

Effects of Illumination Angle and Different Light Sources on Acousto-Optic-based Sensing of Focused Ultrasound

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Abstract. The acousto-optic (AO) effect, characterized by the deflection and modulation of photons by ultrasound, is currently being explored to a certain degree. AO could bring together advantages of optics and acoustics particularly addressing the challenges of spatially accurate deep tissue optical sensing that arise from light scattering. Moreover, AO-based monitoring technique could offer valuable insights into the effects of focused ultrasound (FUS) in its therapeutic applications. This paper investigates different light sources (coherents and noncoherent) for use in AO. In particular, we compare tagging efficiency detected in the context of AO signal amplitude. Additionally, the impact of adjusting the angle of the light source to optimize light penetration into the focal area is examined. Experiments were performed in both transmission and reflection modes utilizing AO phantom models, with a primary emphasis on reflection mode. The findings reveal that coherent light sources produce larger but has greater standard deviation in AO signal, while light emitting diodes (LED) yield comparatively stable signals. Additionally, results from reflection mode experiments indicate an enhancement of AO signals by as much as 40% when the angle of coherent sources is adjusted from perpendicular to 45° within a phantom that simulates the optical characteristics of the average human brain. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: focused ultrasound; tagging efficiency optimization; acousto optic sensing; laser diode and LEDs; coherence & non-coherence light.

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1 Introduction

Acousto-optic sensing (AOS) is a hybrid technique aiming to combine the deep penetration and high spatial resolution of ultrasound with optical sensing to overcome optical scattering limitations while maintaining the original optical contrast [1, 2]. The AO phenomenon was first demonstrated experimentally already in 1930s [3, 4], however, Marks et. al made its biomedical application possible in 1993 [5]. By utilizing non-invasive FUS transducers to modulate a specific area within a medium

by acoustic waves and by illuminating that area with light, the AO effect partially modulates the light by frequency shifting. The key to achieving optimal modulating or tagging efficiency lies in ensuring that photons pass through the focal area efficiently. The separation of these modulated photons enables acoustic resolution in acoustic focus area, facilitating the retrieval of local optical properties within the focus area [1].

AOS exhibits sensitivity to both optical absorption and scattering, offering optical contrast that can dynamically capture changes in tissue properties due to

metabolic and structural alterations. When light interacts with the focal area of ultrasound wave, optical diffraction and frequency shifting occur, enabling a light detector to capture a fraction of this frequency-shifted light as it propagates out of the tissue [2, 4, 6]. The choice of ultrasonic frequencies in AOS is critical, necessitating a balance between achieving deep tissue penetration (with order of cm) and providing spatial accuracy within the mm range. However, deep tissue sensing poses challenges in detecting and separating tagged photons from untagged background photons, leading to a lower signal-to-noise ratio (SNR) in the AOS [7]. In this study, we explored both transmission and reflectance modes, but placing particular emphasis on the practicality of reflectance, similar to the settings commonly employed in human brain hemodynamic measurement by functional near-infrared spectroscopy (fNIRS), where both light source and detector are placed on the scalp commonly at 3 cm source-detector distance. Latter approach overcomes the limitations of transmittance mode, presenting a more practical solution for instance in brain monitoring applications.

In Ref. [8], the influence of varying source angles on tagging efficiency was investigated through simulations. Building on this, the present research expands the investigation experimentally by introducing additional

parameters, such as FUS frequency and pressure, source-detector distances, light source angles, and the use of both coherent and non-coherent light sources. The aim is to quantify FUS-modulated light in terms of AO signal intensity under these conditions.

2 Methods

This section first presents the simulation of FUS wave propagation, followed by MC modeling to simulate the FUS-induced modulation of light. Subsequently, the experimental procedures are described in detail.

2.1 Simulations

The acoustic waves generated by FUS cause pressure changes in the medium which were simulated using the open-source MATLAB toolbox *k-wave* [9], to determine the position, size, and shape of the pressure distribution as a function of the frequencies. Using *k-WaveArray* class model [10], transducers can be defined in any shape and placed inside the computational grid anywhere without being constrained by grid points. The acoustic properties of the simulation medium are detailed in Table 1.

Table 1 Tissues' acoustic and transducer parameters.

Acoustic Parameters [11, 12]					Transducer Parameters		
	V_a	ρ	α_0	y	Frequency (MHz)	ROC (mm)	$\emptyset$ (mm)
Water	1483	999.5	0.0022	2	0.5	57.5	60
Average Brain	1552	1046	0.21	1.18	1	18.5	20
V_a = Ultrasound Velocity ($\frac{m}{s}$)					$\emptyset$ = Diameter		
ρ = density ($\frac{kg}{m^3}$)					ROC = Radius of Curvature		
α_0 = Power-Law Prefactor (dB MHz ^{-y} cm ⁻¹)							
y = Power Law Exponent							

Table 2 FUS and MC simulation parameters.

Input parameter	Value			
Focused area dimension (mm)	1 MHz		0.5 MHz	
	Length	Width	Length	Width
	9	1.3	15	2.5
SDD (mm)	30, 35			
Detector fiber diameter (mm)	0.2			
Light source angle (°) with respect to the Z-axis (depth) and X-axis (*SDD direction)	0° – perpendicular in the experiments, 20°, 45°			
Optical properties of the average brain [17, 18] (850-nm)	μ_a (mm ⁻¹)	μ_s (mm ⁻¹)	g	n
	0.037	9.5	0.82	1.38

*SDD = Source-Detector Distance

The acoustic absorption coefficient was computed according to the frequency-power law, $\alpha = \alpha_0 \omega^y$. The focal pressure distribution and focus size were examined for two transducers, with the brain modeled as an average volume submerged in water as the surrounding medium. The dimensions of the 3D simulation domain were $60 \times 60 \times 80 \text{ mm}^3$, with a grid spacing of $140 \mu\text{m}$.

