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Super-resolution Photoacoustic Microscopy Using Near-field Localization by a Plasmonic Metal Nanoaperture: A Simulation Study

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Abstract— Super-resolution microscopy (SRM) is becoming increasingly important to study nanoscale biological structures. Two most widely used devices for SRM are super-resolution fluorescence microscopy (SRFM) and electron microscopy (EM). For biological living samples, however, SRFM is not preferred since it requires exogenous agents and EM is not preferred since vacuum is required for sample preparation. To overcome these limitations of EM and SRFM, we present a simulation study of super-resolution photoacoustic microscopy (SR-PAM). To break the diffraction limit of light, we investigated a sub-10 nm near-field localization by focusing femtosecond laser pulses under the plasmonic nanoaperture. Using this near-field localization as a light source, we numerically studied the feasibility of the SR-PAM with a k-Wave simulation toolbox in MATLAB. In this photoacoustic simulation, we successfully confirmed that the SR-PAM could be a potential method to resolve and image nanoscale structures.

Index Terms—photoacoustic imaging, super resolution imaging, near-field imaging, plasmonic nanoaperture

I. INTRODUCTION

SUPER-RESOLUTION microscopy (SRM) techniques [1] exceed the diffraction limit of light [2] and enables investigation of biological samples, from individual proteins to entire organelles, at a nanometer scale [3]. Super-resolution fluorescence microscopy (SRFM) [4] is widely used since it can distinguish specific cellular components by using molecule-specific exogenous fluorescence agents [5] even inside a live sample [6]. However, the fluorescence agents may degrade the effective resolution of SRFM [7] because of photobleaching and also are not typically favored for biological samples [8, 9].

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Electron microscopy (EM) is another widely used commercialized SRM technique. Although EM achieves atomic resolution [10], it has many practical limitations for live samples [6]. One of the biggest limitation is that the samples must be placed in a vacuum environment. Thus, the samples need to be fixed, thin-sectioned, and dehydrated for EM imaging. In addition, the EM system is extremely expensive and requires a high maintenance cost.

Photoacoustic (PA) imaging is a hybrid biomedical imaging method to investigate organelles to organs [11-21]. The PA imaging involves two main principles [22]; (1) molecules convert absorbed light into heat which subsequently leads to thermo-elastic expansion of target resulting in PA wave generation, and (2) these generated PA waves are detected and reconstructed to form PA images. The contrast in PA image depends on optical absorption capability of different endogenous molecules, such as DNA/RNA, hemoglobin and melanin. Thus if appropriate laser wavelength is selected, no exogenous agent is required to photoacoustically image these internal targets [19]. Recently, several studies have shown that photoacoustic imaging (PAI) system can acquire super-resolution images of biological samples [23-28]. Photoacoustic nanoscopy using nonlinear PA effects resulted in a super-resolution PA image of single mitochondria with 88-nm lateral resolution [23]. Another technique called photoimprint PAI using photobleaching effects of chromophores acquired a super-resolution PA image of melanoma cells with 90-nm resolution [24]. The lateral resolutions achieved in these two techniques were approximately three times better than that of the conventional PAI [29].

Microsphere aided super-resolution imaging has also been found to be useful in photoacoustic imaging. A numerical simulation study with photonic nanojets generated by using microsphere confirmed the possibility of super-resolution PAI [25-27]. Although all these super-resolution PAI techniques overcame the diffraction limit, they are still limited in resolution and thus cannot investigate samples of size < 10 nm [30]. To overcome this limitation, very recently, super-resolution visible photoactivated atomic force microscopy (pAFM) was developed [28]. The pAFM uses a pulsed laser to generate PA and photothermal signals from a target and achieved ~8 nm lateral resolution with a cantilever tip. Another SRM imaging method is near-field scanning optical microscopy (NSOM) [31]. NSOM uses a tapered optical

Fig. 1. Overall simulation strategy from localized near-fields to photoacoustic (PA) simulation.

fiber tip with an aperture size of 50 nm or less [32]. Although NSOM achieves super resolution (less than 50 nm), it requires contrast agent since it only detects fluorescence signals from the samples. Applying nanoscale size of the aperture, like NSOM, can help to achieve a super-resolution PAI.

