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REVIEW ARTICLE

Plasmon Based Super Resolution Imaging for Single Molecular Detection: Breaking the Diffraction Limit

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Abstract
Developments of super-resolution imaging techniques have considerable interests to detect and image tiny molecular events under a diffraction limit. Stimulated emission and depletion (STED) microscopy, photo-activated light microscopy (PALM) and structured illumination microscopy (SIM) are representative successful novel imaging techniques. Recently, surface plasmons (SP) based super resolution imaging techniques which can achieve super resolution with no deviation from conventional microscopic schematics have been actively investigated. In this paper, we explain the principle of SP phenomena which can apply for bioimaging, and introduce localized SP based super resolution imaging techniques to increase lateral and axial resolution below the diffraction limits. Three different novel techniques based on field localization are introduced to increase lateral resolution. Also, additional three imaging techniques based on extra-ordinary transmission and Förster resonance energy transfer are introduced to increase axial resolution. Consequently, we explore a future direction of SP based imaging researches for 3D spatiotemporal super resolution microscopy.

Keywords Surface plasmon, Super resolution, Diffraction limit, Nanostructures

INTRODUCTION

Varied optical measurement and imaging techniques have been applied to study and analyze cellular dynamics and bio-molecular activities [1-5]. A crucial limitation of optical measurement and imaging for cellular or bio-molecular studies is spatial resolution which is bounded by Abbe's lateral and axial diffraction limits [6]. Breaking the diffraction limits is expected to monitor and analyze cellular and bio-molecular dynamics in nanoscale and detect extremely small molecular events in cellular or subcellular environments. Thus, development of new microscopic techniques to improve spatial resolution under diffraction limits has been desired for a long time for lots of potential investigations.

Recently, numerous microscopies have been suggested as super resolution optical measurement and imaging: stimulated emission and depletion (STED) microscopy [7, 8] which is based on the establishment of narrow excitation light spots, photo-activated light microscopy (PALM) [9] acquired by calculations of the stochastic photo-activation of fluorescent molecules, and structured illumination microscopy (SIM) [10, 11] using the reconstruction of high resolution images from partial images with sinusoidal illumination spatially encoded. Also, a few optical imaging and tomographic systems with the enhancement of sectioned measurement and imaging capabilities have been introduced: total internal reflection fluorescence microscopy (TIRFM) [12, 13], selective plane imaging microscopy (SPIM) [14-16], and tomographic imaging microscopy [17].

Even though those emerging imaging techniques have received significant interests in lateral and axial resolution enhancement capabilities, they still have lots of difficulties to be broadly used and commercialized due to the limits in detection and imaging speeds, experimental and post-processing complexity and high expenses. On the other hands, surface plasmons (SP) phenomena have been relatively little used for imaging enhancements, because the long propagation of SP on film interrupts to image tiny molecular events on a nanoscale [18]. However, applying nanotechnology on surface which can redistribute and localize SP fields can overcome

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the interruption. The approach is beneficial in terms of resolution improvement, as fields localized by using nanostructures can be extremely small, smaller than the diffraction-limits. If an area of localized fields is equal to a size of a single nano fluorescence molecule, we can hypothesis that the fluorescence emission indicates the presence of the single molecule in principle. Thus, single molecular images can be reconstructed through image post-processing.

In this review, we introduce promising SP based super resolution imaging techniques with two approaches which consequently target for three-dimensional super resolution imaging under diffraction limits.

SURFACE PLASMONS

SPs occur in the metallic geometry using gold, silver and aluminum, since the real part of the metal relative permittivity is negative and its magnitude must be bigger than that of the dielectric relative permittivity. The negative value causes π-phase shifts in the parallel direction of electric wave at surface, and the consistent wave crossing at surface is essential to the SPs waveguide. The waveguide presents longitudinal electron oscillations at surface between metal and dielectric layer under a total internal reflection condition. The waveguide produces thin evanescent fields in the range of about 100 to 200 nm on surface.