MC Multi-Layer [13, 14] modeling was implemented and modified in MATLAB to model FUS-modulated light inside biotissue and calculate the tagging efficiency. The medium was represented as a cuboidal layer with dimensions of $75 \times 75 \times 25 \text{ mm}^3$, and simulations were executed using the parameters outlined in Table 2. The focal area with adequate approximation, as stated in Table 2, was modeled in MC simulation as an ellipse with its length and width of focal area considered as major and minor radius, respectively. The simulation study focused on quantifying the number of photons traversing the focal area and reaching the detector. Among the photons interacting with the focal region – either through scattering or direct passage – 70% were classified as tagged photons [15]. The intensity of the photons was determined by adding the weights of all photons that reached the detector and dividing the total weight by the detector area. Simulations were performed separately and in parallel for each of the US frequencies, source angles, and source-detector distances. Upon reaching the detector, the labeled photons were recorded as modulated signals, whereas the remaining photons were classified as unmodulated signals [14]. By varying the illumination angles and source-detector distances (SDD), the optimal configuration for maximizing the tagging efficiency was established. The Monte Carlo simulation, which involved 2 billion photons and spanned 48 h, detected only a few thousand photons. This outcome is attributable to the simulation's use of a 1 mm diameter detector, reflecting the specified of the experimental fiber detector. The MC was verified by MCX results on source-detector separation versus diffuse-reflectance plot [16–18].

2.3 Measurements Setup

Polyvinyl chloride-plastisol and Titanium Dioxide Nanopowder (TiO₂-5430MR) were used as the base materials for the construction of the tissue phantoms in this study. These materials were mixed in a ratio of 1 mL to 3 mg using an electromagnetic mixer and heated for twenty minutes until the oven temperature reached 200 °C. Afterward, the magnetic stirrer was removed from the glass cup, the oven was turned off, and the mixture was allowed to cool naturally to room temperature to solidify. The optical and acoustic properties of this ultrasoft phantom closely resemble those of biological tissue, with an acoustic speed of $1.4 \text{ mm}/\mu\text{s}$ and a density of 1 g/mL , which are comparable to those of soft tissues (approximately $1.5 \text{ mm}/\mu\text{s}$ and 1 g/mL). Its optical absorption and reduced scattering coefficients are approximately 0.02 mm^{-1} and 1 mm^{-1} , respectively, which align well with the characteristics of brain tissue in the near-infrared wavelength range. Therefore, this phantom

can effectively serve as a model for brain tissue in optical and acoustic studies of the brain [19, 20]. Transmission mode measurements were conducted on a cuboidal phantom of $3 \text{ cm} \times 3 \text{ cm} \times 7 \text{ mm}$, whereas reflection mode measurements were performed on a cylindrical phantom standing at 4 cm height with a radius of 5 cm.

The experimental setup for reflection mode is presented in Fig. 1(a) wherein the fibers and transducer are aligned on the XY plane, in which the FUS wave propagation is aligned with the Z-axis. Two commercially available transducers (HIFU-Siansonic) were employed in the study: the TQ60-0560, which operates at a frequency of 0.5 MHz, featuring a curvature radius of 60 mm and an active diameter of 57.5 mm, and the TQ20-1020, operating at 1 MHz, with a curvature radius of 20 mm and an active diameter of 18.5 mm. The pressure at focal area was measured using a hydrophone (NH1000, Precision Acoustics) within a water bath under controlled conditions. The lock-in amplifier (MFLI-500 kHz/5 MHz) generates an internal sinusoidal driving signal, precisely tuned to the center frequencies of the transducers (0.5260 MHz and 1.0299 MHz). This signal is amplified by the power amplifier (240L-RF10KHZ-12MHZ, 40 W) to drive the transducer. The internal sinusoidal driving signal is used as the reference throughout the lock-in detection process to detect modulated light signals. To achieve acoustic impedance matching, the phantom was positioned within a container filled with distilled water. The transducer was submerged and placed above the phantom to ensure that the focal area was located at a depth of 18 mm, which is critical for utilizing AO techniques in brain monitoring, where penetration depths on the order of centimeters are essential. The source fiber and detection fiber were located at varying distances of 30, and 35 mm on the phantom surface. This configuration was chosen to replicate penetration depth observed in the human brain, as supported by relevant literature [18, 21]. Three different light sources were employed, each varying at three angles – perpendicular, 20°, and 45° – with respect to the X-axis and Z-axis. A 100 mW long coherent (CrystaLaser, DL852-100-SO) operating at $\lambda = 852 \text{ nm}$, a 150 mW laser diode (L852P150-Thorlabs) operating at $\lambda = 852 \text{ nm}$, and a 400 mW high-power LED (ARE6-88D1-0GH00) provided by Broadcom Limited operating at $\lambda = 855 \text{ nm}$. An adjustable attenuator and a power meter were employed to maintain a consistent optical power of 40 mW transmitted to the sample surface from the light sources through a multimode fiber cable (M76L02-Ø400). Light scattered within the sample passed through the focal area and was partially modulated by FUS. A portion of the modulated light, along with the diffuse reflectance comprising both tagged and untagged components, was transmitted through the detection fiber (M76L02-Ø400) to a fast photoreceiver (FEMTO-OE-300), which functioned as both a detector and an amplifier. The photoreceiver's output was bifurcated, with one pathway interfacing with a data acquisition (DAQ) card to record unmodulated light. Concurrently, the other pathway was routed to a lock-in amplifier to isolate the modulated light, thereby obtaining the AO signal amplitude. This extracted signal was subsequently transmitted to a computer for comprehensive analysis.