In this work, we present a three-dimensional simulation study of super-resolution photoacoustic microscopy (SR-PAM) using a plasmonic nanoaperture. To overcome the diffraction limit of light, we investigated and confirmed using RSoft Photonics CAD software that sub-10 nm near-field localization can be achieved by focusing femtosecond (fs) laser pulses under the nanoaperture [33, 34]. Using this generated near-field localization, we simulated PA images by implementing the three-dimensional SR-PAM system with the k-Wave simulation toolbox in MATLAB [35]. Through simulation, we measured the lateral resolution and resolving capability of the SR-PAM. Our simulation results demonstrated the feasibility of delineating nanoscale samples with the proposed SR-PAM system.

II. METHODS

A. Overall Simulation Strategy

Overall simulation strategy is depicted in Fig. 1. In the first step, a three-dimensional nanoaperture was modeled to generate a super tiny localized near-field as a light source using the RSoft CAD software. This near-field was imported to the k-Wave Toolbox in MATLAB for PA simulation. With all simulation parameters initialized, the PA simulation is performed to generate volumetric PA data with X and Y directional scanning. In Fig. 1, PA A-line and B-scan data meant one-dimensional and two-dimensional depth-resolved PA data, respectively. Details of data generation are described in the next section.

B. Near-fields Distribution Modeling Induced by Ultrashort Light Pulses

To induce near-field, we modeled an array of an equilaterally shaped triangular gold nanoaperture with a size of $L = 150$ nm and a period of $\Lambda = 500$ nm. A single nanoaperture of the array was shown in Fig. 2(a). The size and period were chosen to avoid plasmonic quadrupoles and near-field inter-aperture coupling, which otherwise would reduce field enhancement on

the gold apertures and ultimately degrade the resolution of PA imaging. The gold nanoapertures were of 15-nm height and modeled on a 30-nm thick ITO layer and a quartz substrate as shown in Fig. 2(b). To induce localized near-fields, we employed a fs Gaussian pulse expressed in Eq. (1):

$$(t) = \frac{1}{\sqrt{\pi}\tau} \exp\left[-\left(\frac{t-t_d}{\tau}\right)^2\right] \sin\left(\frac{2\pi c}{\lambda_0} t\right) \quad (1)$$

where, τ and t_d are the pulse width and delayed time, and c is the speed of light. The Gaussian pulse had an envelope function whose temporal FWHM (full width at half maximum) was 50 fs with a spectral linewidth of 50 nm, center wavelength of $\lambda_0 = 850$ nm and normal incidence on the nanoaperture arrays as shown in Fig. 2(c). Since the pulsed light was homogeneously incident on the entire nanoaperture structure, the spot size of the pulsed light was not defined. The incident pulsed light was polarized along y direction and the center wavelength selected for PA imaging exhibited resonant behavior of gold nanoaperture in the intensity spectra [34, 36, 37].

C. Simulation of Three-dimensional Photoacoustic Data Generation using k-Wave Toolbox in MATLAB

We used the k-Wave toolbox in MATLAB to simulate a super-resolution PA image with enhanced near-field localization obtained in the previous step as a light source. The k-Wave toolbox was used only to generate the PA data while image processing/reconstruction was performed using general functions of MATLAB. We carried out the PA simulation on a graphic processing unit (GPU) to reduce the compute times. The GPU was NVIDIA TitanX which has 3072 CUDA cores and 12-GB RAM.

1) Initialization

The simulation was initialized by generating three-dimensional geometry and importing two types of light source into MATLAB workspace [Fig. 3(a)]. The size of the grid was set to $120 \times 120 \times 1000$ voxels with 1 nm/voxel resolution. A perfectly matched layer was used to absorb outgoing waves from the target while the internal echoes from the surface were blocked. The imported light source was located 1 nm above the target [Fig. 3(b)]. A single element ultrasound transducer was

Fig. 2 Schematic diagram of the three-dimensional simulation geometry for (a-c) near-fields localization on a triangular shape gold nanoaperture using FDTD method. (a) Triangular shape gold nanoaperture on the ITO film with the aperture length L . (b) The nanoaperture placed on an ITO film. Glass used as a substrate. (c) Enhanced near-fields generated by induced broad pulse laser at vertices of the nanoaperture. The red arrow indicating the vertex of the nanoaperture where the localized near-field was induced. ITO film, Indium tin oxide film.