Early understanding of SP dates back to the 1900s when Wood RM and Rayleigh L first discovered uneven distribution of light in diffraction grating and explained theoretically in 1902 and 1907, respectively [19, 20]. The potential of SP for characterization of thin films was initially recognized in 1970s [21]. At the turn of 1980s, the attempts for gas detection and biosensing were introduced [22]. Since then, SP has been receiving considerable attention from various scientific fields due to its potential. According to a recent survey through PubMed, around 10,000 research articles and several reviews have been published on SP based techniques from 2005 to the present. Among them, a SP resonance biosensor is one of the successful approaches, because it stems from the fact that specific interactions between biomolecules can be monitored in real-time with high sensitivity without labeling [23]. Recently, many research groups have been focusing on the feasibility of SPs for developments of imaging systems [24, 25].

LATERAL RESOLUTION ENHANCEMENT TECHNIQUES

Surface plasmon enhanced total internal reflection fluorescence microscopy

TIRFM is an optical imaging method to acquire cellular and molecular dynamics within a thin region of illumination which is less than 200 nm. For the restriction of fluorescence excitation depth, the TIRFM employs evanescent fields, electromagnetic fields exponentially decaying from the glass-sample medium interface. Since the investigation of a surface contact microscope for cell migration analysis in 1956 [26], TIRFM has been generally applied to monitor and analyze cellular and molecular activities in thin cellular surfaces as cell migration and adhesion [27, 28], drug absorption dynamics through cell membranes [29], virus invasion [30], and endocytosis processes [31, 32].

For preserving advantages of axial resolution and improving other factors in optical measurement and imaging, several research groups introduced SP enhanced TIRFM with metallic and metal-dielectric blended layers below the sample. SP are free electron oscillations at the interface between metallic and dielectric materials since signs of the relative permittivity real part in two materials are different [33]. SP in TIRF configuration propagate along the surface and enhance the evanescent electromagnetic field as the excitation light of fluorescence imaging, so SP mediated TIRFM to enhance fluorescence detection sensitivity has been developed. Tang WT and co-authors investigated theoretical analysis of fluorescence signal enhancement of SP coupled fluorescence microscope with the sample above 50 nm gold film [34]. In the point-spread function analysis of this study, SP based fluorescence enhancement offers better detection sensitivity and signal-to-noise ratio compared to conventional TIRF measurements. Silver film based TIRFM

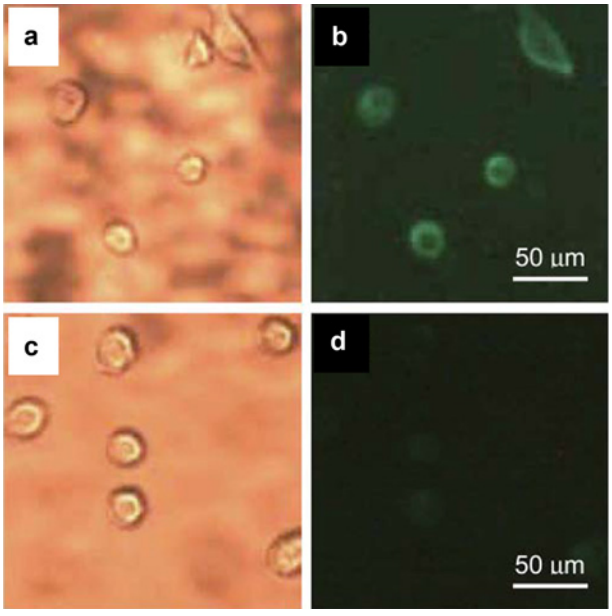

Fig. 1. (a) Bright field and (b) TIRF images of A431 cells with the SP enhancement by the multi-layer dielectric film. (c) Bright-field and (d) TIRF images without the film. The figure is reproduced from ref. [37] with approval by the Optical Society of America.

