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Antennas based near-field scanning optical microscopy (NSOM) for protein imaging beyond diffraction limit

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Abstract

In recent years, super resolution microscopy has been rapidly applied in biological study. Near-field Scanning Optical Microscopy (NSOM) is one of the most powerful optical super resolution microscopy that can be used for protein nanoimaging in living cells. Moreover, optical antennas have also been adapted to NSOM and greatly improved the performance of NSOM. In this mini review, the application of different optical antennas for NSOM has been described, and the use of NSOM for protein nanoimaging has also been highlighted.

Review

Near-field Scanning Optical Microscopy (NSOM) is a typical super-resolution optical microscopy that showed great promising in biological applications [1]. The most conventional NSOM is aperture type NSOM, which employ an aperture-tip as the probe and light source. This kind of NSOM has already been used for protein imaging in living cells. However, the physical size of the aperture tip is large, which usually limited the resolution and hard to control.

To offset the problem, a new concept of optical antennas [2,3] was adapted for the probe design [4]. The optical nanoantennas have great potential to further constrain the light on a sub-20 nm scale and greatly enhance the light-matter interactions [5,6]. The nanoantenna can create highly enhanced local fields, leading to fluorescence enhancement. One simple and powerful nanoantenna is bowtie nanostructure. By using a gold bowtie nanoantenna, a single molecule's fluorescence was enhanced over 1000 times [7]. By adapting this notion for NSOM probe design, a bowtie nanoaperture antennas was fabricated

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at the apex of the tapered fiber probe, which provided a super confined nano spot with intensive local field and broadband response [8]. As a result, the power throughput was improved ~3 orders of magnitude, the signal intensity of single molecule was enhanced ~6 folds [4]. With the bowtie nanoaperture probe, the optical confinement of 20~80 nm was obtained [8,9], which opened the possibility to image the molecules smaller than ~50 nm.

By taking the advantage of nanotip antenna and nanoaperture, another powerful antenna probe for NSOM is tip-on-aperture, in which the local illumination from aperture probe can directly drive the antenna tip to resonance. With this configuration, the enhanced optical field at the end of tip can be confined to suppress the background illumination and thus enhanced the resolution [10]. By using the tip-on-aperture NSOM, it is practical to image individual protein on the cell membrane with a resolution of ~30 nm which is incapable to conventional NSOM [11]. Furthermore, by integration of bowtie nanoaperture with a monopole nanoantenna, a hybrid antenna NSOM probe was fabricated which combines broadband enhanced emission, extreme field confinement (few nanometers), and background-free illumination [12]. This configuration reached 20 nm resolution and angstrom localization precision, and also enabled simultaneous detection and imaging of molecules in two different colors [12], which is expected to enable multiplex nano-imaging of live cells.

The most attractive advantages of NSOM for protein imaging include high resolution which could provide information beyond the diffraction limit that is incapable of for traditional optical microscopies, topography could be provided simultaneously, and compatibility with physiological conditions. With these intriguing and unique advantages, NSOM can provide more convincing and insightful information about the proteins beyond the diffraction limit [13,14]. For example, with a gold-nanoparticle antenna probe, the NSOM could resolve single Ca^{2+} transmembrane protein on erythrocyte plasma membrane which it is incapable of resolution by a traditional confocal fluorescence microscopy due to high protein density (20~30 proteins/μm) and small protein size (<100 nm, far beyond the diffraction limit) [13]. With biocompatible aperture NSOM equipped with a bent double-etched aperture probe, L-type Ca^{2+} ion channel on membrane of fixed H9C2 cells was observed [14]. It was found that the ion channel is localized in small clusters that vary in size from ~<60 to 250 nm, which is beyond the diffraction limit of conventional optical microscopy. According to the fluorescence density and size, it was estimated that most of the clusters are composed of 5-10 channels with random distribution across the cell membrane. Topography combined 3D image, which can clear show the distribution and microenvironment of the channels on cell membrane was also obtained. This result provided most convincing evidence that the Ca^{2+} channels mainly formed 5-10-channels clusters on the cell membrane. More impressively, with the nanoscale imaging to which the conventional methods is inaccessible, NSOM analysis directly provided new insights and enabled many new interesting findings. For example, previous experimental results from conventional protein chemistry and immunology studies suggested the mature IL-22 protein is secreted to the extracellular environment. With NSOM-quantum dot based nanoscale imaging, it was found that IL-22 was bounded to the cell membrane and shared similar distribution patterns with membrane CD3 [15]. These unexpected discoveries from NOSM analysis raised the possibility to redefine the *in vivo* function of many important proteins.

With the high resolution NSOM image, it is also possible to quantify the protein intensity and estimate the stoichiometry more accurately, even at a single molecule level. With a bent-fiber aperture (diameter 40-80 nm) probe equipped NSOM, the β-adrenergic receptor was visualized on the membrane of cardiac myocytes cells. In the light of high resolution NSOM imaging, the stoichiometry of the receptor cluster was estimated to be ranging from 12 to 72 molecules per cluster with diameter from 120 to 160 nm [16,17]. Colocalization results indicated that 15~20% receptors (β_2AR) were pre associated with caveolae (caveolin 3) into signaling islands [16,17].

With high localization accuracy (~few nanometers) combined with high spatial resolution, NSOM holds the potential to discriminate proteins with high proximity, which is suitable to address another important question of protein-protein interaction [16,18,19]. Although this protein-protein interaction can be readily characterized by biochemical means, very little is known about their spatial organization in-situ [20]. Because these proteins are in close proximity and cannot be discriminated by conventional microscopy. With NSOM imaging on live cell surface, it was found that the integrin receptor LFA-1 formed ~85 nm size clusters before activation, while ~30% raft components of GPI-APs proteins formed oligomers close (<200 nm) to LFA-1 [19]. However, ~70% of GPI-APs are monomers and randomly distributed and have no correlation with LFA-1. Upon ligand-mediated LFA-1 activation, the GPI-APs monomers are converted to nanodomains (~20% GPI-APs are in monomers) and reorganized with LFA-1, forming larger functional supramolecular nucleation sites for cell adhesion. The observation of the crucial intermediates (GPI-APs/LFA-1 nanodomains) in the process leading to effective cell adhesion provided a prototype of nanoscale inter-receptor assembly [19].

Conclusion

In conclusion, NSOM, with the rapid development in recent years, its resolution and stability were greatly improved. And also, its power in imaging of proteins in living cells has already been approved. We believed that, with this powerful super resolution tool, many new findings beyond the diffraction limitation will be explored in the near future, which will surely broaden our knowledge about the life.

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