Fig. 3. Schematic diagram of (a) the three-dimensional simulation geometry for photoacoustic imaging using k-Wave toolbox in MATLAB. (b) A cross sectional view along the dashed box in panel a. Two types of numerical targets used for this simulation: (c) a single cuboid target, and (d) a four cuboids target. UST, ultrasound transducer.

placed at $0.97 \mu\text{m}$ away from the center of a target in the z -direction. The reason we shortened the distance between the transducer and the target was to reduce the overall simulation time and GPU memory usage. The time used for one A-line simulation with the distance of $0.97 \mu\text{m}$ was 30 minutes and the GPU memory usage was 4 Gigabytes. As the distance increases, the time and the memory usage increased significantly, so we set the values within the range that can be simulated. The center frequency of the transducer was set to 1 GHz with a 90% bandwidth. The medium was water and assumed to be acoustically homogenous and lossless. Since the free working distance of a 1 GHz transducer in the water is less than $80 \mu\text{m}$ [38], it can be assumed that there is no acoustic loss within the distance. The speed of sound was set to 1500 m/s. To simulate the stable PA wave propagation, the Courant-Friedrichs-Lewy

(CFL) condition [39] is considered carefully. CFL is defined in Eq. (2):

$$CFL \equiv c_0 \Delta t / \Delta x \quad (2)$$

where, c_0 is the speed of sound, Δx is size of the grid, and Δt is the time step size. It is well known that the CFL number is typically equal to or less than 1 [40]. With lower CFL number stability is better but simulation takes a longer time. In this simulation, we chose CFL number to be 0.8 as a good compromise between stability and simulation time. With this CFL number, the time step Δt from Eq. (2) is:

$$\Delta t = \frac{0.8 * \Delta x}{c_{max}} \quad (3)$$

where, c_{max} is the maximum speed of sound in the medium. c_{max} was set to 1500 m/s. Calculated time step was 0.53

Fig. 4. Numerical simulation of localized near-fields on gold nanoaperture using FDTD method. At a vertex of the red dashed box in (a), calculated near-fields projected on (b) X-Y plane and (c) X-Z plane. (d) Intensity profile with full width at half maximum (FWHM) measured along the light blue dashed line in panel c. Transposed near-fields at the vertex of the red dashed box in (e) projected on (f) X-Y plane and (g) X-Z plane. Along the light blue dashed line in panel g, (h) intensity profile with FWHM measured.

picosecond and the number of time steps was 3000. From the propagated PA waves, the waves were down sampled a hundred times to imitate an 18.86 GigaSamples/s data acquisition system.

Cuboid targets were used as the numerical phantoms for the simulation [Fig. 3(c) and (d)]. In all simulations, we used an initial pressure distribution in binary form as the phantoms. Since the targets were binary, there was no initial pressure rise outside the target and the initial pressure rise was 1 inside the target. A single cuboid target [Fig. 3(c)] with a dimension of 1-nm (along X-axis) $\times$ 10-nm (along Y-axis) $\times$ 2-nm (along Z-axis) was used as a resolution target. The next numerical target was made up of the four cuboids [Fig. 3(d)] each with the dimensions of 24-nm (along X-axis) $\times$ 3-nm (along Y-axis) $\times$ 2-nm (along the Z-axis). These four cuboids were placed 3-nm apart to study the ability of the system to resolve adjacent cuboids.

2) Time-domain photoacoustic wave simulation

For all simulation studies, X and Y direction scanning were performed to acquire volumetric PA data. At first, the target was moved along the X-axis with a step size of 1 nm to form a B-scan image while the light source and the detector were fixed. At each step, the PA A-line data were generated and stacked next to each other after applying 1% Gaussian random noise filter and the Hilbert transform. The total number of X steps for the single cuboid target was 21 and for the four cuboids target was 53. For both targets, the Y-axis step size and number of steps were the same and they were 1 nm and 14 times,

respectively. During the scanning, the distance between the target and the source was kept constant at 1 nm.