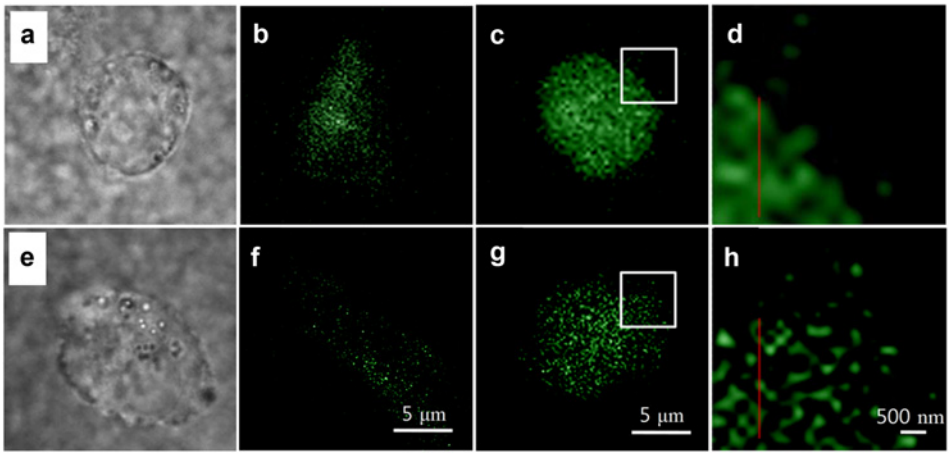

Fig. 2. Optical images acquired after the endocytosis of adenovirus into A549 cells. (a) Bright-field image of the cell and conventional TIRF images of the thin film control: (b) 15 and (c) 30 minutes after the injection of adenoviruses. The same images in (e) bright field and (f), (g) SUPRA-TIRFM on the silver nanoislands sample. (d), (h) magnified images of squares shown in (c) and (g). The figure is reproduced from ref. [39] with approval by Wiley-Blackwell.

was developed to improve fluorescence signal efficiency in visible light for various applications of functional fluorescent indicators [35, 36]. Moreover, TIRFM using two layers of dielectric films was developed to minimize fluorescence signal loss from the reflection by the metal [37]. Both the simulation and fluorescence imaging experiments using fluorescent microbeads and A431 cells exposed by fluorescent quantum dots (Fig. 1) indicate that 300 nm SiO_2 and 20 nm Al_2O_3 thin films offer highly improved fluorescence measurement and imaging sensitivity.

LSP enhanced TIRFM with lateral resolution enhancement

One of the remaining limitations in TIRFM and SP enhanced TIRFM is lateral resolution of optical detection and imaging is bounded by the diffraction limit, so dynamics of biomolecules with the size below the diffraction limit cannot be measured and imaged clearly. For this reason, TIRFM with localized surface plasmon (LSP) based lateral resolution enhancements have been investigated by several research groups. The LSP is collective electron charge oscillating phenomenon in metallic nanostructures and particles which are excited by electromagnetic fields [38]. Highly concentrated near-fields are established by LSPs and the size of localized fields is usually smaller than the diffraction limit. Using appropriate metallic nanostructures and nanoparticles and effective post-processing, TIRFM with LSPs can break the diffraction limit and approach greatly enhanced lateral resolution in optical imaging.

SP enhanced randomly activated (SUPRA)-TIRFM is one of the LSP enhanced TIRFM using randomly distributed silver nanoislands instead of silver films [39]. Like the origin of STED microscopy, SUPRA-TIRFM can provide fluorescence images with better lateral resolution through selective excitation of fluorescent molecules inside LSP fields. In this case, lateral resolution of SUPRA-TIRFM is determined by the sizes of LSP fields, which is below the diffraction limit. Theoretical simulations and experimental fluorescence measurements with fluorescent nanoparticles and adenovirus endocytosis confirmed the concept of SUPRA-TIRFM (Fig. 2).