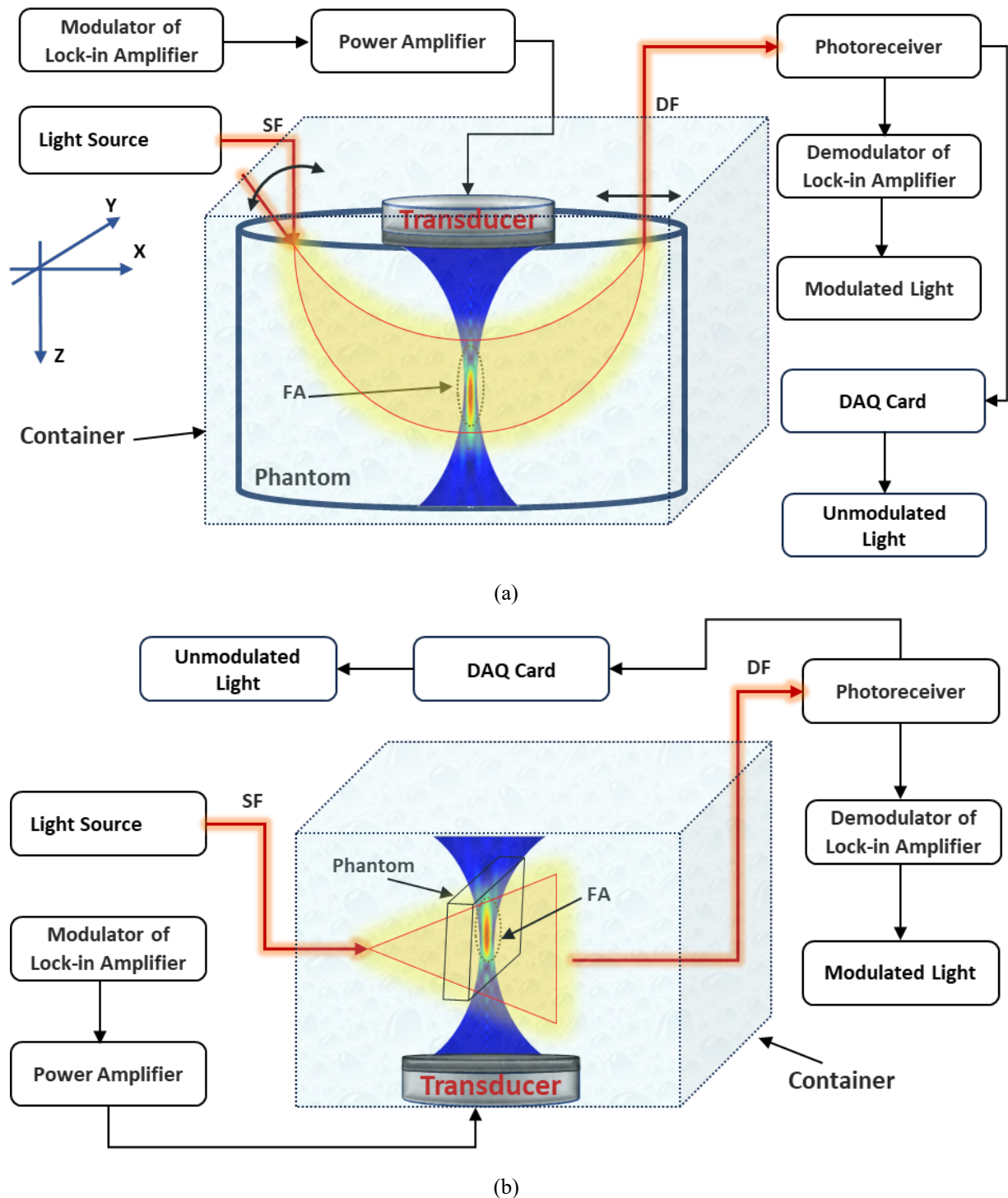


Fig. 1 Experimental configurations for detecting FUS-modulated light. The optical signal is represented by red arrows, while black arrows indicate the electrical signal. Abbreviations used are as follows: SF (source fiber), DF (detector fiber), and FA (focal area). Panel (a) illustrates the reflection mode configuration, and panel (b) depicts the transmission mode configuration.

Fig. 1(b) illustrates the transmittance setup, utilizing the same light sources and transducers as in the reflection mode, but arranged in a different configuration. The light source intensity was limited to 18 mW due to detector saturation. The transducer was fully immersed and positioned at the bottom of a cuvette measuring $23 \times 16 \times 13 \text{ cm}^3$, which was filled with distilled water. A cuboidal phantom $3 \times 3 \text{ cm}^2$ with 7 mm thickness in

the optical transmission direction fixes inside the cuvette, ensuring the focused area aligned with the center of the phantom. The source fiber and photoreceiver fibers were axially aligned with the focal area. The rest of the detection process was the same as in the reflection mode. All measurement data presented is based on a four-minute recording, repeated three times for consistency.

3 Results

This section starts with the simulation results, followed by the presentation of the experimental outcomes, commencing with the transmission configuration and subsequently addressing the reflection mode results.