III. RESULTS AND DISCUSSIONS

A. Near-field Localization on Gold Nanoaperture

With known nanoaperture dimensions and incident light properties, we performed numerical calculation of near-fields using 3D finite difference time domain (FDTD) method for a uniform mesh size of $1 \times 1 \times 1 \text{ nm}^3$. A unit calculation volume was defined as one period in lateral direction, under periodic boundary conditions, and an axial length of 240 nm in z-axis with perfectly matched layer conditions. Localized near-fields were calculated for total of 120 fs with a time step of 0.002 fs. The spatial distribution of integrated near-fields over the total time was calculated under the assumption that a fs pulse had a broader bandwidth than detector bandwidth. Fig. 4(b) and (c) showed the close-up images of localized near-field distribution at a vertex of the red dashed box in Fig. 4(a) on the X-Y and X-Z plane respectively, which were 1 nm above the top surface of the gold nanoaperture. The near-field FWHM [Fig. 4(d)] was 3.3 nm when measured along the dashed line in Fig. 4(c). Transposed distribution of the near-field was depicted in Fig. 4(e-h). The FWHM of the transposed near-field [Fig. 4(h)] was calculated to be 2.2 nm along the dashed line in Fig. 4(g). The 3D calculation results were subsequently imported as light source for PA simulation.

Fig. 5. k-Wave simulation of photoacoustic maximum amplitude projection (PA MAP) image of a cuboid target with two type of near-field localization. (a) PA MAP image of the cuboid target excited by Fig. 4a. (b) B-scan image and (c) normalized line profile along the red dashed line in panel a. (d) PA MAP image of the cuboid target excited by Fig. 4d. (e) B-scan image and (f) normalized line profile along the red dashed line in panel d.

Fig. 6. k-Wave simulation of photoacoustic maximum amplitude projection (PA MAP) image of a four cuboids target excited by the near-field localization of Fig. 4a. (a) PA MAP image of the four cuboids target. Normalized line profile and full width at half maximum of (b) and (c) acquired along the red dashed line A and B in panel a.

B. Photoacoustic Microscopic Imaging with Nanoaperture Using k-Wave Simulation Toolbox in MATLAB

Three different PA simulations were performed in this study. First two simulations were performed to numerically measure the lateral resolution of the proposed SR-PAM. The final simulation was performed to verify the feasibility of the proposed SR-PAM as a nanoscale PA imaging method.

1) Lateral resolution measurement of a simulated super-resolution photoacoustic microscope

In the first simulation, the single cuboid target was excited by the enhanced near-field, as shown in Fig. 4(b), to measure the lateral resolution of the SR-PAM. The PA's maximum amplitude projection (MAP) image of the target was formed by projecting the acquired volumetric PA data onto the X-Y surface plane [Fig. 5(a)]. B-scan image [Fig. 5(b)] and the normalized profile [Fig. 5(c)] were acquired along the red dashed line of Fig. 5(a). The normalized profile was fitted by a Gaussian distribution function. The FWHM of the fitted

Gaussian function was 3.6 nm and this FWHM was considered as the lateral resolution of the SR-PAM system with X-direction scanning in Fig. 2(a). Non-transposed near-field localization was symmetrical to the X-axis but was asymmetrical to the Y-axis, thus the PA MAP image [Fig. 5(a)] was also symmetric to the X-axis and asymmetric to the Y-axis.

In the second simulation, the single cuboid target was again excited but using transposed enhanced near-field, as shown in Fig. 4(e), to measure the lateral resolution. The PA MAP image and B-scan image of the single cuboid target were shown in Fig. 5(d) and (e) and it showed the characteristics of the transposed near-field. The normalized profile along the red dashed line of Fig. 5(d) was fitted with a Gaussian function [Fig. 5(f)] and from the Gaussian distribution, the lateral resolution of the SR-PAM was quantified to be 2.3 nm. This resolution was obtained with scanning along the Y-direction in Fig. 2(a).

Above two simulation results show that the shape of PA MAP image and the lateral resolutions are well-matched with the characteristics of the light sources.

