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Photonic nanojet engineering to achieve super-resolution in photo-acoustic microscopy – a simulation study

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ABSTRACT

Label-free photoacoustic microscopy (PAM) with nanometric resolution is important to study cellular and sub-cellular structures, microcirculation systems, micro-vascularization, and tumor angiogenesis etc. But, the lateral resolution of a conventional microscopy is limited by optical diffraction. The photonic nanojet generated by silica microspheres can break this diffraction limit. Single silica sphere can provide narrow photonic jet, however its short length and short working distance limits its applications to surface imaging. It is possible to increase the length of the photonic nanojet and its working distance by optimizing the sphere design and its optical properties. In this work, we will present various sphere designs to achieve ultra-long and long-working distance photonic nanojets for far-field imaging. The nanojets thus generated will be used to demonstrate super-resolution photo-acoustic imaging using k-wave simulations. The study will provide new opportunities for many biomedical imaging applications that require finer resolution.

Keyword: Photoacoustic nanoscopy, Super-resolution, Sub-diffraction, Photonic nanojet, Microsphere

1. INTRODUCTION

Photoacoustic imaging (PAI) is an *in vivo* 3D imaging modality which has demanding applications in both preclinical and clinical practices.[1-10] The three major embodiments of PAI are microscopy, endoscopy, and computed tomography. Optical resolution photoacoustic microscopy (ORPAM) is a high-resolution version of PAI modality. ORPAM has several advantages over pure optical methods:[11] (i) It makes use of the weak acoustic scattering to break the optical diffusion limit, (ii) both imaging resolution and imaging depth are scalable in ORPAM,[6, 12-14] (iii) It provides optical absorption contrast unlike confocal fluorescence microscopy (CFM, it provides fluorescence contrast), and optical coherence tomography,[15] and coherent Raman microscopy[16-19] (OCT, and CRM provide scattering contrast), (iv) it images optical absorption with 100% relative sensitivity, and its sensitivity is 2 orders of magnitude greater than those of confocal microscopy and OCT, (v) it allows *in vivo* imaging of functional, structural, and molecular information [7, 9, 20, 21].

In ORPAM, both the laser excitation and acoustic detection are focused in a confocal geometry. In ORPAM, the optical focus is finer, and hence it decides the imaging resolution of the system. The size of optical spot limits the imaging resolution in optical microscopy. The diffraction-limited size of optical focus is $DL = 0.51\lambda/NA$, here λ is the optical wavelength and NA is the numerical aperture of the optical objective. Imaging resolution can be enhanced by using short wavelengths and high-NA objectives. ORPAM with resolution $\sim 5\ \mu\text{m}$ using $NA = 0.1$ and $\lambda = 590\ \text{nm}$, [22] $2\ \mu\text{m}$ using $NA = 0.47$ and $\lambda = 532\ \text{nm}$, [23] and $0.22\ \mu\text{m}$ using $NA = 1.23$ and $\lambda = 532\ \text{nm}$ [24] were successfully demonstrated on biological samples. ORPAM could be an important tool for cellular and sub cellular imaging. Photoacoustic imaging of cell nuclei by excitation of DNA and RNA was demonstrated using UV source.[25] Although, using short wavelength and high NA will improve the resolution to some extent, however, the resolution of these systems is limited by the optical diffraction. Enhancing imaging resolution beyond the diffraction limit, to image sub-cellular structures, mitochondria, hemoglobin nanoclusters, nanoparticles etc., is necessary. Some methods have been introduced to break the diffraction limit in photoacoustic imaging. A nonlinear super-resolution photoacoustic microscopy[26] was demonstrated for visualizing 10-nm gold nanoparticles in graphene, and hemoglobin nanoclusters in

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live erythrocytes. A photoacoustic nanoscopy (PAN) by combining optical saturation technique and polynomial fitting was demonstrated for imaging mitochondria in NIH 3T3 fibroblasts.[27] A sub-diffraction limited lateral resolution was demonstrated in photo-imprint photoacoustic microscope (PI-PAM) using double exposure.

Microspheres were used to break the diffraction limit in optical microscopy. This approach is simpler, low-cost, allows far-field imaging, and no modifications in the conventional system is necessary. 50-nm optical imaging was achieved in white light microscopy using SiO₂ microspheres.[28] When microsphere is illuminated by a light beam, a narrow (sub-wavelength waist), intense, non-evanescent, and low-diverging jet like optical beam is generated on the shadow side of the microsphere. Such nanojet can break the diffraction limit in optical microscopy by greatly reducing the size of the excitation volume, and the signal generated in the excitation volume can still be collected in the far-field. The super-resolution is due to photonic nanojet generated by the microsphere. Super-resolution imaging using microsphere was successfully demonstrated many optical modalities, for example, white light microscopy, coherent anti-stokes Raman scattering microscopy,[29] fluorescence microscopy,[30] Raman microscopy,[31] etc.