However, aperiodic nanostructures do not offer exact locations of LSP fields, so appropriate post-processing is not possible using them. On the other hands, LSP fields at expected locations can be obtained with periodic nanostructures. Thus, exact kernel for post-processing can be only provided with periodic nanostructures. Images of fluorescence molecules moved on periodic nanostructures were reconstructed by post-processing, nanoscale localization sampling (NLS), to

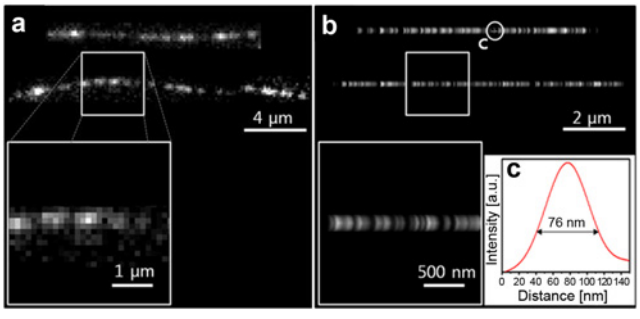

Fig. 3. NLS based lateral resolution enhancement in optical imaging. (a) Control image of microtubules traveling on a 10 nm thick gold film captured by a conventional TIRF microscope (b) Lateral-resolution-improved image of microtubule by the NLS based reconstruction using a nanohole array of 300 nm diameter and 1 μm period. (c) Fluorescence intensity profiles across the line in the circle of image (b). The figure is reproduced from ref. [40] with approval by Wiley-Blackwell.

obtain enhanced lateral resolution [40]. Near-field distributions on the periodic nanostructures were estimated by electromagnetic field simulations. After that, highly enhanced fluorescence images of microtubules transported on the periodic nanostructures were achieved by the NLS (Fig. 3). The optimum lateral resolution of images was estimated to 76 nm, which is below the diffraction limit.

Simple field localization using nanostructures remains a limitation that localized fields totally depend on the geometry of nanostructures and resolution cannot be improved less than the size of localized fields. Moreover, the simple localized field is not sufficient to image molecular dynamics and can be executed under suboptimal excitation conditions. Thus, schematic changes of SP enhanced TIRF microscopy, which is abbreviated as plasmonics based spatially activated light microscopy (PSALM), have been investigated to overcome the issues [41]. (Fig. 4). Localized fields on nanostructures are spatially switched by changing of light incidence as presented in Figs. 4a and 4b. With nanograting patterns and two different optical paths, the significant resolution enhancement is clearly shown in the x direction, which is orthogonal to nanogratings. Especially, Fig. 4c presumably presents 100 nm sized aggregated fluorescent beads that are apart by single nanograting ridge. The peak-to-peak distances of nanograting were estimated to be around 90 to 100 nm. These results show good agreement with the distance between neighboring localized fields generated by two different optical paths. The approach has lots of feasibilities to improve resolution with increment of optical paths. For instance, with 8-channels PSALM, imaging resolution will be obtained theoretically down to 20 nm. However, precise controls of optical paths and clear and strong localized fields are needed to achieve the goal.