3.1 Results of Simulation

The pressure distribution generated by FUS transducers is shown in Fig. 2. The pressure amplitude exhibited a range of 0.3 to 0.5 MPa, which remains well within the permissible limits established by the FDA for medical applications [16]. At 3 dB amplitude, the focal length and width are 9 mm and 1.3 mm for 1 MHz, and 15 mm and 2.5 mm for 0.5 MHz, respectively.

Fig. 3 shows map illustrating the trajectory of photons and the history of their intensity that clearly indicates a decrease in the number of photons reaching detectors as

the distance between the source and detector increases. However, detected photons can penetrate deeper, up to about 18 mm.

Initial simulation results were presented in Ref. [8]. Fig. 4 illustrates that the tagging efficiency decreased as the FUS frequency increased from 0.5 MHz to 1 MHz; however, the number of untagged photons increased. This phenomenon occurs because, as the frequency rises, the focal area becomes smaller, limiting the number of photons that can interact with the focused region. In comparison to 1 MHz shown in Fig. 4(a) and 4(b), in 0.5 MHz, a greater number of tagged photons reached detectors D1 and D2 at the 0.5 MHz frequency, resulting in a larger accumulation of weights, as illustrated in Fig. 4(c) and 4(d). Additionally, lower FUS frequency generates larger particle displacements for the same ultrasound pressure, leading to higher tagging efficiency [15].

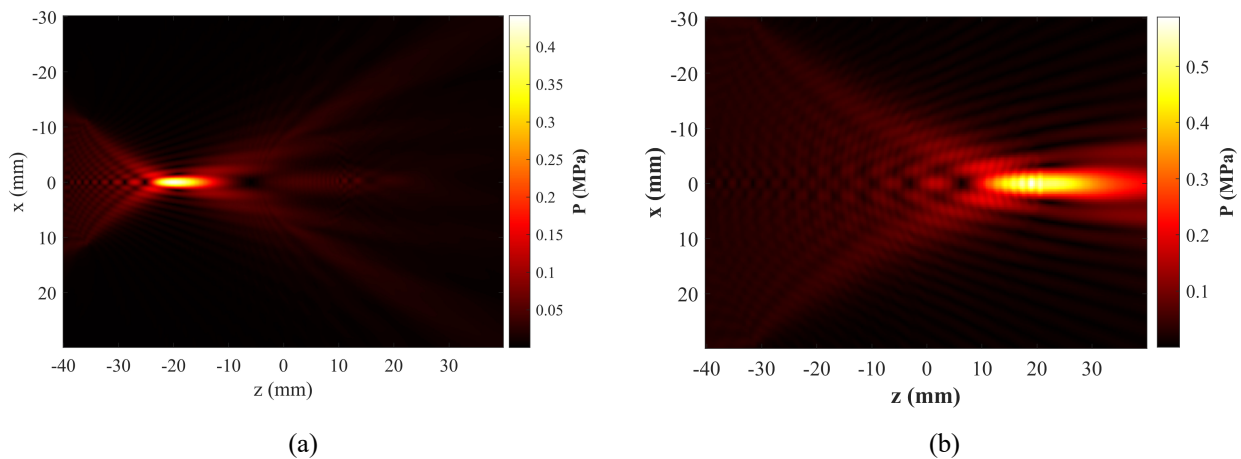


Fig. 2 Magnitude pressure calculated for FUS propagation in the medium and pressure distribution in the focal region at two frequencies: (a) 1 MHz and (b) 0.5 MHz. The FUS source is positioned at coordinates $(x, y, z) = (0, 0, 0.0399)$ and is directed along the +Z-axis, producing a peak pressure amplitude of approximately 0.5 MPa. The acoustic properties of the medium are specified in Table 1, with a grid spacing of 140 μm utilized in the simulation.

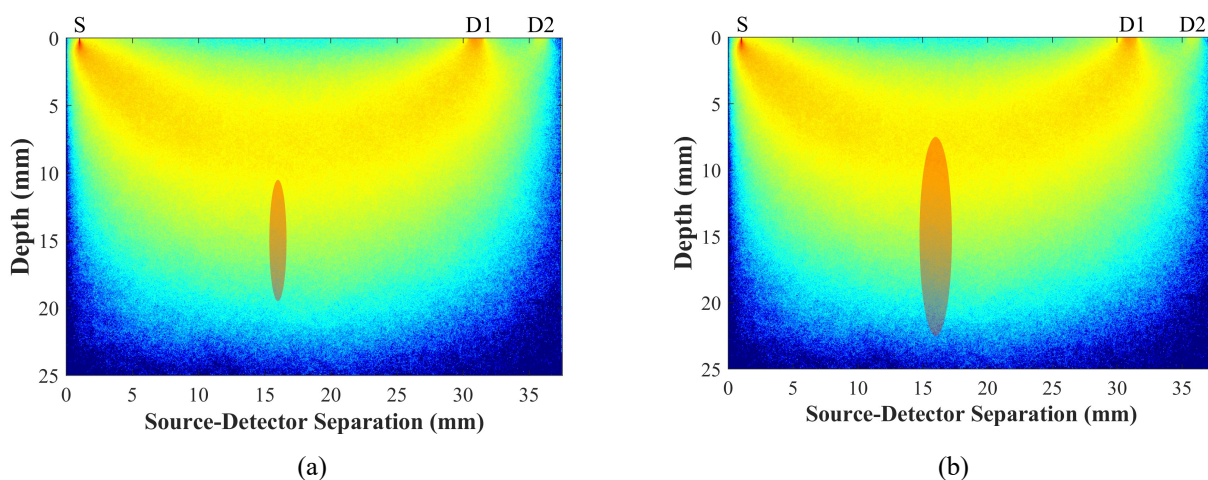


Fig. 3 Photon trajectory mappings (logarithmic color maps) at 850 nm wavelength are depicted for two source-detector distances: S-D1: 30 mm, and S-D2: 35 mm, relative to the focus area generated by FUS. (a) At frequency of 1 MHz, while panel (b) displays them at 0.5 MHz.