Super-resolution imaging in ORPAM using microsphere was demonstrated through simulations.[32] These studies showed that: (i) It is possible to generation of PNJ by a round microsphere under optical focusing in water, (ii) waist of PNJ depends on several parameters such as incident wavelength, numerical aperture of the objective, refractive index of the microsphere, and diameter of the sphere. Later another simulation study showed that it is possible to control the important parameters of PNJ such as length, width, peak intensity, working distance etc., by changing the sphere design.[33] The study also compared PNJ parameters for various sphere designs such as round microsphere (RM), truncated microsphere (TM),[34] microsphere with concentric rings (MCR),[35] multi-layer microsphere (MLM),[36, 37] and truncated multi-layer microsphere (TMLM). TMLM design could generate ultra-long PNJs. In this work, though k-wave simulations we will demonstrate the use of ultra-long photonic nanojets for imaging deep objects. The photoacoustic images of a 60-micron deep object generated under Gaussian beam excitation, short-PNJ excitation, and ultra-long PNJ excitation were compared.

2. METHODS

COMSOL Multiphysics software for generating fluence maps and k-wave tool box for photoacoustic image were used in this work. For both COMSOL and k-wave simulations, a desktop with 64-bit windows 10 operating system, 3.7 GHz processor, and 16 GB RAM was used.

2.1 Generating fluence maps

A detailed description of COMSOL simulation geometry used for generating the focused Gaussian beam and photonic nanojets can be found in Ref.[33] A 2D FEM simulation geometry based on electromagnetic theory was designed. A focused beam was simulated by a Gaussian beam with a full width at half maximum (FWHM) beam waist diameter of $\lambda/2NA$ or an $(1/e^2)$ radius of $\lambda/2.35NA$ in intensity, where λ - incident wavelength, and NA - numerical aperture. The measured FWHM waist diameter of the focusing Gaussian beam obtained with $\lambda = 800$ nm and NA = 0.1 was ~ 3958 nm. A 5 μm -diameter sphere was placed inside the Gaussian focus. Keeping the diameter same (5 μm), different spheres were designed and tested for PNJ properties: (i) Round microsphere (RM): This design was widely used for generating photonic nanojets. (ii) Truncated microsphere (TM): previous simulation studies showed that PNJ parameters were significantly influenced by truncating the round microsphere (RM). (iii) Microsphere with concentric rings (MCR): Both experimental and simulation studies showed that round microsphere with concentric rings on its shadow side surface could modulate the nanojet. (iv) Multi-layer microsphere (MLM): It was shown that using two-layer microsphere configuration it is possible to enhance the length of the nanojet significantly. PNJ with FWHM length of 22λ (~ 14 μm), and PNJ with decay length of 100λ (~ 40 μm) were achieved using two-layer microsphere. (v) Truncated Multi-Layer Microsphere (TMLM): The two-layer spheres were truncated to study the effect of its cutting thickness on the PNJ parameters. We found that truncating the MLM design significantly enhanced the effective length, and focal length compared to all other designs. The three different spheres used for MLM design were truncated by thickness 0.25D, 0.5D, and 0.75D (D- diameter of the sphere). A table summarizing the properties of PNJs generated from these five different designs can be found in Ref.[33]

2.2 Generating photoacoustic B-scan images

A numerical phantom as shown in Fig. 1 was used for the study. The phantom consists of square targets with side length $1\ \mu\text{m}$, separated by a distance $1.5\ \mu\text{m}$. Seven such targets were placed with $10\ \mu\text{m}$ gap between each pair of targets to create an object with depth of $60\ \mu\text{m}$. The targets were embedded inside a non-absorbing medium. To generate photoacoustic B-scan image, the light illumination was fixed at the center of the scanning area and object was scanned across the light focus. The object was moved $5000\ \text{nm}$ across the light field with a step size of $50\ \text{nm}$. A point detector (UST) was used to collect the A-lines. The center frequency and the fractional bandwidth of the detector were $500\ \text{MHz}$ or $1000\ \text{MHz}$ and 80% , respectively. Such high frequency USTs were used previously for high-resolution photoacoustic imaging. To collect PA signal over a length of $100\ \mu\text{m}$, a total of 1340 time steps with each step $50\ \text{ps}$ were used. The A-line PA data was stacked after taking Hilbert transform (HT) to form a B-scan image. The medium, in which targets were embedded, was considered acoustically homogeneous and there was no absorption or dispersion of sound. The simulations assumed a sound speed of $1500\ \text{m/s}$.

Fig. 1 Schematic of the simulation geometry for photoacoustic microscopic imaging using k-wave in MATLAB. Here UST-ultrasound transducer (point detector) with central frequency $500\ \text{MHz}$ or $1000\ \text{MHz}$.