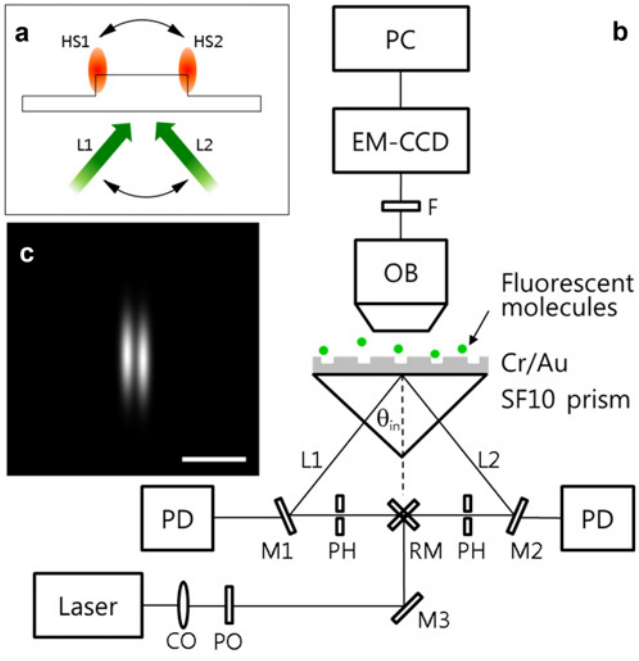

Fig. 4. Plasmonics based spatially activated light microscopy (a) Concept illustration of PSALM. Switching of light incidence between L1 and L2 produces spatial switching of localized fields between HS1 and HS2. (b) Experimental configuration (CO, collimator; PO, polarizer; M, mirror; RM, rotation mirror; OB, objective; and F; filter). (c) Image of fluorescent beads taken by PSLAM. The figure is reproduced from ref. [41] with approval by the Optical Society of America.

AXIAL RESOLUTION ENHANCEMENT TECHNIQUES

Most of techniques introduced in section 2 are based on TIRF microscopy, so they can obtain a single section axial image within a penetration depth of evanescent field, typically 100 nm from surface. Although multiple sectioning was achieved by depth-resolved angle scanning TIRFM such as multi-angle TIRFM [42] and multiple grating based subtractive penetration depth TIRFM [43], it is still limited to the penetration depth. Therefore, several studies have been investigated high-resolution optical sectioning and tomographic imaging techniques for axial resolution enhancement without the limitation of depth-of-detection.

Extraordinary transmission based axial resolution enhancement

Since classical Bethe's theory of light through subwavelength structures and Ebbesen's experimental study of extremely enhanced light transmission through a nanoscale aperture, the extraordinary transmission (EOT) through subwavelength nanoapertures on metallic surface was studied theoretically and experimentally [44, 45]. Several studies indicated that the EOT and EOT based zero-mode waveguides could be applied to chemical and biological sensing with the enhancement of sensitivity and spatial volume [46, 47]. Also, super resolution imaging applications using nanoscale fields generated by the subwavelength apertures were investigated [48, 49].

EOT based axial imaging (EOT-AIM) is optical tomographic imaging with high-resolution axial sectioning [50]. The EOT-AIM is based on EOT by the linear arrays of subwavelength sized nano-apertures and depth-of-illumination of EOT can be adjusted by the design of each nano-aperture in the linear arrays. Numerical calculations using rigorous coupled wave analysis were progressed to estimate EOT field distributions through subwavelength aperture arrays (Fig. 5a). Subwavelength aperture arrays optimized by simulation results were fabricated on a glass by electron beam lithography. In the experimental study of EOT-AIM, the intensities of light produced by the subwavelength aperture arrays were measured