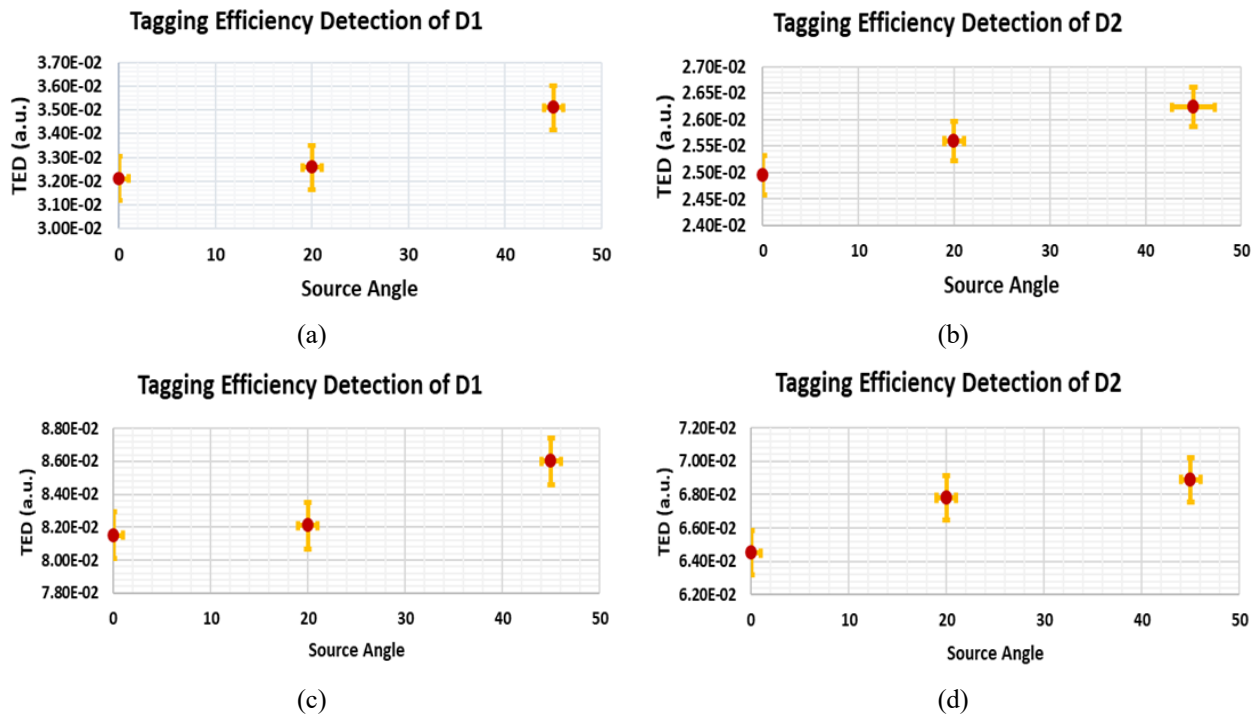


Fig. 4 Illustration of the detected tagging efficiency for three distinct angles (perpendicular, 20°, and 45°) and two SDDs. Panels (a) and (b) depict the results obtained using a 1 MHz FUS transducer, while panels (c) and (d) showcase outcomes for a 0.5 MHz frequency. The SDD is 30 mm in panels (a) and (c), and 35 mm in panels (b) and (d).

3.2 Results of Experiments

Results of AO measuring at transmission mode are presented in Fig. 5. FUS pressures in the focal region were measured ranging between 300 and 500 kPa. As FUS pressure increased, the amplitude of AO signal in both coherent and non-coherent light also increased; however, phase modulation in the coherent light introduced greater fluctuations in the signal. Upon deactivating FUS power, the AO signal rapidly diminished to levels comparable to the noise floor, primarily attributed to shot noise and environmental factors. Likewise, when the laser was turned off while maintaining FUS transducer activity, the AO signal also decreased to a similar noise level. This confirms that the AO signal arises from the interaction between ultrasound waves and light. The unmodulated light registered approximately 1 V, whereas the AO signal amplitude varied from 10^{-5} to 10^{-3} V. The amplitude of the AO signal demonstrated a proportional increase across three distinct FUS pressure levels at one-minute intervals. For coherent light sources, this increase followed a polynomial trend, whereas for noncoherent light, it demonstrated a linear relationship. Fig. 5(a) shows the AO signal generated by the 1 MHz FUS transducer, which produced lower intensity signal compared to the 0.5 MHz transducer, as illustrated in Fig. 5(b). This enhancement in light modulation at lower FUS frequencies, under constant pressure, can be attributed to the inverse relationship between particle displacement and ultrasound frequency. Furthermore, the larger focal area produced by the 0.5 MHz transducer likely increases

the interaction volume between light and FUS, thereby enhancing the probability of modulated light detection.