2) Feasibility of a simulated super-resolution photoacoustic microscope as a nanoscale image method

In this third simulation, the numerical target with the four cuboids was imaged using the enhanced near-field, shown in Fig. 4(a), to study the ability of system to resolve closely spaced objects. The PA MAP image in Fig. 6(a) showed that the cuboids placed 3 nm apart were clearly resolved. To quantify the resolving capability of the SR-PAM, two normalized lateral line profiles along dashed red lines A and B of Fig. 6(a) were acquired and fitted by a Gaussian distribution as shown in Fig. 6(b) and (c). The FWHMs from the dashed red lines of A and B Gaussian fittings were 1.4 nm and 2.0 nm, respectively. The two profiles, as stated previously in the previous paragraph, were not only symmetric and asymmetric with respect to the X-axis and Y-axis, respectively, but also the FWHM of the two profiles were different. These results confirm that the generated PA signal and the PA MAP image clearly reflect the characteristics of the near-field localization.

The numerical simulation results of the SR-PAM system presented in this work show the potential of the system to refine the lateral resolution beyond the diffraction limit. To our knowledge, our approach, which uses the light source from the enhanced near-field localization generated by a plasmonic metal triangular shaped nanoaperture, is the first attempt to acquire PA signals. Using this system with appropriate laser wavelength, we expect to be able to observe nanoscale biological samples such as viruses, proteins, and molecules without any agent. Especially, using near-infrared (NIR) pulse laser source, this system can play a major role in research for finding or developing NIR fluorescence proteins with chromophores in cells because NIR fluorescence proteins have been actively studied since they are suitable for long-term studies [41]. One drawback with our approach is the limited penetration depth compared to the conventional OR-PAM. Since the focal length of the light source is limited to < 10 nm, this system can only work at the near-field. Therefore, extending the limited penetration depth of the SR-PAM system for larger depth application will be a challenge to overcome.

Another challenge for applying our proposed system to practice is that the distance between the nanoaperture and the sample should be very close. However, we can apply the similar approaches which conventional AFM or NSOM with short pulse laser use. The probe tip (like our nanoaperture) in AFM and NSOM can be very close to the sample at ~ nm distance during the whole scanning by using shear-force-based distance detection in combination with an electronic feedback system controlling the piezoelectric scan stage. Using these techniques, they successfully obtained cell and surface images in the near-field region [42]. We will also be able to develop our proposed system in a similar direction to adjust the distance between the sample and the nanoaperture to ~ nm. Additional challenges in implementing our system are: (1) possibility of reduced spatial resolution due to deformation of the nanoaperture structure from photothermal annealing [43], and (2) possibility of reduced performance in near-fields due to difficulty in fabrication of uniform and reproducible nanoaperture [34]. In the near future, we expect the short time of the near-field localization and novel lithographic techniques will overcome these challenges [44]. As a next step, we will implement the proposed SR-PAM system and experiment with single cell membrane structure. Especially, membrane proteins perform a variety of functions vital to the survival of organisms[45], therefore it would be valuable research.

IV. CONCLUSION

In this study, we numerically achieved sub-10 nm lateral resolution in SR-PAM imaging. This system overcame the limitations of existing system for super resolution microscopy by not using contrast agents, like SRFM, and not using vacuum, like EM, while achieving sub-10 nm lateral resolution. Our near-field localization simulation results showed that the near-field of sub-10 nm spot size was achieved with equilaterally shaped triangular periodic gold nanopost aperture with a size of $L = 150$ nm and a period of $\Lambda = 500$ nm. Consequently, we could achieve lateral resolution of 2.3 nm which is about 35x improvements over other SR-PAM systems and can resolve objects that are 3 nm apart. Therefore, we can conclude that the simulation of the SR-PAM using the near-field localization can be a potential tool to delineate nanoscale biomedical applications [46-48]. This SR-PAM method is not only simple and cost effective, it is also beneficial to biological samples since it usually requires no contrast agents and special conditions, like vacuum. In the near future, we plan to fabricate a triangular-shaped plasmonic nanoaperture and obtain PA signals from nanoscale biological samples to experimentally establish the results obtained in this simulation study.

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