3. SIMULATION RESULTS

Properties of photonic nanojets from various sphere designs were discussed in Ref.[33] The Gaussian focus generated with $\text{NA} = 0.1$ and $\lambda = 800\ \text{nm}$ is shown in Fig. 2(a), and its FWHM waist diameter measured to be $\sim 3958\ \text{nm}$. Single round microsphere can generate strong, narrow, and short nanojet. The fluence map generated when a Gaussian beam (with waist size $3958\ \text{nm}$) passed through a round microsphere with $5\ \mu\text{m}$ and refractive index 1.45 (SiO_2 sphere) is shown in Fig. 2(b). The truncated multi-layer microsphere (TMLM) design with cutting thickness $0.75D$ generated ultra-long PNJs. The ultra-long nanojet generated with TMLM design (with refractive index of the sphere- 1.45 , refractive index of the outer layer- 1.51 , and $0.75D$ cutting thickness) is shown in Fig. 2(c). The fluence maps for Gaussian focus and photonic nanojets shown in Fig. 2 were then used for illuminating the object (Fig. 1) in k-wave simulations in MATLAB to generate photoacoustic B-scan image.

The numerical phantom described in Fig. 1 was sequentially scanned by the fluence maps shown in Figs. 2(a-c). Figures 3(a-f) show the 2D B-scan ORPAM images of the target excited with the Gaussian focused beam with FWHM $3958\ \text{nm}$ [Fig. 2(a)] and excited with photonic nanojets with FWHM $809\ \text{nm}$ [Fig. 2(b)], $1459\ \text{nm}$ [Fig. 2(c)],

respectively. The images shown in Figs. 3(a-c) were acquired using 500 MHz UST. From the ORPAM image shown in Fig. 3(a), it is clear that the Gaussian focus could provide information of all the targets at depth but it could not resolve the gap between the targets. It is because the Gaussian spot size (3958 nm) is larger than the separation between the targets (1500 nm). Figure 2(b) shows that the narrow PNJ (809 nm) generated by round microsphere could resolve the gap (1500 nm) between the targets but it could not image the targets beyond 10 micron-depth. It is because the nanojet is too short for imaging deep targets. From Fig. 2(c), it is clear that the ultra-long PNJ ($\sim 67 \mu\text{m}$) generated using TMLM design could image all the targets and also could resolve the gap (1500 nm) between the targets. Here, the gap between the targets is resolved. Similar simulations were repeated by using 1000 MHz UST and the corresponding ORPAM images are shown in Figs. 3(d-f). From these results, it is clear that the imaging depth of super-resolution ORPAM can be enhanced with the help of proper microsphere.

Fig. 2 COMSOL generated fluence maps: (a) focusing Gaussian beam in water with $\lambda = 800 \text{ nm}$, $\text{NA} = 0.1$, photonic nanojet generated when the Gaussian beam (Fig.2a) focused on (b) round microsphere (RM) with $D = 5 \mu\text{m}$, $n_1 = 1.45$, (c) Truncated Two Layer Microsphere (TMLM) with $D = 5 \mu\text{m}$, $n_1 = 1.45$, $n_2 = 1.51$.

4. CONCLUSIONS AND DISCUSSIONS

Photonic nanojet generated by microsphere has already proven its potential in white light microscopy by imaging 50 nm-features and in fluorescence microscopy by imaging subcellular structures like centrioles, mitochondria and chromosomes, and also in Raman microscopy by imaging sub-diffraction features. The simulation studies in these papers[32, 33] promise that by using proper microsphere, conventional microscopy could be transformed in to nanoscopy at virtually no extra cost. The nanojet approach can find applications for imaging cellular structures, microvasculature, and microcirculation systems, etc. It may be used to image biological entities, such as bacteria, large virus particles, labeled nucleic acids, molecules etc. In this paper, we discussed different microsphere designs that could

generate ultra-long PNJs. We observed that round microsphere can generate strong, and ultra-narrow nanojet, it can be used for high-resolution imaging of surface structures. By simple truncating the round microsphere one can achieve elongated photonic nanojet for imaging deep objects. ORPAM images of a deep targets obtained with high-frequency USTs with central frequency 500 MHz and 1000 MHz are shown in this work. The microsphere approach is simple, cost-effective, and just by using different microspheres one can change the resolution of the imaging system.

Fig. 3 k-wave generated photoacoustic images: (a-c) 500 MHz UST, (d-f) 1000 MHz UST. Images (a,d) are obtained with Gaussian focus [Fig. 2(a)], images (b,e) are obtained with short photonic nanojet [Fig. 2(b)], and images (c,f) are obtained with ultra-long photonic nanojet [Fig. 2(c)] excitation.

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