Fig. 6 illustrates the AO signal amplitude in reflection mode, using a 1 MHz FUS transducer. In transmission mode, the LED produces an AO signal comparable with CL and LD, whereas, in reflection mode, no discernible AO signal was detected when using the LED. Examination of the SDD was conducted at 30 mm in Fig. 6(a), (b), and (c) and 35 mm in Fig. 6(d), (e), and (f). Fig. 6(a) and (d) with the source angle perpendicular to the surface of the phantom, Fig. 6(b) and (e) with a 20° angle of source illumination, and Fig. 6(c) and (f) with a 45° angle of source illumination.

Analysis of the AO signals revealed an enhancement of approximately 20% at the minimum pressure level and up to 30% at the maximum pressure level for coherent light sources when the illumination angle was adjusted from perpendicular to 20°. In contrast, at a 45° angle of illumination, the AO signal demonstrated increases of up to 40% and 60% at the minimum and maximum pressure levels, respectively. These results underscore the significant impact of illumination angle on the modulation of light in reflection mode. In the following, Fig. 7 present the results obtained using a 0.5 MHz FUS transducer. Due to the diameter of the 0.5 MHz transducer exceeding 60 mm, it was not feasible to utilize it in the reflection mode configuration.

Instead, a side mode configuration was employed to assess the impact of the angle of the light source. In this arrangement, the transducer was positioned laterally relative to the phantom, with the source and detector placed above the phantom and oriented perpendicularly to each other. Similarly to the reflection mode setup with

the 1 MHz transducer, the SDDs, and the focal area were situated approximately 18 mm deep inside the tissue, as observed from the perspective of the paired fibers. As anticipated, the AO signal exhibited greater magnitude compared to the 1 MHz transducer. Analysis of the AO signals revealed a notable enhancement, with an approximate 30% increase observed at the minimum

pressure level and up to a 40% increase at the maximum pressure level for coherent light sources when the illumination angle was adjusted from perpendicular to 20°. Conversely, when illuminated at a 45° angle, the AO signal displayed a remarkable increase, reaching up to 50% and 70% at the minimum and maximum pressure levels, respectively.

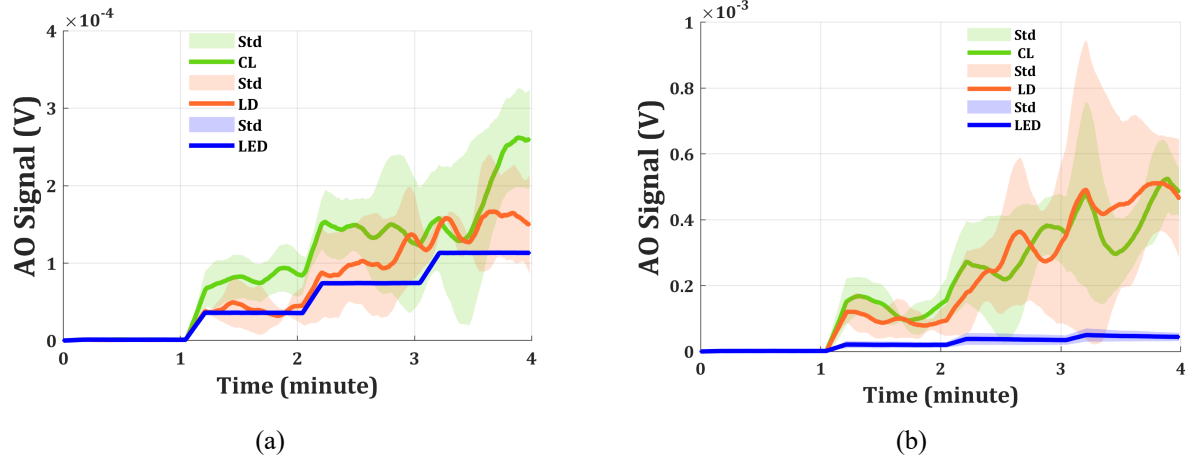


Fig. 5 The AO signal generated by FUS, which reflects the amplitude of the AO signals in transmission configurations is presented. This data was collected in the time domain and includes measurements taken at three distinct pressure levels during the first minute of FUS inactivity. Three different light sources were utilized: a crystal laser (CL) as a long-coherent source, an LD, and an LED. The curve illustrates the AO signals, while the shaded region represents the standard deviation. (a) 1 MHz frequency FUS, and (b) 0.5 MHz frequency FUS.

