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Perspective

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Illuminated up close: near-field optical microscopy of cell surfaces

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Abstract

Invented in the 1990s, near-field optical microscopy (NSOM) was the first optical microscopy method to hold the promise of finally breaking the diffraction barrier in studies of biological samples. This promise, though, failed to materialize at that time, largely owing to the inability to image soft samples, such as cell surfaces, without damage. However, steady technical improvements have now produced NSOM devices that can routinely achieve images of cell surfaces with sub-100 nm resolution in aqueous solution. Further, beyond just optical information, these instruments can also provide simultaneous topographic, mechanical, and/or chemical details of the sample, an ability not yet matched by any other optics-based methodology. With the long recognized important roles of many biological processes at cell surfaces in human health and disease, near-field probing of cell surfaces is indeed now well poised to directly illume in biomedicine what has, until recently, been unknowable with classic light microscopy.

From the Clinical Editor: This paper presents a novel and important near-field microscopy-based method directly enabling the imaging of cell surfaces with sub-100nm resolution. Unlike other optics-based methods, the presented technique can also provide topographic, mechanical and chemical details of the samples.

Key words: Super resolution; Cell membrane; Nanodomains

Since its invention nearly four hundred years ago,[1] optical microscopy has played an irreplaceable role in unravelling the structural underpinnings of biological systems, from the initial discovery of individual cells by van Leeuwenhoek to the organization of cells in complex tissues to the delineation of chromosomal territories within individual nuclei.[1-3] However, up until recently, what has been possible to resolve with conventional light microscopy has remained stubbornly limited by diffraction to about 250 nm laterally and several times worse axially.[4] With this limitation, structural features that are in closer proximity to each other than these distances cannot be spatially distinguished. As many subcellular structures are themselves smaller than 250 nm, there has thus been a long-standing interest in biology in techniques or technologies that can supersede this limitation and resolve what is unknowable using classical light microscopy.

In the last few decades, we have indeed witnessed unprecedented developments in this regard: there are now approaches that, although still limited by diffraction, can nonetheless attain a resolution of ~100 nm in all three directions and others that overcome the diffraction barrier altogether, yielding resolutions down to ~20 nm laterally and ~50 nm axially.[5-7] These approaches fundamentally differ from classical microscopy by using either novel illumination or detection schemes. The most successful of these methods include structured illumination microscopy (SIM), stimulated emission depletion (STED), photo-activated localization microscopy (PALM), and stochastic optical reconstruction microscopy (STORM).[5-7] SIM works through patterned illumination that encodes the higher resolution spatial details of the sample in multiple images that can then be recovered with extensive post-exposure analysis. STED also works with "patterned" illumination but here the pattern is produced by a pair of beams designed to drastically reduce the emission volume to nanoscopic dimensions, which is then used to scan the sample to obtain the image. By contrast, PALM and STORM take advantage of a property of many fluorescent molecules to photo-switch: the molecules are first induced into a non-emitting,

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dark state and then a very low fraction of these molecules are stochastically photo-activated to emit. The individual fluorophores are then localized, which can be achieved down to less than 10 nm, as this is not limited by diffraction but instead by the number of detected photons.[8] Iterative accumulation of sufficient localizations allows a reconstruction of the full image at a much higher resolution than conventional diffraction-limited microscopy.

Although such technologies continue to evolve, their power is already evident with several seminal contributions to our understanding of biological super-structures, including the discovery of a linearly periodic arrangement of actin-spectrin structures along the axons of neurons[9] and the delineation of the multilayer molecular architecture of the focal adhesion core.[10]

These techniques have thus justly attracted a great deal of attention from a broad spectrum of biologists. In light of this great interest, we note that another super-resolution method, near-field optical microscopy (NSOM),[11-16] should be of especial interest to the biomedical community, owing to its unique capabilities to image biological processes at the cell membrane, the site of many medically important events. Like STED, this method breaks the diffraction barrier by reducing the size of the region of molecules that fluoresce. However unlike STED, this is achieved in NSOM by simply bringing a very small aperture at the end of a tapered glass fiber in extremely close proximity (~10 nm) to the sample surface (Figure 1). In this near-field regime, the illumination intensity is limited laterally as well as axially, thus bypassing the diffraction limit in all three dimensions, and bringing the lateral resolution to below 20 nm in raster-scanned images.