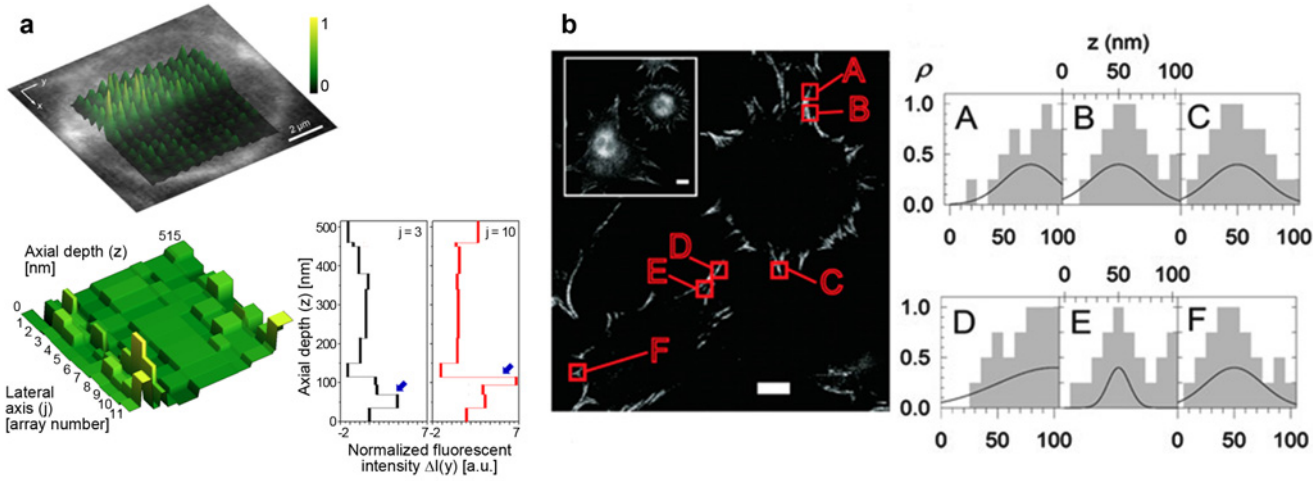

Fig. 5. Transmissive optical tomographic methods. (a) Fluorescence intensity image and intensity profiles along depth axis of a RAW264.7 cell measured by EOT-AIM, which is overlayed with a wide-field image of the same cell. The profiles at j = 3 and 10 (array numbers) are also shown on the right. (Ref. [50], Wiley-Blackwell) (b) Axial distribution estimation of the adhesion protein paxillin in fibroblast cells by SpecON. The figure is reproduced from ref. [54] with approval by the National Academy of Sciences.

using 10 μM fluorescein uniformly coated on the arrays. After that, super-resolved axial fluorescence measurements can be achieved through the estimation of fluorescence intensity differences between neighboring apertures. EOT-AIM provides between 20 and 125 nm axial resolution through the subwavelength aperture arrays using Fluorescein and FITC-conjugated cholera toxin subunit B (CT-B) (Fig. 5b). Authors claimed that EOT-AIM has lots of feasibilities for various microfluidic devices applications and additional imaging improvements with optimization of structures.

Fluorescence energy transfer based axial super-resolved imaging

Förster resonance energy transfer (FRET) is a dipoles coupling energy transfer mechanism between two molecules or materials [51]. By the measurement of FRET efficiency of two fluorescent molecules, the molecules within a certain distance could be quantitatively estimated. The method has been employed for understanding various chemical and biological processes [52]. Chizhik AI and co-authors investigated axial nanoscopy using FRET between fluorescent molecules and metallic substrates [53]. Fluorescence lifetime images indicate the distance from the gold surface and fluorescent molecules attached on live cell membranes by this metal-induced energy transfer (MIET), and three-dimensional reconstruction of the cell membranes was achieved (Fig. 6a). The MIET based axial position estimation of cell membranes has 3 nm axial resolution in the range of 100 to 200 nm.

On the other hand, Elsayad K and co-authors introduced

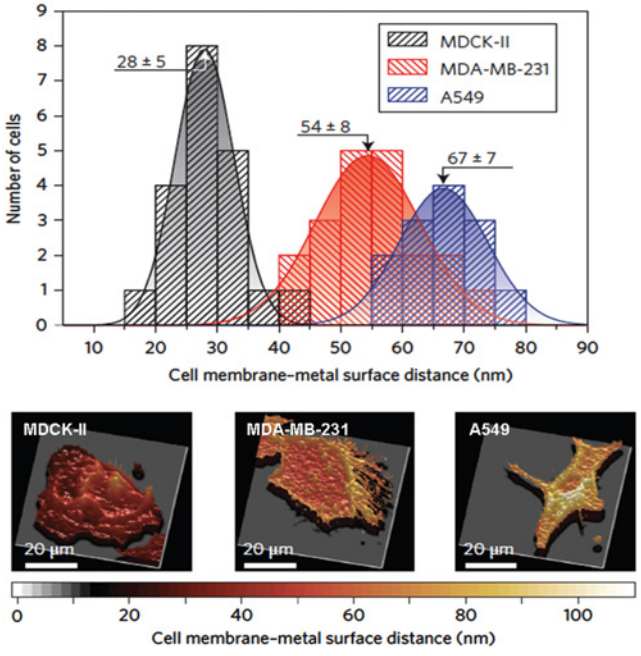

Fig. 6. Average distance from the surface and MIET-based reconstructed 3D images of cell membranes of the MDCK-II, MDA-MB-231, and A549 cells. The figure is reproduced from ref. [53] with approval by Nature Publishing Group.

