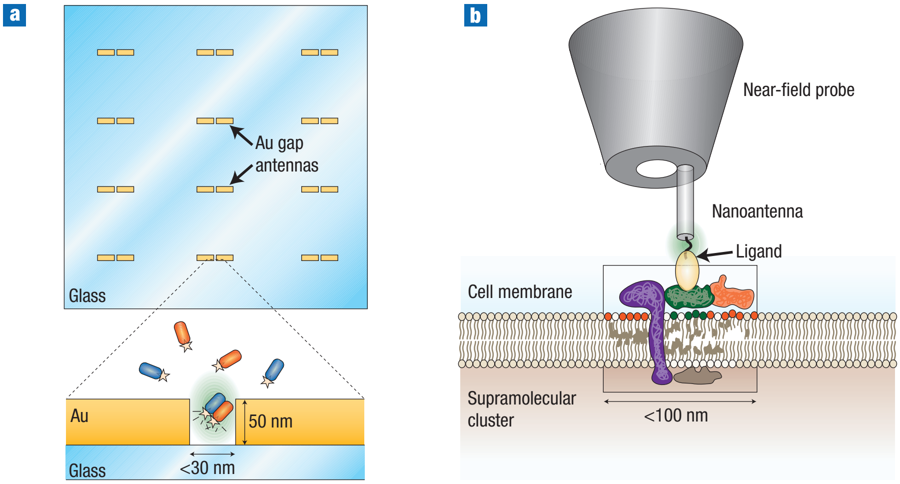

Figure 2 Potential applications of optical nanoantennas. **a**, Sensing molecular interactions at high concentrations using gold gap nanoantennas. The reduced observation volume combined with field enhancement enables detection of weak interactions at the individual level. **b**, Imaging molecular interactions within a dense supramolecular cluster on the cell membrane. The ligand attached to the nanoantenna activates the cell receptor and triggers cellular signalling. Topography, biochemical recognition and fluorescence images at the nanometre scale are simultaneously obtained.

have been explored. One of the most successful methods uses metal planar nanostructures on glass substrates, either stripes or bow ties with gap dimensions as small as 30 nm (refs 9–11). For instance, impressive work reported in ref. 10 demonstrated generation of white-light-supercontinuum radiation in the antenna feedgap, on illumination with picosecond laser pulses. Moreover, the emission from the antennas was more than 1,000 times stronger than from similar metallic structures without a feedgap. Local intensity enhancement of the same order has also been reported using gold bow-tie nanoantennas[11].

The extraordinarily enhanced emission and extremely small excitation volume achieved using these structures would enable, for instance, the study of single-(bio)molecule interactions that only occur when the interacting molecules are at very high concentrations. In 2003 high-concentration (micromolar) single-molecule spectroscopy was demonstrated using a combination of zero-mode waveguides and fluorescence correlation spectroscopy[12]. Exploiting optical antennas to boost the field confined in small areas will further increase the limit of detection by orders of magnitude, enabling the monitoring of extremely weak chemical or biological reactions (Fig. 2a). It may even be possible for these resonant optical antennas to be used for single-molecule Raman spectroscopy. Arrays of optical nanoantennas could be used on substrates to which single cells are attached. The feedgaps would work as 'hot spots', where local fluorescence correlation spectroscopy could be performed. This technique could be particularly suitable for investigating slowly diffusing supramolecular complexes on the cell surface. Different gap sizes could provide distinct effective confinement volumes, from which the characteristic diffusion time of receptors and their association and dissociation rates could be investigated in much greater detail than is possible at present.