This technique in fact significantly pre-dates the development of the aforementioned super-resolution methods, being developed on the heels of the transformative scanning tunneling microscope.[17,18] The validity of the NSOM concept was well demonstrated at that time, at least for imaging in air or vacuum, and the potential of such a technique in biomedicine was also appreciated almost immediately.[18,19] However, initial applications in biological research turned out to be rather disappointing, owing to the difficulty of maintaining the probe at a fixed distance from the sample in solution (see below).[15] Because of these difficulties, early optimism faded and enthusiasm for this methodology quickly waned. Still, this technology continued to evolve owing to the persistent efforts of a few labs. It is the purpose of this Perspective to bring attention to recent breakthroughs in this methodology and to provide a succinct account of the nanoscopic information that can presently be achieved with these devices.

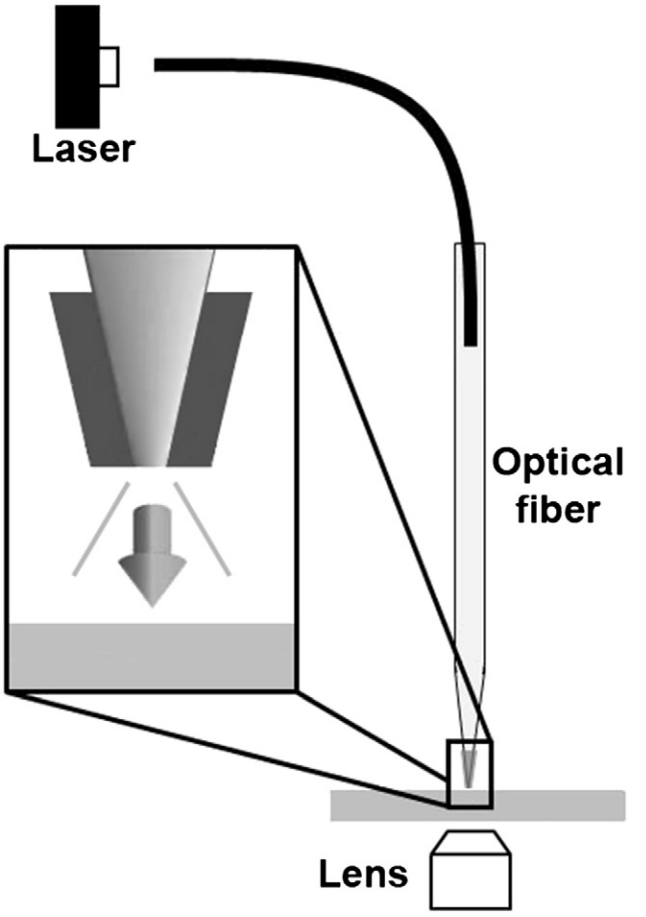

Figure 1. Schematic diagram of NSOM. A tapered optical fiber with a metal-coated aperture is brought within nanometers of the sample, which severely limits sample illumination. Images are obtained by raster-scanning the fiber at a constant probe-sample distance.

Improving the original NSOM design: effective shear-force feedback in solution

The most commonly used method in NSOM is rather straightforward. Optical fibers are pulled to form tapered probes between 20 and 100 nm in diameter, and then coated with an optically opaque metal (typically aluminum) to establish a transmitting aperture at the apex. As the tapered end has dimensions much smaller than the illuminating wavelength, these probes can exhibit low light throughput. In particular, as the size of the fiber diameter first approaches and then becomes much smaller than the wavelength, light propagation becomes significantly perturbed by the small dimensions of the conduit: the number of propagating modes decreases as the fiber diameter reduces until the fiber is too small to support even a single mode, at which point the field decays exponentially.[13,20] Those light modes that do not propagate are either reflected back into the fiber or are absorbed by the aluminum, which can lead to severe heating when the power is excessive.[13] Despite these heating effects and this low efficiency of light transmission, these probes have indeed proven reasonably effective for many applications.[13,18,19,21]

However, these probes are also extremely delicate and any forceful contact with the sample surface causes irreparable damage to the probe or the sample. This is indeed what led to the early disappointments when attempting to image biological samples in solution, a requirement for any technique engaged to resolve most biologically relevant features. In the original setup, the probe-sample distance was maintained by the "shear-force" detection scheme [22,23] In this approach, a piezoelectric device drives the optical fiber into lateral oscillation, and shear forces between the tip and sample lead to a reduction in the amplitude, which is monitored to maintain a constant probe-sample distance. In air or vacuum, where there are high-quality factor (Q) resonances, very small shear