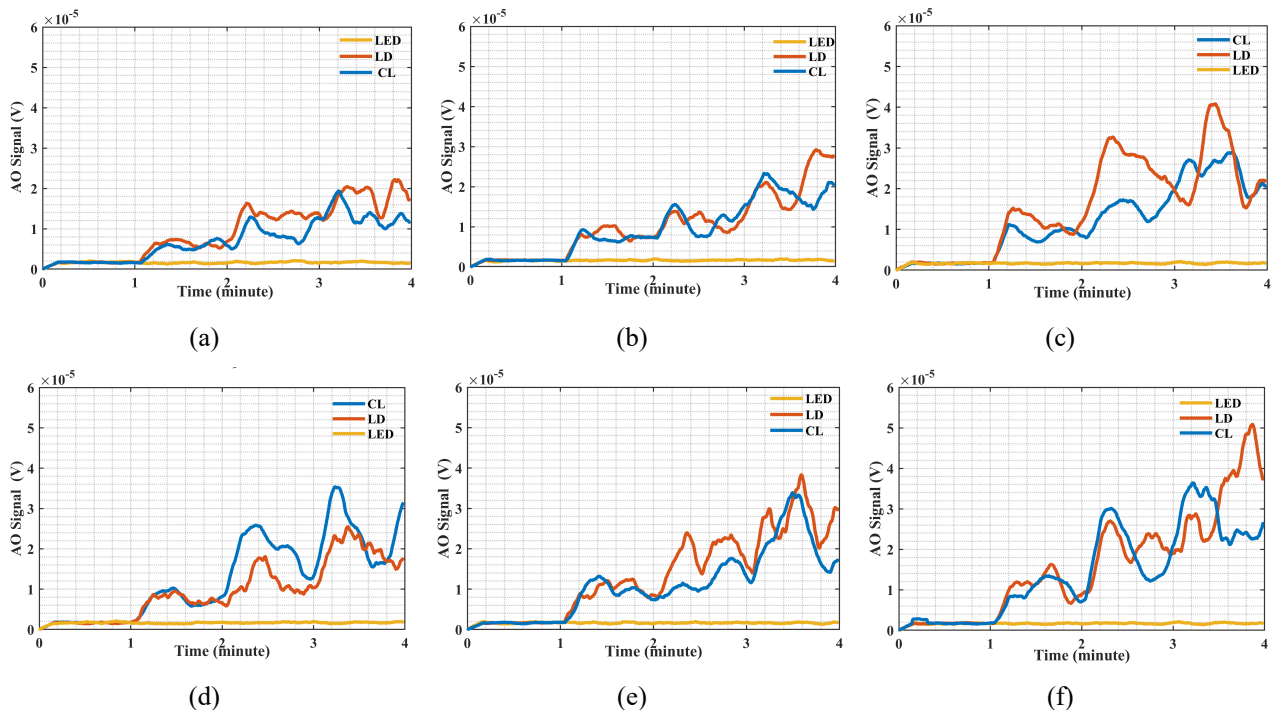


Fig. 6 The amplitude of AO signals, indicating light modulated by 1 MHz FUS transducer in reflection mode. Measurements were taken at SDD of 30 mm on the bottom and 35 mm on the top. Three different light source angle configurations were used: panels (a) and (d) with the source positioned perpendicularly to the phantom surface, panels (b) and (e) with a 20° angle relative to the X-Z plane, and panels (c) and (f) with a 45° angle relative to the X-Z plane. The blue, red, and yellow curves represent AO signals for crystal laser (CL), laser diode (LD), and (LED) illumination light sources, respectively.

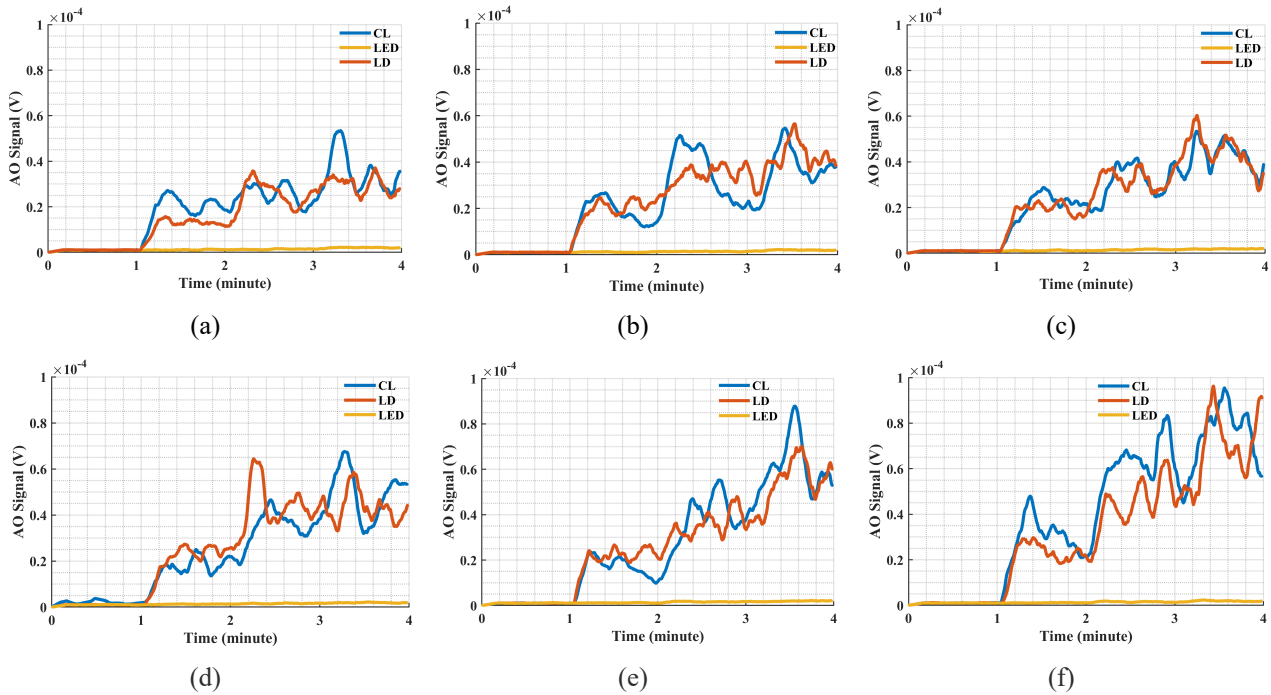


Fig. 7 The amplitude of AO signals, indicating light modulated by 0.5 MHz FUS transducer in reflection mode. Measurements were taken at SDD of 30 mm on the bottom and 35 mm on the top. Three different light source angle configurations were used: panels (a) and (d) with the source positioned perpendicularly to the phantom surface, panels (b) and (e) with a 20° angle relative to the X-Z plane, and panels (c) and (f) with a 45° angle relative to the X-Z plane. The blue, red, and yellow curves represent AO signals for crystal laser (CL), laser diode (LD), and (LED) illumination light sources, respectively.