nanosectioned fluorescence imaging using the changes of emission spectrum via axial position of fluorescent molecules from a plasmonic substrate [54]. To be detailed, the orthogonal condition of fluorescent molecules on the plasmonic substrate can be reflected to spectral distributions of emitted fluorescence by photophysics related to FRET. An optimized

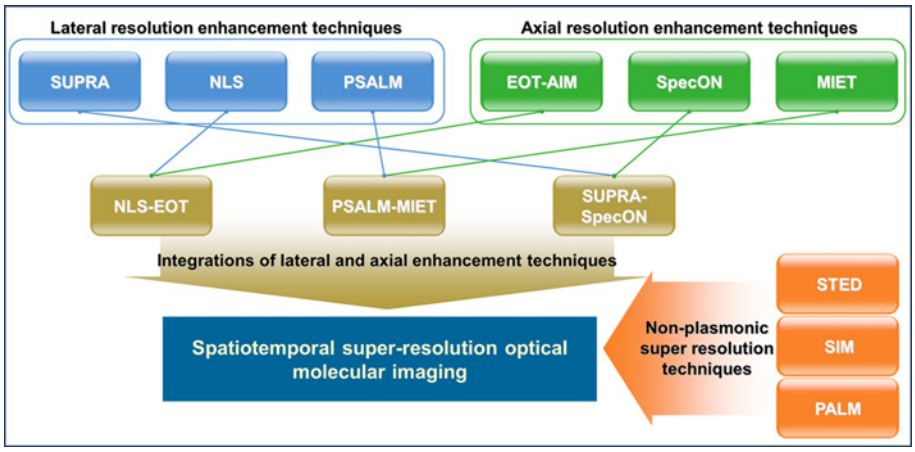

Fig. 7. Technical diagram for plasmonic techniques for super resolution imaging for single molecular detection and future directions of integration of multi-dimensional resolution enhancement tools for spatiotemporal super resolution optical molecular imaging.

thin metal-dielectric film (12 nm silver and 7 nm silicon nitride (Si_3N_4) bi-layers on quartz substrate) was found with measurements of maximum spectral changes decided by axial positions of Alexa 488 molecules. After the optimization, spectrally coded optical nanosectioning (SpecON) was achieved with 5 to 10 nm axial resolution in the range of 10 to 150 nm from the substrate (Fig. 6b) in cell experiments of NIH 3T3 cells stained by Alexa Fluor 488-paxillin.

SUMMARY

As we introduced SP enhanced optical imaging techniques in previous sections, the techniques can provide extreme improvements of lateral or axial resolution. However, each technique cannot achieve super resolution in a three-dimensional (3D) area. In fact, a 3D super resolution imaging technique might be a final goal for precise imaging and tracking of molecular dynamics. Moreover, high temporal acquirements in the technique are also important, since most of molecular dynamics in biological environments are fast and immediate. Thus, convergence of several imaging techniques can be one of the solutions to satisfy those needs. Several research groups have started to investigate integration of different novel imaging techniques for 3D super resolution imaging techniques [55, 56].

A future direction in developments of SP based optical imaging techniques also should target to acquire 3D super spatiotemporal resolution. The summarized technical diagram including various plasmonic techniques for lateral and axial resolution enhancements suggests future directions (Fig. 7). The convergence of plasmonic resolution enhancement techniques with other optical super resolution imaging techniques can produce future microscopic platforms with tremendous spatiotemporal resolution improvements.

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CONFLICT OF INTEREST STATEMENTS

Choi J declares that he has no conflict of interest in relation to the work in this article. Lee S declares that he has no conflict of interest in relation to the work in this article. Kim K declares that he has no conflict of interest in relation to the work in this article.

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