Although planar structures would be suitable for monitoring (bio)chemical reactions and transient dynamic interactions *in vitro* and *in vivo*, true nanometric optical microscopy would have to come from free-standing structures in combination with scanning-probe methods. Along these lines, gold nanoparticles attached to glass tips have been exploited as nanoantennas[13,14], and the fabrication of bow-tie-shaped antenna probes using atomic force microscopy cantilevers is also being explored[15]. With regard to biological nano-imaging, single calcium channels on erythrocyte plasma membranes have recently been visualized for the first time using a gold nanoparticle-based optical antenna[16]. The spatial resolution obtained was 50 nm, although improvements to the antenna geometry and illumination schemes could improve this to 10 nm. These elegant experiments have the additional value of being performed in aqueous conditions, opening the door to a multitude of physiologically relevant experiments, including elucidating the complex relationship between the cell membrane and cell function. Yet, the concept is still far from fully mastered and requires further improvements.

In the approach reported in ref. 16, a substantial amount of background signal is generated by the focused laser beam used to irradiate the optical antenna. Also, although the work is performed in physiological conditions, at present the approach requires the cells to be fixed, which prevents dynamic studies.

A different excitation scheme that suppresses background illumination has been proposed[17] and, more recently, applied to antenna concepts[18]. In these tip-on-aperture antennas, the main idea is to take advantage of the local illumination properties of aperture-type NSOM to drive the antenna to resonance. The antenna is thus a metallic tip on top of a normal nano-aperture. In the initial experiment, reported in ref. 17, the tip was considered mainly as a nanoscale scattering tip. Yet, simultaneous topography and fluorescence imaging of labelled DNA with 10-nm resolution was convincingly demonstrated[17]. Using a similar experimental approach, but exploiting the resonant properties of the metallic tip (tuning the antenna dimensions to the wavelength used), and using optimal excitation conditions, single-molecule detection with 30-nm resolution and virtually no background was demonstrated[18,19].

If, instead of precisely carving an antenna from the surrounding aluminium of a conventional NSOM probe, an easier method can be found to grow the tips using modern deposition techniques, optical nanoantennas could be used in a large number of biological applications. Investigating multiple components on the native cell membrane at physiologically relevant packing densities, and correlating membrane topology (also with nanometre resolution owing to the sharp tip) with structural information such as lipid rafts, membrane ruffles or caveola, are only a few examples of the many topics that could be addressed. Finally, the nanoantenna could be functionalized with specific ligands (in very much the same way as is performed in atomic force microscopy). This would make it possible to simultaneously record topography, biochemical recognition and fluorescence imaging at the nanometre scale, and all at the individual-molecule level (Fig. 2b).

Other configurations using metallic tips, such as infrared spectroscopy NSOM[20] or tip-enhanced Raman spectroscopy[21], which provide chemical recognition by means of molecular-vibrational 'fingerprints', would also benefit from free-standing nanoantennas. As already suggested, sharper tips should enable recognition of chemical composition and the molecular conformation of single proteins or even their subunits[20].

The rapid development of nano-optics technology means that a bright future in biological nano-imaging and (bio)chemical sensing lies ahead of us. However, caution is required, especially when addressing the biological community, as these techniques are still far from being routinely implemented in a biology lab. Serious challenges are still foreseen regarding the reproducible and precise fabrication of resonant antennas, and there is a need to fully understand specific antenna effects before practical applications in the biological field are possible. Finally, for wide-spread use of nanophotonic technologies in biology, development of user-friendly experimental set-ups will be imperative.

Optical nanoantennas are not the only route to super-resolution. In fact other forms of microscopy have recently demonstrated their enormous potential in biology. These methods rely on the specific photophysical properties of fluorescence probes in conjunction with tailored methods of illumination to achieve very high-resolution imaging[22–24]. As these techniques entirely rely on very specific fluorescent labels, they might not reach the full spectrum of biological applications. For instance, they may be limited to conditions where genetic transfection of autofluorescence proteins is not possible, or the sets of fluorescent labels may be incompatible with the biomolecules under investigation. On the other hand, multicolour versions of these techniques and the first demonstrations in living cells are very promising for the future. Clearly, the long-awaited super-resolution optical imaging is now at hand, and breakthroughs in biological understanding should soon emerge. Whether these discoveries will be achieved using optical nanoantennas or super-resolution fluorescence-based microscopy will probably be of less importance to biologists than a ready-to-use, flexible instrument.

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