4 Discussion and Conclusion

The numerical simulations of FUS pressure in the focal region, targeting average brain tissue, revealed pressures ranging between 300 and 500 kPa, consistent with experimental measurements. This pressure range is suitable for applications, such as, blood brain barrier (BBB) opening for brain drug delivery, as reported [22]. The results indicate that for a 0.5 MHz transducer, the focal dimensions were elongated compared to the 1 MHz transducer. This elongation is likely due to the greater transmission efficiency at lower frequencies. MC simulations of FUS-light interactions confirmed that the 0.5 MHz transducer exhibited higher tagging efficiency, attributed to the larger focal area it generated compared to the 1 MHz transducer. The close relationship between FUS pressure, frequency, and modulation efficiency was evident, with higher frequencies resulting in lower AO modulation under identical FUS pressures. This occurs because particle displacement is inversely proportional to FUS frequency [15], leading to reduced modulation amplitude at higher frequencies.

Simulation results further demonstrate that at a wavelength of 850 nm, and with FUS frequencies of 0.5 or 1 MHz, increasing the source angle from perpendicular to 45° led to increased tagging efficiency. However, increasing the SDD resulted in a decrease in tagging efficiency. Notably, the 0.5 MHz transducer consistently produced higher tagging efficiency values, trends that were corroborated by the experimental measurements.

In our study, the simulation and phantom experiments were designed with distinct objectives in mind. Although they may seem independent, they are intended to complement one another by addressing aspects of the research question. We aimed to closely match the optical properties between the phantom and the input simulation. However, due to certain practical limitations, slight differences remain. For instance, the phantom had optical absorption and scattering coefficients of approximately 0.02 mm⁻¹ and 10 mm⁻¹, respectively, while the corresponding values in the simulation were 0.037 mm⁻¹ and 9.5 mm⁻¹. By considering that in AO techniques the scattering coefficient plays a more critical role than the absorption coefficient, these differences can be considered small and do not have much significant influence. Furthermore, the detector size, ultrasound transducer shape, and frequencies were kept same and consistent between the simulation and the experiment. The source-detector distances were maintained at 30 mm and 35 mm in both cases. Additionally, three light source angles (0° – perpendicular in the experiments, 20°, and 45°) were investigated in the reflection mode configuration.

The numerical experiments provide a controlled, idealized environment to test theoretical assumptions, while the phantom experiments introduce more realism necessary for practical applications. Taken together, these approaches reinforce the findings: for instance, the pressure in the focal area ranged from 0.25 to 0.5 MPa in the simulations and from 0.3 to 0.5 MPa in the

experiments. Moreover, the experimental results in Fig. 6 indicate that a source angle of 45° yields the maximum AO signal, which is further supported by the simulation results in Fig. 4, showing that tagging efficiency is maximized at the same angle.

Several techniques for detecting ultrasound-modulated light have been developed, each with specific advantages and limitations. In this study, we employed a relatively low-complexity setup that is highly sensitive and can potentially be adapted for preclinical, and eventually clinical, applications. The setup's sensitivity allows it to detect FUS-modulated light in a human head model configuration. This configuration, where both the light source and detector fibers are positioned on the same side of the phantom, could be advantageous for transcranial high-intensity focused ultrasound (HIFU) therapy monitoring. The reflection setup, involving paired source and detector fibers, is convenient and adaptable for future *in vivo* and preclinical studies.

Across all measurements, increasing the FUS pressure corresponded to an increase in AO signal strength, reflecting a direct relationship between pressure at the focal point and signal amplitude. This trend is consistent with Fig. 5 to Fig. 7, which illustrate a linear relationship for non-coherent light sources and a more complex trend for coherent light sources. In transmission mode, the AO signal remained relatively stable for LED sources but exhibited significant fluctuations under coherent light, likely due to phase modulation effects. These fluctuations result from speckle patterns generated by variations in refractive index and particle displacement, leading to random phase interference. In contrast, the AO signal for LED sources remained stable, primarily due to intensity modulation. This stability can be explained by the pressure-induced density changes in the medium, which modulate the optical properties, and

subsequently, the intensity of the transmitted light [1, 6, 23, 24]. Wherein, experimental results confirmed the fundamental theory of AO technique, demonstrating that in non-coherent light, FUS modulates the intensity of the light.

Although the AO technique faces challenges, particularly when compared to more mature modalities like photoacoustic imaging, it remains a promising field for development. Our results show that adjusting the source illumination angle can enhance the AO signal by up to 40% using a single detector. This study represents the first experimental investigation of the effect of source angle on FUS-modulated diffusing light aiming for brain monitoring to optimize photon illumination and detection at the focal area. Three different source angles – perpendicular, 20°, and 45° relative to the X-Z plane – were evaluated. Specifically, coherent light at 20° angle showed 20% increase in AO intensity, while at 45° angle resulted in a 40% increase, both compared to the perpendicular angle.

In conclusion, these findings advance the understanding of AOS by demonstrating optimization of AO signal amplitude through light illumination angles and detection strategies. Future research will focus on enhancing the system's stability for planned *in vivo* experiments, in order to further develop it for selected clinical applications.

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Disclosures

The authors declare that they have no conflict of interest.

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